# MARKERS RELATED TO TOLERANCE OR SENSITIVITY TO LATE WILT DISEASE IN MAIZE

By

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# ABSTRACT

**Sayed Hassan Ahmed Agag: Markers Related to Tolerance or Sensitivity to Late Wilt Disease in Maize. Ph.D. Thesis, Plant Pathology Department, Faculty of Agriculture Ain Shams University, 2022 .**

Late wilt in maize caused by *Magnaporthiopsis maydis* is one of the most important diseases in Egypt. To find molecular markers related to tolerance or sensitivity to late wilt, sixteen isolates of *M. maydis* were isolated from diseased plants from different governorates and identified using a specific primer. On PDA medium all isolates have similar growth form, growth elevation and growth margin but different in mycelial color, the faster isolate was S3 while the lowest was A2 . The pathogenicity test was carried out in greenhouse using single cross hybrid Pioneer 3062 . Isolates F13 and F14 were the most virulent while the lowest was Mi5. By using six RAPD primers the 16 isolates classified into five clusters. Twenty five single cross hybrids of maize were screened against late wilt disease in two seasons 2018 and 2019 in greenhouse and field experiments. The field experiment was only able to combine so the higher infection was 67.31% for hybrid P3084 while the lowest was zero for hybrid P3433. By using disease rating scale, the 25 hybrids can be classified into 12 tolerant hybrids, seven moderately tolerant hybrids and six sensitive hybrids .Three vegetative traits and six yield traits were measured during field experiment in two seasons 2018 and 2019. There was a negative significant relationship and medium correlation obtained between plant height, ear height, stalk diameter, ear diameter, 100 kernels weight and grain yield per hybrid and late wilt infection while there was no correlation obtained between ear length, number of row per ear and number of kernels per row and late wilt infection. Two different molecular markers, nine ISSR primers, five SCoT primers as dominant markers and 129 SSR primers as co-dominant markers were used to study the molecular diversity between five tolerant maize hybrids and five sensitive one. The final cluster analysis based on ISSR

,SCoT and SSR markers classified the tolerant and sensitive hybrids into four clusters .

DNA fingerprinting for the five tolerant and five sensitive hybrids was carried out by using 80 SSR primers whereas 8 SSR primers can be used as DNA finger printing for tolerant hybrid P3737, one SSR primer can be used as DNA finger printing for tolerant hybrid P3433, one SSR primer can be used as DNA finger printing for sensitive hybrid ARC 168 and one SSR primer can be used as DNA finger printing for sensitive hybrid P31G. A 600 bp locus was found in all sensitive hybrids only when using ISSR primer (AC)9A which specialized for sensitivity to late wilt disease and it can be used as molecular marker for sensitivity to late wilt .Two inbred lines ,tolerant inbred G5 and sensitive inbred G9 were evaluated against late wilt in field experiment in disease nursery in agricultural research center for two seasons 2018,2019. F1 was selfed to produce F2 which phenotyping against late wilt by using disease severity scale .Among 207 tolerant individuals of F2, 34 individuals were selfed to produce F3 families which phenotyping against late wilt. By using bulked segregant analysis, the most 11 tolerant individuals from 34 individuals of F3 and the most 11sensitive individuals from F2 were bulked of their DNA to produce two bulks , tolerant bulk and sensitive bulk. Among 129 SSR primers there were 29 primers polymorphic only between the two inbred lines which used to electrophoresis of the parental lines and the two bulks. SSR primer umc 1277 located in chromosome 9 bin 7 was only the SSR primer which correlate between tolerant inbred line and tolerant bulk which can be used as marker assisted selection to late wilt . The F2 segregation showing the typical phenotypic ratio 3 tolerant: 1 sensitive and also these ratio suggest that one single gene is controlling the tolerance to late wilt in maize and showing complete dominance.

**Keywords:** Late wilt Disease, *Magnaporthiopsis maydis*, Molecular markers, Bulked segregant analysis and Marker assisted selection.

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| | |
|---|---|
| AFLP | Amplified Fragment Length Polymorphism |
| BSA | Bulked Segregant Analysis |
| ITS | Internal Transcribed Spacer |
| ISSR | Inter-Simple Sequence Repeats |
| MAS | Marker Assisted Selection |
| NCLB | Northern Corn Leaf Blight |
| PDA | Potato Dextrose Agar |
| QTL | Quantitative Traits Loci |
| RAPD | Randomly Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |
| SCA | Saprophytic Competitive Ability |
| SCAR | Sequence Characterized Amplification Regions |
| SCoT | Start Codon Targeted Polymorphism |
| SSR | Simple Sequence Repeats |