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Identification of Pea (*Pisum sativum* L.) Accessions Resistant to Fusarium Wilt Using Integrated Disease Severity Indices and Multivariate Classification

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KEY WORDS:
Fusarium wilt resistance; Pisum sativum; disease severity index; area under the disease progress curve (AUDPC); multivariate analysis.

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ABSTRACT

Fusarium wilt, caused by *Fusarium Oxysporum* f. sp. *pisi*, represents one of the most significant challenges to global pea (*Pisum sativum* L.) production, leading to substantial yield losses under favorable conditions for disease development. Conventional control approaches, such as crop rotation and chemical treatments, have shown limited long-term effectiveness, thereby reinforcing the importance of breeding for durable genetic resistance. This study aimed to identify promising pea genotypes that exhibit resistance to Fusarium wilt through an integrated evaluation of disease progression and multivariate variability. A total of 84 genotypes were tested under greenhouse conditions using naturally infested soil. Disease symptoms were recorded weekly, and cumulative disease severity was quantified using a standardized rating system. Based on symptom development, the genotypes were initially classified into resistant, moderately resistant, and susceptible groups. Multivariate analyses revealed clear phenotypic differentiation among these groups. Several genotypes consistently displayed reduced disease severity, indicating their potential value for future breeding programs. Statistical analyses validated the classification using dimensionality reduction and discrimination techniques. This study demonstrates that integrating disease severity data with multivariate analyses provides an effective and reproducible framework for the early identification of resistant genotypes. The promising genotypes identified herein serve as valuable genetic resources for developing resistant cultivars that support the sustainability of legume production under increasing biotic stress conditions.

تحديد التراكيب الوراثية من البازلاء المقاومة لذبول الفيوزاريوم باستخدام مؤشرات شدة المرض وتحليل التباين المتعدد

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الخلاصة

يُعد ذبول الفيوزاريوم، الناتج عن *Fusarium Oxysporum* f. sp. *pisi* ، من أهم التحديات التي تواجه إنتاج البازلاء (*Pisum sativum* L.) على مستوى العالم، حيث يتسبب بخسائر جسيمة في ظل الظروف الملائمة للمرض .لم تُظهر طرق المكافحة التقليدية، مثل التدوير الزراعي والمعالجات الكيميائية، فعالية طويلة الأمد، مما يُعزز من أهمية التحسين الوراثي لمقاومة المرض .استهدفت هذه الدراسة تحديد التراكيب الوراثية الواعدة من البازلاء التي تُظهر مقاومة لذبول الفيوزاريوم، من خلال تقييم تكاملي لتطور المرض وتحليل التباين المتعدد . تم اختبار 84 تركيبًا وراثيًا تحت ظروف الدفيئة باستخدام تربة مصابة طبيعيًا .تم تسجيل الأعراض أسبوعيًا، واحتُسبت شدة المرض التراكمية باستخدام مقياس معياري .استنادًا إلى تطور الأعراض، تم تصنيف التراكيب إلى مجموعات أولية كمرشحين مقاومين، معتدلين، وحساسين .كشفت التحليلات المتعددة عن تمايز ظاهري واضح بين هذه المجموعات .أظهرت عدة تراكيب انخفاضًا ثابتًا في شدة المرض، مما يشير إلى إمكانية استخدامها في برامج التربية المستقبلية .أكدت التحاليل الإحصائية صحة التصنيف من خلال تقنيات تقليل الأبعاد والتصنيف .تُظهر هذه الدراسة أن دمج بيانات شدة المرض مع التحليل المتعدد يوفر إطارًا فعالًا وقابلاً للتكرار للتعرف المبكر على التراكيب المقاومة .تُعد التراكيب الواعدة المحددة في هذه الدراسة موادًا وراثية قيّمة لتربية أصناف مقاومة تدعم استدامة إنتاج البقوليات تحت ظروف الإجهاد الحيوي المتزايدة.

الكلمات المفتاحية: مقاومة ذبول الفيوزاريوم؛ البازلاء؛ مؤشر شدة المرض؛ المساحة تحت منحنى تطور المرض(AUDPC) ؛ التحليل المتعدد المتغيرات.

INTRODUCTION

Pea (*Pisum sativum* L.) is a high-value legume crop and an important source of plant-based protein worldwide (Fahim, Attia, & Kamel, 2019). In addition to its economic significance, pea cultivation contributes to agricultural diversification and global food security. According to data from the Food and Agriculture Organization (FAO), global pea production reached 48 million tons in 2023 (FAO, 2023). In Indonesia, the Ministry of Agriculture reported a 15% increase in national pea production in 2024, with total yields exceeding 100,000 tons (Sanaky, 2024). However, despite this upward trend, Fusarium wilt continues to limit yields, with affected plants exhibiting stunted growth, basal leaf chlorosis, wilting, and eventual plant collapse. This disease, caused by *Fusarium Oxysporum* f. sp. *pisi* (Fop), frequently interferes with pea production (Chakrapani *et al*., 2023). The disease disrupts water and nutrient transport in the vascular system, causing wilting, necrosis, and yield losses that may reach 100% under favorable conditions (Deng *et al*., 2022). Current disease management strategies still rely on conventional methods, such as fungicide applications and crop rotation (Awal *et al*., 2024). Nevertheless, these conventional approaches present several limitations, including high costs, negative environmental impacts, and inconsistent long-term effectiveness. Therefore, developing Fop-resistant pea varieties represents a more sustainable and ecologically sound solution. Identifying accessions with high resistance is a crucial first step in breeding programs aimed at enhancing disease resistance.

Several studies have evaluated pea resistance to Fop using various methods and treatments. For instance, Bani *et al*. (2012) assessed pea resistance to Fop race 2 by measuring disease incidence, disease rating over time, and the area under the disease progression curve (AUDPC). Similarly, Haglund and Kraft (1979) examined the resistance response of different pea cultivars to Fop and identified significant variation in susceptibility. Additionally, Sharma *et al*. (2018) applied PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) to detect Fop presence in pea plants, a technique useful for early selection of resistant cultivars. Other studies have also tested Fusarium wilt resistance in fewer pea genotypes using conventional disease scoring approaches, demonstrating resistance variation but without comprehensive statistical classification (Dhar *et al*., 2011; Sharma Brij and Sharma Vinod, 2013; Bani *et al*., 2018). However, previous evaluations typically involved a limited number of pea genotypes and primarily relied on single-parameter assessments such as disease rating or disease incidence, which restricts the ability to comprehensively characterize resistance variability within large germplasm collections.

Disease severity assessment is widely used as a quantitative method to evaluate disease progression based on symptom scales. While this approach offers high reliability, it is often limited to descriptive statistics and lacks deeper insights into variation patterns. Advanced statistical tools such as Principal Component Analysis (PCA) and biplots offer an improved framework to explore variation patterns and relationships among accessions based on their disease response. Additionally, centroid analysis enhances PCA results by enabling more objective clustering in a multidimensional space, facilitating identification of resistance distribution patterns (Mustakim, 2017). Venn diagrams also serve as an additional visualization tool to classify accessions with similar responses. In a study by Aslam *et al*. (2013), PCA was successfully applied to assess resistance in various *Cicer arietinum* L. genotypes against Fusarium, allowing the identification of superior-resistant genotypes.

The purpose of this study was to evaluate genetic resistance variation among 84 pea accessions against *Fusarium Oxysporum* f. sp. *pisi* by integrating temporal disease severity assessment with AUDPC quantification and PCA-based multivariate classification. The integration of disease assessment and multivariate analysis not only provides valuable insights for breeding programs but also offers a systematic approach for evaluating plant genetic resources for disease resistance thoroughly. This study was designed to reveal the extent of genetic variability in resistance to Fusarium wilt among diverse pea accessions and to identify highly resistant candidates that can be prioritized for breeding. Additionally, the findings are expected to provide a stronger scientific basis for resistance classification and support more effective selection strategies in future pea improvement programs aimed at developing cultivars with durable and reliable protection against *Fusarium Oxysporum* f. sp. *pisi*.

MATERIALS AND METHODS

Plant Materials and Experimental Design

This study employed an augmented experimental design to evaluate resistance levels in 84 accessions of pea (*Pisum sativum* L.) against *Fusarium Oxysporum* f. sp. *pisi*

(Fop). Each accession comprised ten plants, resulting in a total of **840 individual plants grown** under greenhouse conditions at Brawijaya University, Malang, Indonesia, from September to December 2024. Environmental parameters were **maintained within controlled ranges**, including a temperature of 25–30°C, relative humidity of 60–80%, and a 12-hour photoperiod.

Plants were cultivated in individual plastic pots (30 cm diameter × 30 cm height), each containing 5 kg of **homogenized growth medium** composed of field soil, rice husk, and farmyard manure in a 2:1:1 ratio. Pots were arranged 30 cm apart to facilitate adequate aeration and minimize inter-plant competition. **Watering was performed every two days with 500 mL of water per pot.** Fertilizers were excluded to avoid confounding nutrient effects.

Although augmented designs typically involve untreated controls, the failure to establish reliable non-inoculated controls in this study necessitated a comparative approach among accessions. Resistance was evaluated using multiple indicators, including the Disease Severity Index (DSI), the Area Under the Disease Progress Curve (AUDPC), and multivariate classification.

Pathogen Inoculation and Growth Conditions

Pathogen inoculation was performed using naturally infested soil collected from agricultural fields in Malang known to be prone to Fusarium wilt. Donor plants displayed typical symptoms of Fop infection, including basal chlorosis, root necrosis, vascular discoloration, and wilting, in accordance with established diagnostic standards (Charchar and Kraft, 1989;Chiwina *et al*., 2023). These symptoms were consistently reproduced in previous trials using the same source, **thereby supporting the reliability of symptom-based identification.**

Although molecular confirmation was not conducted, the high host specificity of Fop to *Pisum sativum* along with consistent symptomology across trials provided adequate validation. Following protocols adopted in previous semi-natural infection studies (Shahiba *et al*., 2024;Pugliese *et al*., 2013), infested soil was mixed with sterilized field soil at a 1:4 ratio. Seeds were sown directly into the prepared medium, and watering frequency was maintained to ensure moisture retention without leaching the inoculum. **Digital sensors were used to monitor temperature and humidity throughout the experiment.**

Figure 1. Characteristic symptoms of Fusarium wilt in a donor pea plant used as the soil inoculum source are shown, including severe leaf chlorosis, wilting, basal stem discoloration, and root necrosis**, which are pathognomonic for** *Fusarium Oxysporum* f. sp. *pisi* infection in *Pisum sativum*.

Disease Assessment

Disease severity was monitored weekly over five weeks on days 28, 35, 42, 49, and 56 after planting. Scoring was performed using a standardized 0–5 scale adapted from Charchar and Kraft (1989), where 0 = no symptoms, 1 = initial chlorosis and stipule curling, 2 = progressive yellowing, 3 = wilting and stunting, 4 = vascular discoloration, and 5 = complete plant collapse. The AUDPC was calculated to quantify cumulative disease progression, capturing both intensity and temporal development. This metric is widely recognized as a reliable indicator of quantitative resistance.

Statistical Analysis

Statistical analysis was conducted using RStudio version 4.2.2. One-way ANOVA was applied via the agricolae package to evaluate differences in AUDPC among resistance categories. Tukey’s Honest Significant Difference (HSD) test was used for post hoc comparison at a 5% significance level. Assumptions of normality and homogeneity were verified using the Shapiro–Wilk and Levene’s tests, respectively.

Accessions were categorized into three resistance levels based on empirically determined AUDPC thresholds: resistant (≤100), moderate (101–200), and susceptible (>200).

Accessions were categorized into three resistance levels based on empirically determined AUDPC thresholds: resistant (≤100), moderate (101–200), and susceptible (>200). The Area Under the Disease Progress Curve (AUDPC) was calculated using the trapezoidal method, following the formula: $AUDPC = \Sigma[(Xi + Xi+1)/2] \times (ti+1 - ti)$ (Bani *et al.*, 2012).

In this formula, X_i represents the estimated proportion of disease severity at assessment date i, X_{i+1} is the estimated proportion of disease severity at date $i+1$, and $(t_{i+1} - t_i)$ denotes the number of days between the two consecutive assessment dates. This approach quantitatively integrates both the severity and duration of disease symptoms, providing a comprehensive measure of resistance response over time. Principal Component Analysis (PCA) was conducted using the prcomp() function on standardized data to explore clustering and variation structure. The first two principal components accounted for more than 70% of the total variance, with PC1 strongly associated with disease severity. Linear Discriminant Analysis (LDA) using the MASS package further validated the resistance classification, showing clear phenotypic separation. Visualizations were generated using ggplot2 and factoextra packages.

RESULT AND DISCUSSION

Figure 2 illustrates the severity of Fusarium wilt symptoms in pea plants at 49 days after planting (DAP), with six disease categories ranging from 0% (healthy plants) to 100% (total plant death). Plants classified as 0% severity (Figure 2a) exhibited no visible symptoms of Fusarium wilt, maintaining green leaves and healthy stems. Plants with mild symptoms (Figure 2b) showed early signs of infection, such as pale yellow-green discoloration and slight downward curling of leaf edges. As the severity increased, moderate symptoms (Figure 2c) were characterized by progressive leaf yellowing, beginning from the base of the stem. More advanced disease stages involved significant wilting and stunting (Figure 2d), drying of lower leaves (Figure 2e), and complete plant collapse or necrosis (Figure 2f, 100% severity). To quantify these visual observations, ANOVA analysis (Table 1) was performed to determine whether the differences in disease severity across accessions were statistically significant. The results indicated a highly significant variation ($p < 0.001$) among accessions, confirming the genetic influence on resistance to Fusarium wilt. These symptom classes, from asymptomatic green plants to complete wilting and necrosis, are consistent with established descriptions of Fusarium wilt in peas, where leaf yellowing, downward curling, progressive wilting, vascular discoloration, and eventual plant death are reported as characteristic features of severe infection. This visual scoring approach has also been widely adopted in previous Fusarium wilt studies in pea plants, supporting the validity of the presentation of results in this study (Usman *et al*., 2025).

Figure 2. Visual representation of Fusarium wilt severity in pea plants at 49 days after planting (DAP). Disease severity was scored on a standardized 0–5 scale: (a) No visible symptoms (score 0), (b) Early leaf chlorosis and stipule curling (score 1), (c) Progressive leaf yellowing from base to apex (score 2), (d) Wilting and stunting (score 3), (e) Lower leaf drying and vascular discoloration (score 4), and (f) Complete wilting and plant death (score 5). These categories reflect increasing levels of host susceptibility to *Fusarium Oxysporum* f. sp. *pisi*.

Table 1: Analysis of Variance (ANOVA) for Disease Severity (DS) and AUDPC

Source	Df	Sum Sq	Mean Sq	F Value	P-Value
Between Groups	2	150731.05	75365.52	115.34	1.99×10^{-24}
Within Groups	81	52926.75	653.42		

The evaluation of Fusarium wilt severity in pea accessions using a 0–5 disease severity scale provided insights into the variation in resistance responses across the tested genotypes. Plants that scored 0 (Figure 2a) exhibited complete resistance, showing no symptoms of Fusarium wilt, suggesting the presence of strong defense mechanisms such as reinforced cell walls, antimicrobial compound production, or hypersensitive response (HR). According to Bhatnagar Sakshi (2017), resistant plants often display higher peroxidase and polyphenol oxidase activity, which strengthens vascular tissue protection against pathogen invasion.

Plants with scores of 1–2 (Figures 2b–2c) showed mild symptoms, including early leaf chlorosis and slight wilting, which could indicate partial tolerance. This moderate response may result from localized defense signaling that slows disease progression (Sun and Zhang, 2021). In contrast, plants scoring 3–4 (Figures 2d–2e) exhibited severe wilting, necrosis, and impaired growth, reflecting high genetic susceptibility to Fusarium wilt. At the end, plants scoring 5 (Figure 2f) experienced complete plant death, characterized by vascular system collapse and systemic necrosis, which aligns with the findings of Barbosa and Benedetti (2007) that describe how *Fusarium Oxysporum* occludes xylem vessels with biofilms and toxins, leading to water transport failure.

The disease scoring results highlight the importance of phenotype-based selection in identifying Fusarium-resistant accessions. Accessions with low disease severity (scores 0–1) can be valuable genetic resources for breeding programs aimed at enhancing Fusarium wilt resistance in peas.

Figure 3 presents the weekly progression of disease severity (DS) in pea accessions over five weeks. In the first week, DS values were relatively low for most accessions, with only PSAT56-036 (24%) and PSAT22-009 (14.28%) showing signs of early infection. However, by the second week, disease progression intensified, particularly in PSAT56-038, which reached 52% severity, indicating heightened susceptibility to *Fusarium Oxysporum* f. sp. *pisi*. From the third to fourth week, multiple accessions exhibited a sharp increase in disease severity, culminating in total plant mortality (100% DS) in some cases. Accessions such as PSAT65-003 and PSAT56-038 exhibited a rapid progression, reaching 80% severity by the third week and complete collapse by the fourth week. Meanwhile, resistant accessions, such as PSAT44-042 (3.3%) and PSAT57-002 (0%), maintained low disease severity throughout the experiment. By the fifth week, most accessions reached peak disease severity, marking the final infection stage. This contrasting pattern reflects differences in host defense efficiency, where susceptible accessions experience rapid pathogen colonization of vascular tissues, while resistant accessions effectively restrict fungal proliferation and delay symptom expression over time. Such vascular wilt patterns have also been documented in previous pathosystems, where penetration and rapid xylem colonization of *Fusarium Oxysporum* in susceptible cultivars led to systemic wilting, whereas resistant cultivars restricted xylem invasion and maintained vascular integrity (Li *et al*., 2015). This aligns with the infection dynamics observed in the present study, in which accelerated vascular disruption explains the rapid transition from initial symptoms to complete plant collapse in highly susceptible accessions.

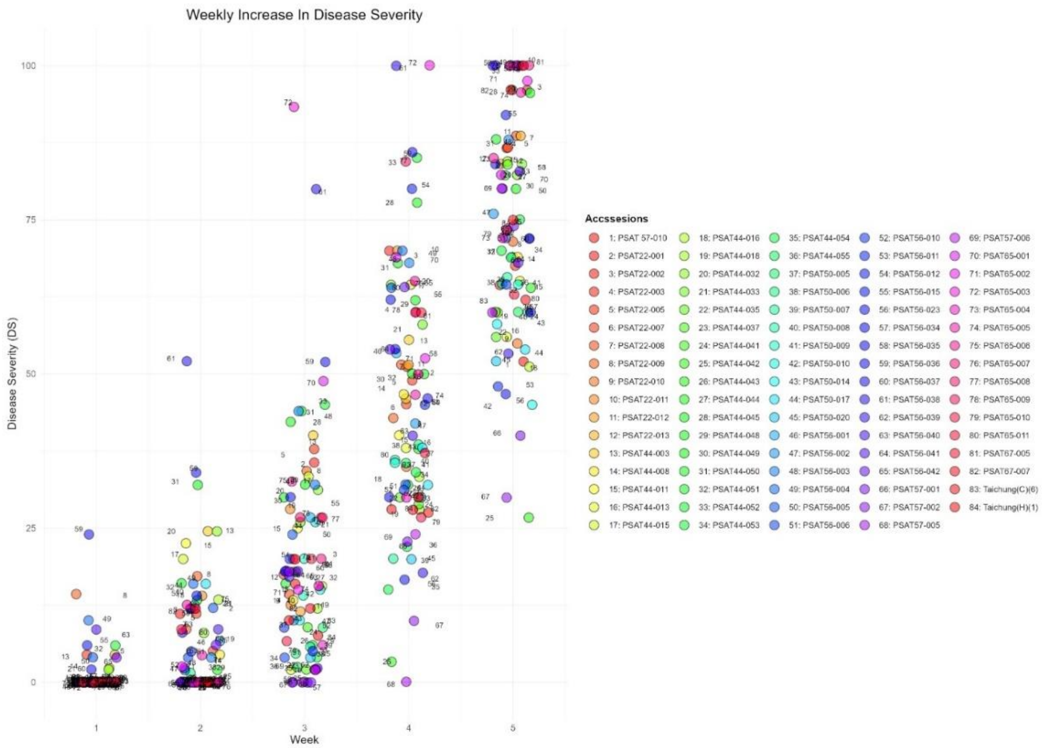

Figure 3. Weekly progression of disease severity in pea accessions over a five-week period. Data represent average severity scores per week across 84 accessions based on a 0–5 visual scale. The trend highlights differential responses to Fusarium wilt infection among resistant, intermediate, and susceptible groups.

The progression of disease severity (DS) over five weeks reflected significant differences among accessions in their ability to withstand Fusarium wilt. In the first week, DS values remained low across most accessions, with only PSAT56-036 (24%) and PSAT22-009 (14.28%) displaying early signs of infection. By the second week, disease progression intensified, particularly in PSAT56-038 (52%), indicating greater pathogen susceptibility due to the rapid breakdown of host defenses (Zuriegat, Zheng, Liu, Wang, & Yun, 2021). From weeks three to four, several accessions experienced a sharp increase in disease severity, with some reaching 100% DS, resulting in complete plant mortality. Highly susceptible accessions such as PSAT65-003 and PSAT56-038 exhibited accelerated symptom development, likely due to the accumulation of pathogen-derived toxins that impair the xylem transport system (de Quadros, de Freitas, Simioni, Ferreira, & Stadnik, 2020).

In contrast, resistant accessions PSAT44-042 (3.3%) and PSAT57-002 (0%) maintained low DS values throughout the experiment, suggesting effective defense mechanisms such as antimicrobial compound production and hypersensitive response (HR). These findings align with previous studies indicating that resistant plants often exhibit stronger lignification and oxidative enzyme activity to prevent pathogen colonization (Xue *et al*., 2017).

The Area Under the Disease Progress Curve (AUDPC) values obtained from 84 pea accessions indicate the distribution of plant resistance levels against Fusarium wilt into three main categories: resistant, intermediate, and susceptible (Figure 4). The analysis revealed that 21 accessions exhibited resistance to the pathogen, while 20 accessions were classified as susceptible. The majority of accessions, comprising 43 accessions, fell into the intermediate resistance category.

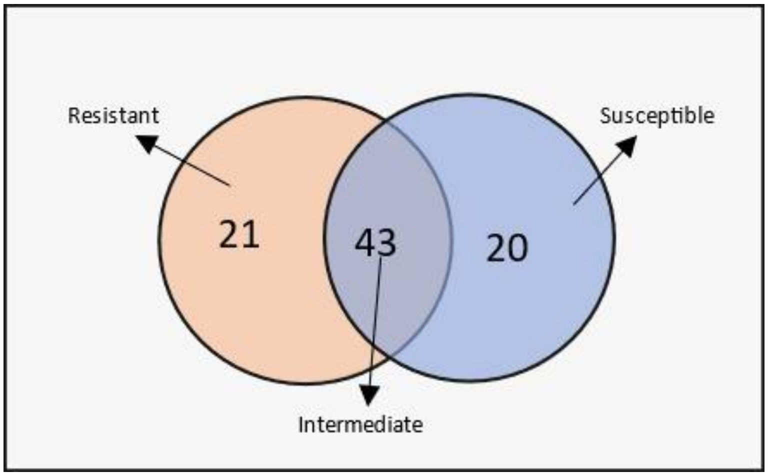

Figure 4. Frequency distribution of 84 pea accessions categorized by resistance level to *Fusarium Oxysporum* f. sp. *pisi* based on Area Under the Disease Progress Curve (AUDPC) values. Accessions were classified as resistant (AUDPC ≤ 100), moderate (101–200), or susceptible (AUDPC > 200).

The distribution presented in Figure 4 highlights significant genetic diversity within the tested pea population. The 21 resistant accessions likely possess genetic traits conferring resistance to Fusarium. This resistance is associated with mechanisms such as cell wall reinforcement, hypersensitive responses, or the production of antimicrobial compounds that restrict pathogen spread. In contrast, susceptible accessions exhibit a lack of effective defense mechanisms, allowing the pathogen to rapidly infect vascular tissues. The genetic factors underlying this susceptibility may include the absence of defense pathway activation or heightened sensitivity to pathogen virulence factors (Galindo-González & Deyholos, 2016).

To categorize the 84 pea accessions based on their response to *Fusarium Oxysporum* f. sp. *pisi* infection, Area Under the Disease Progress Curve (AUDPC) values were analyzed. Three resistance categories were established: resistant (AUDPC ≤ 100), moderate (AUDPC = 101–200), and susceptible (AUDPC > 200). This classification scheme enabled a structured grouping of accessions based on the severity and progression of disease symptoms.

Figure 5 illustrates the distribution of AUDPC values across the three resistance categories. Resistant accessions displayed significantly lower AUDPC values, indicating slower disease progression, while susceptible accessions exhibited the highest values, reflecting rapid symptom development and severe infection. Intermediate accessions fell between these two extremes, showing a moderate disease response

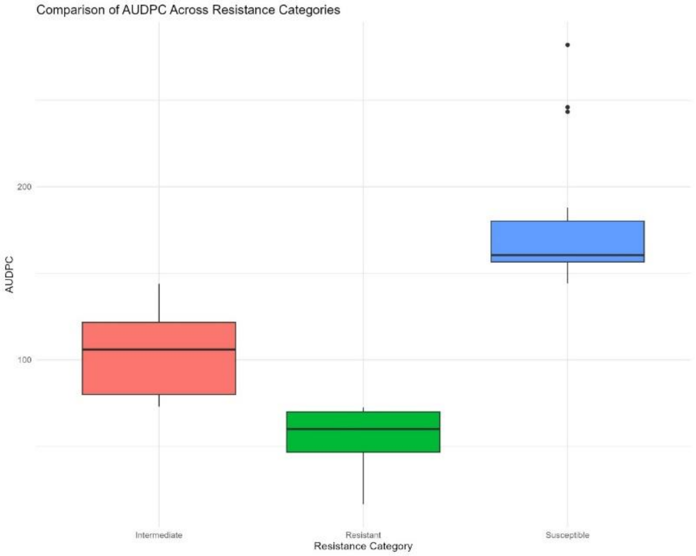

Figure 5. Boxplot showing the distribution of AUDPC values across resistance categories. Resistant accessions displayed significantly lower AUDPC values compared to moderate and susceptible groups (ANOVA, p < 0.001), confirming consistent differences in disease progression.

To validate the differences among these categories statistically, one-way ANOVA followed by Tukey's Honest Significant Difference (HSD) test was conducted. The results (Table 2) confirmed that the mean differences in AUDPC values between all three groups were statistically significant ($p < 0.001$), providing strong support for the reliability of this classification system. This statistical grouping formed the basis for further multivariate analyses, including Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), aimed at validating and visualizing the distribution of resistance traits within the accession panel.

Table 2: Tukey HSD Test for Multiple Comparisons Among Resistance Categories

Group Comparison	Mean Difference	Lower Bound	Upper Bound	P-Value
Resistant Vs. Intermediate	-49.14	-65.39	-32.89	7.79×10^{-10}
Intermediate Vs. Susceptible	71.37	54.86	87.89	2.50×10^{-11}
Resistant Vs. Susceptible	120.51	101.45	139.58	2.50×10^{-11}

The statistical grouping of accessions into resistant, moderate, and susceptible categories was supported by Tukey HSD results, which revealed significant differences in

AUDPC values among the three groups. Accessions classified as resistant consistently exhibited lower AUDPC values, reflecting delayed symptom development and reduced disease intensity. This indicates a more efficient defense response, potentially involving cell wall reinforcement, oxidative enzyme activation, or localized hypersensitive response that limits pathogen spread (Spanic *et al*., 2011;Kumar *et al*., 2021).

In contrast, susceptible accessions showed significantly higher AUDPC values, indicative of rapid vascular colonization and systemic breakdown. This suggests limited or delayed activation of defense signaling, allowing the pathogen to compromise xylem integrity and accelerate wilt progression. Intermediate accessions displayed moderate disease responses, likely reflecting partial resistance mechanisms governed by quantitative trait loci (QTLs) with additive or incomplete effects (Pandey, Sarkar, & Sharma, 2006). These findings reinforce the validity of AUDPC-based classification and highlight its value in early-generation screening. Accessions with significantly lower AUDPC values, as confirmed by post-hoc comparison, represent promising candidates for use in breeding programs targeting durable resistance to *Fusarium Oxysporum* f. sp. *pisi*. Such a pattern is consistent with previous comprehensive AUDPC-based screening of Pisum spp., where accessions exhibited a broad spectrum of disease responses under Fop inoculation, confirming the quantitative nature of resistance and validating AUDPC as a robust tool for early-generation identification of resistant genotypes (Bani *et al*., 2012).

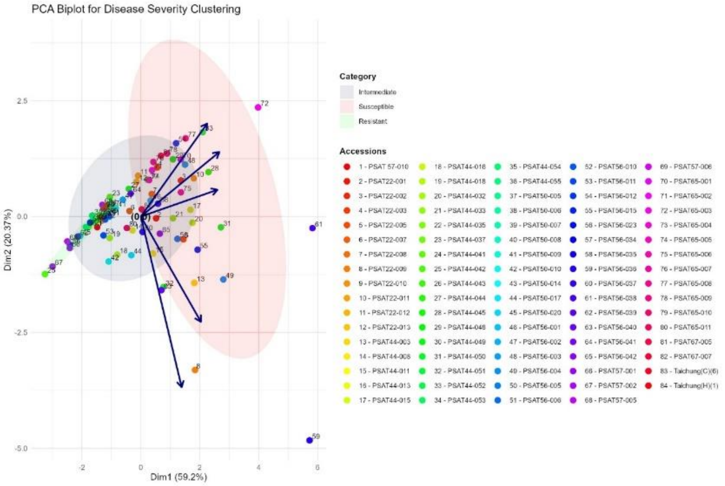

Figure 6. Principal Component Analysis (PCA) biplot based on AUDPC values of 84 pea accessions. Dim1 (PC1) and Dim2 (PC2) represent the two principal components explaining the highest variation (59.2% and 20.37%, respectively). Ellipses indicate 95% confidence intervals for each resistance category.

The Principal Component Analysis (PCA) was conducted to evaluate the variation in disease severity across 84 pea accessions based on AUDPC values (Figure 6). The first two principal components (PC1 and PC2) explained approximately 80% of the total variance, with PC1 contributing 59.2% and PC2 accounting for 20.37%. This indicates that PC1 serves as the primary axis differentiating resistant, intermediate, and susceptible accessions, whereas PC2 captures minor variations that are not directly related to disease severity.

The Principal Component Analysis (PCA) revealed that PC1 (59.2% variance) serves as the primary axis differentiating resistant, intermediate, and susceptible accessions, while PC2 (20.37% variance) contributes to secondary variations likely influenced by environmental or morphological factors. Resistant accessions, such as PSAT44-042, PSAT57-002, and PSAT57-005, were positioned at the low PC1 extreme, confirming their low AUDPC values and strong resistance. The clustering of these accessions suggests that genetic resistance mechanisms, such as cell wall lignification and hypersensitive response (HR), play a crucial role in limiting pathogen spread (Smirnova & Kochetov, 2016).

Table 3: Correlation Between Principal Components (PC1 and PC2) and AUDPC

Principal Component	Correlation With Audpc (R)	P-Value
PC1	0.993	< 0.001
PC2	0.09	0.413

PC1 exhibited a very strong positive correlation with AUDPC ($r = 0.993$, $p < 0.001$) (Table 3), confirming that accessions with higher PC1 values experienced greater disease severity, while those with lower PC1 values demonstrated stronger resistance. Resistant accessions such as PSAT44-042, PSAT57-002, and PSAT57-005 were positioned on the left side of the PCA biplot, indicating low AUDPC values and high stability across different environments. In contrast, susceptible accessions like PSAT56-038, PSAT56-036, and PSAT65-003 were located on the far right of the biplot, reflecting high AUDPC values and strong susceptibility to Fusarium wilt. This separation pattern along PC1 occurs because this component predominantly explains variation in disease progression driven by *Fusarium* colonization in vascular tissues, where genotypes with stronger defense responses exhibit slower symptom development and lower AUDPC. Similar findings have been documented in other crop species, where PCA-based analyses identified PC1 as the principal axis separating resistant and susceptible genotypes based on AUDPC and disease severity traits. This pattern is consistent with previous PCA-based evaluations reported by Chen *et al*. (2009) and collectively supports the use of PC1 as a reliable, biologically meaningful indicator for early-generation screening of Fusarium wilt resistance. Figure 7 further illustrates the relationship between PC1 and disease severity, confirming that PC1 is the dominant determinant of resistance classification.

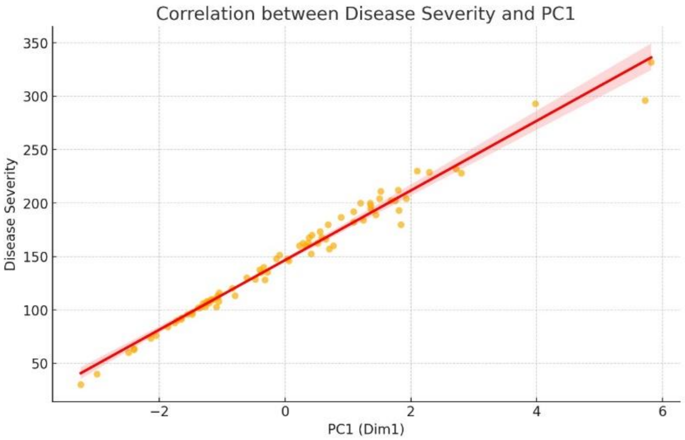

Figure 7. Pearson correlation between Principal Component 1 (PC1) and AUDPC values across 84 pea accessions. A strong positive correlation (r = 0.993, p < 0.001) indicates that PC1 effectively captures variation in disease severity, supporting its role in resistance classification.

On the other hand, PC2 contributed only 20.37% of the total variance (Figure 6) and showed a very weak and non-significant correlation with AUDPC (r = 0.090, p = 0.413) (Table 3). This suggests that PC2 does not significantly influence disease severity but instead captures secondary variations that may be related to environmental factors, plant architecture, or physiological traits. Figure 8 demonstrates the lack of a clear pattern between PC2 and AUDPC, reinforcing that PC2 does not contribute meaningfully to resistance classification.

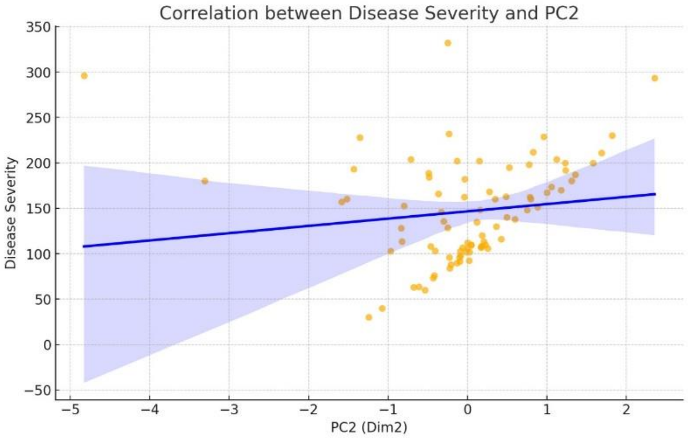

Figure 8. Pearson correlation between Principal Component 2 (PC2) and AUDPC values. PC2 showed a weak and non-significant correlation (r = 0.090, p = 0.413), suggesting it captures variation unrelated to disease severity, possibly due to environmental or morphological differences.

Conversely, susceptible accessions, including PSAT56-038, PSAT56-036, and PSAT65-003, were positioned at the high PC1 extreme, aligning with higher AUDPC values and increased disease severity. The strong correlation between high PC1 values and high AUDPC scores ($r = 0.993$, $p < 0.001$) indicates that PC1 effectively captures the variance associated with disease progression. These results reinforce previous findings that high disease susceptibility is often linked to weak structural defense and increased pathogen colonization in vascular tissues (Pluta *et al*., 2012). Intermediate accessions, such as PSAT44-016, PSAT44-018, and PSAT50-017, were distributed between resistant and susceptible clusters, suggesting partial adaptation to disease stress. This positioning aligns with previous studies indicating that quantitative resistance mechanisms in plants moderate disease progression rather than completely inhibiting infection (Montoya-Martínez *et al*., 2024). The distribution of these accessions also suggests that their disease responses may be influenced by both genetic and environmental interactions, making them potential candidates for targeted agronomic management strategies.

To further validate the clustering of accessions, a centroid coordinate analysis was conducted for each resistance category (Figure 9). The results revealed that resistant accessions had a centroid at (PC1: -0.73, PC2: 0.09), confirming their low disease severity and stable resistance (Table 4). Intermediate accessions were centered at (PC1: 0.25, PC2: 0.28), indicating partial adaptation to disease pressure, while susceptible accessions had a centroid at (PC1: 1.43, PC2: 0.40) (Table 4), reflecting high disease severity and strong environmental dependence. The centroid positioning supports the interpretation that PC1 effectively classifies accessions based on their resistance to Fusarium wilt, while PC2 accounts for minor, non-disease-related variations.

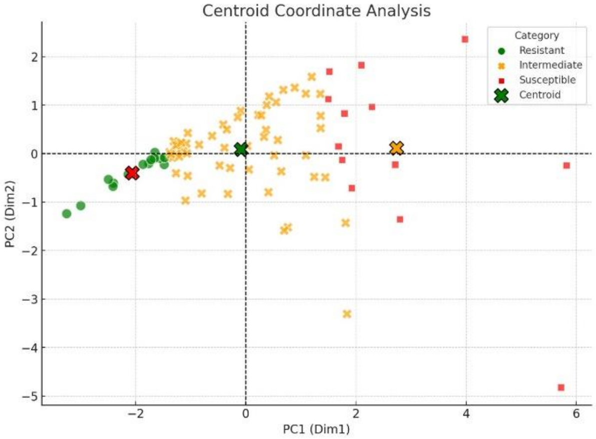

Figure 9. Centroid coordinates of PCA scores for each resistance category. Resistant, moderate, and susceptible groups showed clear separation along PC1, confirming its discriminatory power. PC2 contributed minor variation within groups but did not influence resistance classification.

PCA results confirmed the classification of accessions into resistant, intermediate, and susceptible groups. Resistant accessions demonstrated stable resistance and are promising candidates for breeding programs aimed at improving disease resistance.

Intermediate accessions may require tailored agronomic strategies to enhance resilience. Susceptible accessions, which displayed high disease severity, necessitate alternative control strategies to mitigate the impact of Fusarium wilt in affected growing conditions. These insights highlight the effectiveness of PCA in differentiating resistance levels and provide a valuable framework for future selection of resistant pea genotypes.

Table 4: PCA Centroids for Resistance Categories

Resistance Category	Pc1 Centroid	Pc2 Centroid
Intermediate	0.250249433	0.285544218
Resistant	-0.734366004	0.096867577
Susceptible	1.433726708	0.404534161

The centroid analysis further supports the distinct clustering of resistance categories, with resistant accessions centered at (-0.73, 0.09), intermediate accessions at (0.25, 0.28), and susceptible accessions at (1.43, 0.40). This separation validates PC1 as a reliable predictor of disease resistance, as accessions with lower PC1 values consistently exhibited greater resistance to Fusarium wilt. Meanwhile, PC2 showed a weak and non-significant correlation with AUDPC ($r = 0.090$, $p = 0.413$), confirming that PC2 does not significantly contribute to resistance classification and likely represents minor variations related to environmental or morphological differences (MajidiMehr, El Gataa, Abyar, & Nourinejad, 2024).

These findings align with previous studies emphasizing the utility of PCA in plant resistance analysis, as PC1 has been shown to effectively distinguish resistant and susceptible genotypes in systemic plant diseases (Elhaik, 2022). The strong correlation between PC1 and AUDPC-based disease severity classification reinforces that PCA can serve as an additional tool for resistance screening, complementing traditional selection methods such as AUDPC scoring and genetic marker-assisted selection. Given these results, integrating PCA into breeding programs can provide a more quantifiable, statistically validated approach to identifying superior Fusarium wilt-resistant accessions.

To further validate the resistance classification, a Linear Discriminant Analysis (LDA) was conducted based on disease response metrics. The resulting LDA biplot (Figure 10) displayed a clear separation among the three resistance categories: resistant, moderate, and susceptible. Accessions classified as resistant were predominantly positioned in the negative region of LD1, forming a compact and well-defined cluster. In contrast, susceptible accessions were distributed across the positive LD1 axis, exhibiting broader variation along LD2, which likely reflects differential progression of disease severity. Moderate accessions were concentrated near the center, partially overlapping with both groups, indicating intermediate responses. The separation of these clusters confirms that the classification scheme derived from PCA and AUDPC values is statistically supported and biologically meaningful, demonstrating distinct resistance patterns across accessions.

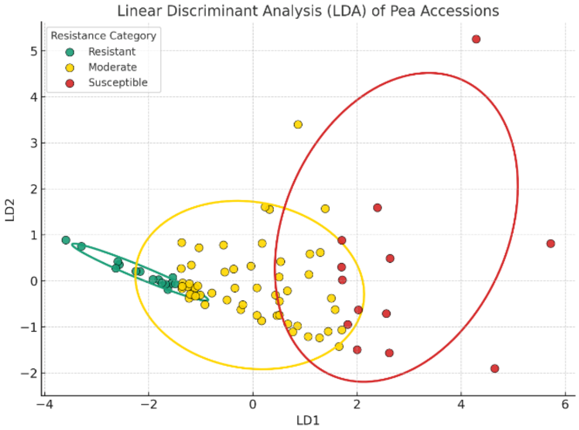

Figure 10. Linear Discriminant Analysis (LDA) plot showing separation of 84 pea accessions based on resistance classification. Accessions are grouped into resistant (green), moderate (yellow), and susceptible (red) categories. The plot demonstrates clear separation along LD1 and LD2, validating the robustness of PCA- and AUDPC-based classification.

The LDA results further strengthen the interpretation of resistance classification by demonstrating that the three predefined categories—resistant, moderate, and susceptible—can be statistically distinguished based on disease response traits. The distinct spatial separation observed in the LDA plot suggests that the underlying phenotypic responses to Fusarium wilt are sufficiently consistent and structured, allowing supervised learning models to classify accessions with minimal overlap. These findings indicate that resistance is not randomly distributed but is genetically or physiologically determined (Yahaya *et al*., 2016;Kushalappa *et al*., 2016).

The ability of LDA to clearly separate resistance categories supports its use as a complementary tool for screening and decision-making in breeding programs. Specifically, it provides a predictive framework that can be integrated into early selection pipelines, enabling breeders to prioritize genotypes based on disease metrics that are objectively quantifiable and reproducible. Together with PCA and AUDPC-based classification, this layered analytical approach provides a robust and multidimensional validation of Fusarium wilt resistance among pea accessions. The identification of resistant pea accessions, particularly PSAT44-042, PSAT57-002, and PSAT57-005, provides a valuable genetic reservoir for breeding programs targeting *Fusarium* wilt resistance. These accessions exhibited consistently low AUDPC values, stable phenotypic responses, and distinct positioning in both PCA and LDA analyses. Such stability indicates the potential presence of quantitative resistance loci that could be harnessed through recurrent selection, backcrossing, or marker-assisted selection (MAS) once linked markers are identified (Miedaner, 2016;Tar'An *et al*., 2003).

In practical terms, resistant genotypes identified in this study can serve as donor parents in crossing programs aimed at introgressing disease resistance into elite but susceptible varieties. Moreover, moderate accessions may serve as intermediate genetic backgrounds to combine resistance with other agronomic traits. The classification framework presented—based on AUDPC and multivariate analyses—also provides a quantitative, reproducible method for early-generation screening in controlled environments, accelerating selection cycles in resistance breeding pipelines. Beyond breeding applications, the resistant accessions identified here could be directly evaluated under field conditions to validate their performance in diverse agroecological zones. This would facilitate the release of Fusarium-resistant cultivars tailored to specific environments. Additionally, the quantitative framework used in this study can serve as a model for evaluating resistance in other legume crops, supporting integrated disease management efforts in smallholder farming systems

CONCLUSION

This study successfully identified 21 Fusarium wilt-resistant pea accessions from 84 evaluated genotypes through an integrated assessment of disease severity indices and multivariate analyses. The resistant accessions PSAT57-010, PSAT44-013, PSAT44-035, PSAT44-041, PSAT44-042, PSAT44-043, PSAT44-054, PSAT44-055, PSAT50-007, PSAT50-020, PSAT56-023, PSAT56-034, PSAT56-039, PSAT57-001, PSAT57-002, PSAT57-005, PSAT57-006, PSAT65-004, PSAT65-010, Taichung (C)(6), and Taichung (H)(1) consistently exhibited low AUDPC values and minimal symptom progression. Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) further verified the phenotypic separation of resistant, intermediate, and susceptible groups, demonstrating the robustness of the classification framework. The strong correlation between PC1 and disease severity confirmed the reliability of multivariate approaches for resistance screening. These resistant accessions therefore represent valuable genetic resources for breeding durable Fusarium wilt resistance. Future work should include multi location field trials and molecular mapping to elucidate the genetic basis of resistance and support marker assisted selection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article. All authors have contributed equally and have no financial or personal relationships that could have inappropriately influenced or biased the content of this study.

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