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RESEARCH ARTICLE



# Assessment of hybrid corn genotypes in the suppression of turcicum leaf blight disease progression in corn under preventive-based protection

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## ABSTRACT

Turcicum leaf blight (TLB) is one of the important diseases in corn (*Zea mays* L.) plants in Indonesia and causes yield losses about 27% to 90%. One of the controls to suppress TLB is using resistant cultivars. This study aims to evaluate the resistance of hybrid corn genotypes to TLB and determine its effect on the epidemic components of the disease. There are four hybrid corn genotypes HCG11, HCG12, HCG13, and HCG14 that were tested with comparison cultivars used are P36, PAC339, NK8103, BISI18, and Anoman. Observation of TLB severity was carried out when plants were 60, 70, 80, and 90 d after plant. The study was prepared using a randomized group design consisting of nine treatments and repeated three times. The observational data were statistically analyzed and continued with least significant different (LSD) test. Analysis of disease progression models in the field was carried out based on goodness of fit tests, area under disease progress curve (AUDPC) and path analysis. The results showed that only the HCG11 genotype showed a somewhat resistant reaction to TLB disease with disease severity of 33.94%, significantly lower than the comparison cvs. PAC339, BISI18, and Anoman in the 5% LSD test. The epidemic component of the disease also showed that the HCG11 genotype had the lowest AUDPC value and infection rate with the highest protection index against TLB disease. Path analysis showed that relative humidity, temperature, precipitation, and wind speed had a significant effect on the progression of TLB disease.

Key words: Disease epidemic, disease severity, *Exserohilum turcicum*, infection rate.

## INTRODUCTION

Turcicum leaf blight (TLB) or northern corn leaf blight (NCLB) is a leaves disease of the corn (*Zea mays* L.) plants that is common in Indonesia, caused by the fungus *Exserohilum turcicum* (Pass.) The TLB causes yield losses of 27% to 90% (Ferguson and Carson, 2007; Muiru et al., 2010; Wang et al., 2012). In India, TLB infection causes yield loss of 91% (Pant et al., 2001; Ishfaq et al., 2014; Nwanosike et al., 2015). The TLB reduced yields if infection occurs during the flowering phase (Carson, 1995).

The progression of this disease is influenced by the cultivation system, cultivar resistance, and weather/climate. Based on this, the progression of effective and efficient control methods for TLB disease needs to continue to be pursued. The TLB disease management requires an integrated approach consisting of the use of resistant cultivars optimal agronomic practices, and the use of biofungicides (Shukla et al., 2012; Wang et al., 2015). Several cultivation techniques that can be adopted are crop rotation, optimal use of populations and spacing, balanced fertilization and land sanitation (Bergamin Filho and Amorim, 2011; Kurosawa et al., 2018).

The use of cultivars with potential genetic resistance to major maize diseases has been widely studied and is believed to be the most effective, efficient and economical control measure (Ferguson and Carson, 2007; Ishfaq et al., 2014; Ayiga-Aluba et al., 2015; Mirsam et al., 2021). However, there is high variability in the level of resistance of maize cultivars to this TLB disease (Bhandari et al., 2017; Mubeen et al., 2017). Therefore, knowledge about the type of control as well as the heritability of the traits involved in the expression of resistance is very important in the development of resistant cultivars so that it can provide guidance for carrying out more efficient selection activities in subsequent breeding program work (Vieira et al., 2009).

Control by using resistant cultivars needs to be accompanied by an understanding of the epidemic components that influence the disease progression. This strategy was used as a preventive measure to control the TLB epidemic during the 1970s (Ullstrup, 1972). A complex understanding of the factors that influence the spatial and time scales will be the basis for predicting the occurrence of disease epidemics in supporting successful disease management (Milgroom and Peever, 2003; Maryono et al., 2020). Therefore, data related to the influence of hybrid corn on components of major corn disease epidemics such as disease intensity, disease spread patterns, infection rates, protection index, and TLB disease progression model need to be studied further. This research aims to evaluate the resistance of hybrid corn genotypes to TLB disease and determine its effect on the components of the disease epidemic.

## MATERIALS AND METHODS

The research for Turcicum leaf blight (TLB) disease was carried out in the Sinjai highlands, Bontolemngan Village (5°16'04" S, 120°00'35" E), West Sinjai District, Sinjai Regency, South Sulawesi Province, Indonesia. In this area there is a rainy season from April to October and a dry season from October to April. This area has two types of climates (Schmidt and Ferguson, 1951), namely climate types D2 and D3. Most of the West Sinjai area is in a zone with a climate type D2, namely areas that experience 3-4 wet months and 2-3 dry months. Some of them are in a zone with a type D3 climate which has 3-4 wet months and 3-5 dry months. Average rainfall ranges from 2000 to 4000 mm  $yr<sup>-1</sup>$  with rainy days varying between 100-160 rainy days per year. The average air humidity ranges from 64% to 87%, with an average air temperature of 21.1 °C (BPS-Statistics Indonesia, 2022).

#### Evaluation of the resistance of hybrid corn cultivars to Turcicum leaf blight

Evaluation of the resistance hybrid corn cultivars to TLB used are randomized block design with nine treatments (cultivars) and was repeated three times. The cultivars were four hybrid maize (*Zea mays* L.) genotypes (HCG11, HCG12, HCG13, and HCG14) and five control cultivars (P36, PAC339, NK8103, BISI18, and Anoman).

The research began with planting spreader rows for TLB disease (Anoman) in three rows each around the test plot and between the experimental blocks. Three weeks after planting, the spreader rows were sprayed with a suspension of conidia of the pathogenic fungus that causes TLB (*E. turcicum*) in the afternoon with a spore density 6×10<sup>4</sup> conidia mL<sup>-1</sup>. The *E. turcicum* isolate used came from the Cereal Disease Laboratory collection. The test genotype was planted after the spreader rows plants were attacked by ≥ 70% TLB disease. The test genotypes were planted in 4 rows each 5 m long, with a spacing of 70×20 cm. Two seeds were planted in each hole and given carbofuran to prevent pest attacks. Plants were fertilized using urea (46% N) 300 kg ha<sup>-1</sup>, NPK Phonska (15% N; 15% P<sub>2</sub>O<sub>5</sub>; 15% K<sub>2</sub>O) 200 kg ha<sup>-1</sup>, half the dose of urea (150 kg ha<sup>-1</sup>) at the age of 10 d after planting (DAP) and half the urea (150 kg ha<sup>-1</sup>) was given at 30 DAP. Observations of TLB disease attacks were carried out at plant ages of 60, 70, 80 and 90 DAP using the modified Sharma scale (Sharma, 1983) (Table 1). The disease scale converted into the attack percentage formula as follows:

$$
DS(\% ) = \frac{\Sigma(n \times v)}{Z \times N} \times 100
$$
 (1)

where DS is disease severity; n is number of plants attacked in each category; v is scale value for each affected plant; Z is highest scale value; and N is number of plants observed at each attack.

Average disease severity at the last observation was used as a standard to determine the level or criteria for resistance of hybrid corn genotypes. The resistance criteria used were based on Indonesian Food Plant Cultivar Release Procedure standards, namely very resistant (0%-5%), resistant (> 5%-20%), moderately resistant (> 20%-40%), susceptible (> 40%-60%), and very susceptible (> 60%). Then the observation data was analyzed statistically and continued with least significance difference (LSD) at the 5% level ( $\alpha$  = 0.005).

The DS value was then used to calculate the DS reduction value. The highest DS value was used as a control value. The DS reduction formula used is as follows:

DS Reduction (
$$
\%
$$
) =  $\frac{\text{DS control-DS treatment}}{\text{DS control}} \times 100$  (2)



### Table 1. Scoring of Turcicum leaf blight.

## Analysis of disease progression models and infection rates

Analysis models of TLB disease progression in the field were carried out based on goodness of fit tests on the three most widely used models, namely monomolecular, logistic and Gompertz (Neher and Campbell, 1992; Xu, 2006). Model selection through the transformation of the collected disease proportion data (x), respectively into  $\ln(1/(1-x))$  for the monomolecular model,  $\ln\{x/(1-x)\}$  for the logistic model, and  $\{-\ln(-\ln x)\}$ for the Gompertz model. These new data are linearly regressed against the time (t) of disease progression. Furthermore, the goodness of fit model was tested by looking at the coefficient of determination ( $R^2$ ) and mean squared error (MSE). The best model was selected with the condition that the  $R^2$  is the largest and the squared MSE is small (Xu, 2006). Moreover, this data is also used to calculate the infection rate (r). The calculation of r is based on the results of selecting a disease progression model using the following formulas:

Monomolecular model: 
$$
r_m = \frac{1}{t} \left( \ln \frac{1}{1 - x_t} - \ln \frac{1}{1 - x_0} \right)
$$
 per unit of time (3)

Logistic model:

$$
r_1 = \frac{1}{t} \left( \ln \frac{x_t}{1 - x_t} - \ln \frac{x_0}{1 - x_0} \right) \text{ per unit of time} \tag{4}
$$

Gompertz model:

 $r_g = \frac{1}{r} - \ln(-\ln(x_t)) + \ln(-\ln(x_0))$  per unit of time (5)

where  $x_t$  is the proportion of disease at time r;  $x_0$  is proportion of disease at the start of observation (t = 0); t is time; r is disease infection rate.

#### Analysis of the area under the disease progress curve and protection index values

The area under disease progress curve (AUDPC) value is obtained based on the disease severity in a certain observation period. The AUDPC describes the level of disease progression over a certain period. The AUDPC value is calculated using the Equation 6 (Mehmood and Khan, 2016):

AUDPC = 
$$
\sum_{i=1}^{n-1} \left( \frac{x_i - x_{i+1}}{2} \right) (t_{i+1} - t_i)
$$
 (6)

where n is the number of observations; x is TLB disease severity, and  $(t_{i+1} - t_i)$  is the time interval between observations. Meanwhile, the protection index is calculated based on the AUDPC value using Equation 7 (Caulier et al., 2018):<br>Protection index (%) =  $\left(1 + \frac{\text{AUDPC treatment}}{\text{AUDPC control}}\right) \times 100$  (7)

#### The influence of weather factors on TLB disease based on path analysis

The influence of weather factors on the TLB disease progression was tested using path analysis. This analysis can describe the direct and indirect influence of weather factors on disease progression and can determine which weather factors have the most influence on disease progression (Salcedo et al., 2020). In this analysis, TLB disease severity is the dependent variable (Y) while weather factors are the independent variable (X). Weather data analyzed includes relative humidity (RH), temperature, rainfall and wind speed taken from the website of the Meteorological, Climatological and Geophysical Agency (BMKG; https://www.bmkg.go.id/en.html).

## RESULTS

## Resistance levels of corn genotypes to severity of TLB disease

The results of observations of the TLB disease severity in each genotype showed that there were differences in the level of resistance to the disease. The severity of TLB disease at the first observation at 60 d after planting (DAP) showed that the percentage of attacks was still relatively low in the attack range 3.03%-21.82%. An increase in TLB disease attacks began to appear at the age of 70 DAP, where the PAC339 comparison cultivar showed quite high and even TLB disease severity in each replicate, with an average attack of 41.82%, while the disease severity in the four test genotypes was still < 30% (Table 2).

The result of observation on 80 DAP genotype HCG11 showed an increase in disease severity but still showed a good resistance reaction. Meanwhile, the comparison cvs. PAC339, BISI18, and Anoman showed high disease severity between 29.09%-53.33%. Furthermore, at the last observation of plant age at 90 DAP, only the HCG11 genotype showed a moderate resistant reaction to TLB disease with disease severity of 33.94% significantly lower than the comparison cvs. PAC339, BISI18, and Anoman at the 5% LSD level test. The three hybrid corn genotypes HCG12, HCG13, and HCG14 showed a susceptible reaction with disease severity ranging between 47.27%-52.12%. Symptoms and signs of TLB disease can be seen in Figure 1.

Table 2. Severity of Turcicum leaf blight (TLB) disease in tested hybrid corn genotypes. <sup>a</sup>Infection rate was significantly lower than the comparison 'P36' at the 5% LSD test level. <sup>b</sup>Infection rate was significantly lower than the comparison 'PAC339' at the 5% LSD test level. <sup>c</sup>Infection rate was significantly lower than the comparison 'NK8103' at the 5% LSD test level. <sup>d</sup>Infection rate was significantly lower than the comparison 'Bisi18 at the 5% LSD test level. <sup>e</sup>Infection rate was significantly lower than the comparison 'Anoman' at the 5% LSD test level. DAP: Days after planting; MR: Moderately resistant; S: susceptible; VS: very susceptible.





Figure 1. Symptoms and signs of Turcicum leaf blight (TLB) disease in corn. Bar scale 20  $\mu$ m.

## Disease progression model and infection rate values

The TLB disease progression model analysis was selected based on the highest coefficient of determination value. The TLB disease progression in the HCG11, HCG12, and HCG14 genotypes follows the monomolecular model, while the disease progression in the HCG03 and Anoman genotypes follows the Gompertz model (Table 3). The coefficient of determination and regression equation values for the HCG11, HCG12, HCG13, HCG14, and Anoman genotypes were 0.890 (Y = 0.0128x - 0.6863), 0.993 (Y = 0.0177x - 0.9388), 0.997 (Y = 0.0416x - 3.3849), respectively, 0.998 (Y = 0.0218x - 1.2326), and 0.999 (Y = 0.0554x - 3.9782).

The severity of TLB disease in each test genotype can be confirmed through the infection rate (r). The TLB disease progression in the HCG11, HCG12, HCG13, HCG14, and Anoman genotypes were observed to show different infection rate (r) values, namely 0.013, 0.018, 0.042, 0.022, and 0.055 respectively (Table 3). The lowest infection rate value was found in the HCG11 genotype, this is in line with the lowest severity of leaf blight disease.

Genotypes	Disease progression model	Infection rate	Regression equation	$R^2$
HCG11	Monomolecular	0.013	$Y = 0.0128x - 0.6863$	0.890
HCG12	Monomolecular	0.018	$Y = 0.0177x - 0.9388$	0.993
HCG13	Gompertz	0.042	$Y = 0.0416x - 3.3849$	0.977
HCG14	Monomolecular	0.022	$Y = 0.0218x - 1.2326$	0.998
Anoman	Gompertz	0.055	$Y = 0.0554x - 3.9782$	0.999

Table 3. Model of Turcicum leaf blight (TLB) disease progression in hybrid corn genotypes.

## AUDPC value and protection index

The results of the analysis showed that there are differences in the AUDPC values and protection index for each hybrid corn genotype. The AUDPC values for the HCG11, HCG12, HCG13, HCG14, and Anoman genotypes were 827.27, 1257.47, 1130.27, 1148.6, and 1715.31, respectively, while the protection index values were 51.77%, 26.69%, 34.11%, 33.04%, and 0.00%. The HCG11 has the lowest AUDPC value with the highest protection index value, while the Anoman has the highest AUDPC value and the lowest protection index. This illustrates that the TLB disease progression in the hybrid corn genotype HCG11 is the slowest compared to other test genotypes, especially the Anoman (Figure 2).



Figure 2. Effect of hybrid corn genotype on the area under the disease progression curve (AUDPC) and protection index: HCG11 (a), HCG12 (b), HCG13 (c), HCG14 (d), P36 (e), PAC 339 (f). A<sub>1</sub>: AUDPC of the first time interval; A<sub>2</sub>: AUDPC of the  $2^{nd}$  time interval; A<sub>3</sub>: AUDPC the  $3^{rd}$  time; A<sub>4</sub>: AUDPC of the 4<sup>th</sup> time interval.

## Influence of weather factors on TLB

Path analysis based on the correlation between weather factors and TLB disease severity shows a positive path coefficient value. This shows that relative humidity, temperature, rainfall and wind speed have a significant effect on the TLB disease progression both directly and indirectly. The path coefficient values for relative humidity, temperature, rainfall, and wind speed are 0.471, 0.682, 0.187, and 0.226 respectively (Figure 3).

The results of path analysis also show that there is a correlation between weather factors (relative humidity  $\leftrightarrow$  temperature  $\leftrightarrow$  rainfall  $\leftrightarrow$  wind speed) in influencing the TLB disease progression, both positive (+) and negative (-) correlations. Weather elements that show a positive correlation in influencing the progression of TLB disease severity can be seen in the correlation between relative humidity  $\leftrightarrow$  rainfall, relative humidity  $\leftrightarrow$ wind speed, temperature  $\leftrightarrow$  rainfall, temperature  $\leftrightarrow$  wind speed, and rainfall  $\leftrightarrow$  wind speed with the respective correlation coefficient values. respectively 0.077, 0.707, 0.077, 4.441, and 0.364. Meanwhile, negative correlation is only shown in the correlation between relative humidity  $\leftrightarrow$  temperature with a correlation coefficient value of (-0.313) (Figure 3).



Figure 3. Diagram of path analysis results of the influence of weather factors on the Turcicum leaf blight (TLB) disease progression. The value on the path that connects weather factors and types of corn disease is the path coefficient. The value on the path connecting humidity, temperature, rainfall, and wind velocity is the correlation coefficient.

## **DISCUSSION**

The initial symptoms of TLB disease were found on the lower leaves in the form of elongated oval spot. Then, the spot grew bigger and turned brown. The symptoms of TLB disease are different from the symptoms of leaf blight caused by *Bipolaris maydis* in the lowlands. The leaf blight symptoms formed by *H. turcicum* attack were larger and longer than the blight symptoms caused by *B. maydis* (Figure 1). According to Navarro et al. (2021) the initial symptom of *H. turcicum* infection was small spot, then the spot turned increasingly elongated in an elliptical shape and developed into necrosis called blight, the color was grayish green or brown. The morphological characters of *H. turcicum* showed that the conidia were shaped like long coils, sometimes slightly bent, insulated, and brown (Figure 2). The TLB disease, like most other leaf diseases in corn, is a late season disease where the most severe disease development occurs during post anthesis (Anwer et al., 2022). Therefore, the disease severity observations of leaf blight infection were carried out during that period.

Evaluation of the resistance of hybrid corn genotypes showed that only the HCG11 genotype reacted moderately resistant to TLB disease with a disease severity of 33.94%. The level of resistance shown by the HCG11 genotype was the same as the resistance level of the P36 resistant cultivar. Meanwhile, the susceptible cvs. PAC339, NK8103, Bisi18, and Anoman showed a very weak resistance level, namely reacting susceptible to very susceptible with disease severity levels ranging from 41.82%-75.15%. The difference in the severity of TLB disease in each test genotype showed a relationship between the resistance level of the hybrid corn genotype and the virulence level of the pathogen that invaded plants in the field. In addition, supportive agroecosystem conditions and abundant inoculum sources cause changes in the resistance level from resistant to susceptible. The disease develops optimally at moderate temperatures, relative humidity from 90% to 100%, low luminosity, abundant inoculum sources, and long dew periods (Carson, 2016; Galiano-Carneiro and Miedaner, 2017; Badu-Apraku et al., 2021). Setiawan et al. (2016) also reported that TLB has the potential to occur in areas with low air temperatures and high humidity at night. This pathogenic fungus releases many conidia during the day after a warm night with relative humidity above 90%. The optimum temperature for conidia formation is 20-26 °C. The infection process takes 6-18 h at 18-17 °C.

The transmission of TLB pathogen infection in this study spread well. These cultivars meet the criteria for susceptible and resistant comparison genotypes (Table 1). This phenomenon is in line with several study reports that TLB development is strongly influenced by cultivar resistance, cultivation systems and weather (Carson, 1995; Treikale et al., 2014; Akinwale and Oyelakin, 2018). In addition, the decline in the resistance level of hybrid corn genotypes to TLB disease may be caused by the epistasis phenomenon. Corn resistance to TLB is controlled by many genes (polygenic), thus many genes interact with each other during crosses between progenitors (Muiru et al., 2010; Castiano et al., 2012; Sartori et al., 2015; Wathaneeyawech et al., 2015). The decline in resistance level of corn genotypes can also be influenced by climatic conditions at the time of testing.

This pathogen can infect plants from the germination phase to harvest. Further spread of the disease in crops occurs due to the presence of conidia produced in abundance on leaf lesions. In general, TLB disease is known to occur sporadically, depending on environmental conditions and the resistance level of the corn genotype (de Rossi et al., 2015). Losses due to reduced yields are greater when plants are infected during the flowering and grain filling phases (Semangun, 2008). The TLB causes the death of leaf tissue, as well as reducing the amount of chlorophyll from which carbohydrates, fats and proteins are produced in plants. Reddy et al. (2014) reported that there was a decrease in photosynthesis rate of around 91% when the severity of TLB disease in corn exceeded 50%.

Based on observations in the field, *H. maydis* fungus was able to survive as mycelia and conidia on the corn crop residue. Conidia could also turn into thick-walled resting spores called chlamydospores. During warm, moist weather in early summer, new conidia were produced on mature corn and carried by wind or rain to the lower leaves of young corn. Infection and disease development require conditions of high humidity, high rainfall, and moderate temperatures. According to Galiano-Carneiro and Miedaner (2017), infection by germinating conidia occurs when free water is present on the leaf surface for 6-18 h and the temperature is between 18-17 °C. Under favorable conditions, lesions develop and produce new spores within 7-12 d on susceptible cultivars, which can cause the disease to spread rapidly. Secondary spread from the lower leaves upwards is mainly caused by rain splashes, while the wind plays a role in the long-distance movement of spores and the spread of disease from one plant to another (Munkvold and White, 2016).

Differences in AUDPC values for TLB disease in tested genotypes that occur over time can describe the dynamics of TLB disease severity and determine the period when the highest severity of TLB disease occurs. In Figure 3, it can be seen that the highest increase in AUDPC value occurred in the plant age period of 70 - 90 DAP. This indicates that the highest level of TLB epidemic occurred in that period. Mirsam et al. (2023) reported that the incubation phase of this pathogen starts from infiltration until the appearance of the initial symptoms of TLB disease, ranging from 45 to 60 DAP. The TLB disease epidemics depend on the ability of *H. turcicum* to infect, grow and develop, and sporulate in corn tissue (Aliyi et al., 2018). The influence of climatic conditions on the TLB disease progression has been widely reported. The *H. turcicum* infection process is strongly influenced by light, dew temperature, dew period, plant growth, and inoculum concentration (Sharma, 2023). The duration of the dew period and dew temperature are the most important environmental factors influencing infection and sporulation (Mirsam et al., 2023).

Analysis of TLB disease progression models on four hybrid corn genotypes showed that there were two selected models, namely the monomolecular and Gompertz (Table 3). The HCG11 genotype had the lowest TLB infection rate value following the monomolecular model, while 'Anoman' (control) had the highest TLB infection rate value following the Gompertz model. The differences in TLB disease progression models in each hybrid corn genotype can be used as the basis for measures to delay infection at the start of planting which has a very important role in reducing disease intensity. Campbell and Madden (1990) proposed that several disease progression models have been used to characterize disease progression over time for polycyclic diseases using logistic and Gompertz models. These models describe disease escalation and predicted attack rates early in the epidemic.

Path analysis showed that all observed climate factors (temperature, humidity, and precipitation) were positively correlated with disease severity. Correlation results between temperature and disease severity contradicted several previous reports. Reddy et al. (2013) reported that there was a negative correlation between disease severity and maximum temperature and a positive correlation with high relative humidity. In addition, Nwanosike et al. (2015) also reported a significant positive correlation between relative humidity and disease severity and a significant negative correlation between minimum and maximum temperature and TLB disease severity.

## **CONCLUSIONS**

The HCG11 genotype showed a moderate resistant reaction to Turcicum leaf blight (TLB) disease with disease severity of 33.94% lower than PAC339, BISI18, and Anoman in the 5% LSD test. The hybrid corn genotypes HCG12, HCG13, and HCG14 showed a susceptible reaction with disease severity ranging between 47.27%- 52.12%. Weather factors such as relative humidity, temperature, rainfall, and wind speed have a significant effect on the TLB disease progression both directly and indirectly with path coefficient values of 0.471, 0.682, 0.187, and 0.226, respectively. The results of this study emphasize the importance of considering fitness components, such as cultivar resistance level and weather conditions, in determining the severity of TLB disease. In addition, information about the characteristics of pathogen populations can also increase the accuracy of TLB forecasting with disease progression simulation models.

#### Author contribution

Conceptualization: H.M., S., S.K., M.Az., M.F.A. Methodology: H.M, S, M.Aq., M.Az. Software: H.M., S., M.Aq. Validation: H.M., M.Az. Formal analysis: H.M., M.Az. Investigation: H.M., S., M.Az. Resources: M.Aq., M.Az. Data curation: H.M., S., M.F.A. Writing-original draft: H.M., M.Az. Writing-review & editing: H.M., S.K., M.Aq., A.T.M., R.N.I., Y.N., M.F.A. Visualization: H.M., M.Az., M.Aq. Supervision: Y.N., M.Aq. Funding acquisition: M.Az. All co-authors reviewed the final version and approved the manuscript before submission.

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