Application of multivariate analysis to electrical penetration graphs using manual and automate waveform recognition from the planthopper *Peregrinus maidis* feeding on susceptible and resistant maize

DINTINGER Jacques, REYNAUD Bernard, BUDUCA Cécile, LECOQ Julien, TURPIN Patrick, GRONDIN Martial, CHIROLEU Frédéric

Cirad UMR 53 PVBMT Cirad/Université de La Réunion 3P Ligne Paradis 97410 Saint Pierre La Réunion Fr

Peregrinus maidis **feeding behaviour plays a key role in the transmission of *Maize mosaic virus* (MMV) and *Maize stripe virus* (MStV) to maize plants. Therefore, information about stylet penetration activities in plant tissues is of basic importance when trying to screen for potentially useful sources of resistance to transmission. Behavioural studies by EPG is an accurate technique in detecting the presence of this resistance and may help to identify the plant tissues where it is mainly expressed. After we identified EPG patterns correlated with the different feeding activities of *P. maidis* on maize, the objective was to characterize the behavioural components of the insect feeding process which were affected by plant resistance to transmission. This study was performed by using arbitrary (manual analysis) and automated (EPG-SOFT analysis) recognition of different EPG phases from susceptible and resistant genotypes. Then, EPG data were analyzed by multivariate methods in order to classify the genotypes, to predict to which group (susceptible or resistant) each one has the greatest chance to belong, and to determine which EPG variables, and therefore which feeding phases, are the more involved in the resistance-susceptibility status of the genotype.**

MATERIAL AND METHODS

- Two sets of data were obtained from two separate EPG experiments on maize inbred lines 37-2 (resistant to transmission) A211, MP705, Rev81 (slightly resistant to transmission), Hi40, found to be completely resistant to MMV, and B73 which is the susceptible check. In experiment 1, the different sequences of EPG recordings were manually identified by an operator, whereas, in experiment 2, they were automatically identified by software 'EPG-SOFT'. In both experiments, recordings were performed during 8 hours. The number of replicates obtained per maize genotype was 10 in experiment 1 and 40 in experiment 2.

- The following different EPG phases were distinguished :

-EPG phase 0: non probing
-EPG phase 1: stylet pathway
-EPG phase 2: active ingestion in xylem vessels
-EPG phase 3: passive ingestion and/or watery salivation in sieve tubes (in experiment 2, EPG phase 3 was subdivided in three different signal classes : 3A corresponded only to passive ingestion, 3C to watery salivation, and 3B to both activities)

- Parameters calculated from EPG data and used for analyzing planthopper–plant interactions :

-nc: frequency of occurrence of considered EPG phase
-dc: total duration of considered EPG phase
-dm: mean duration of considered EPG phase
-da: time to 1st considered EPG phase from start recording

- Principal component analysis was performed on all EPG variables or a subset of EPG variables selected by stepwise linear regression.

EPG equipment and electronic set up : females of *P. maidis* feeding on young test plants were connected to a DC amplifier Giga 8 (Wageningen University) linked to a microcomputer for acquiring numerical data at 100 Hz sample frequencies and displaying them on a screen every 10s-time interval. In experiment 2, the whole process of EPG waveforms analysis was carried out with the software package EPG-SOFT version 1.0 (Reynaud et al., 2002).

RESULTS AND DISCUSSION

In both experiments, axis 1, represented a phloem + to phloem – axis, separating susceptible and resistant genotypes. In contrast, the biological significance of axis 2 looked to be different in the two experiments.

- In experiment 1 (*Fig. 1*), resistant lines 37-2, A211, and MP705 were positioned on phloem – side (strongly correlated with dc0 and nc1 parameters, relative to non probing and stylet pathway phases), whereas susceptible lines B73 and Hi40 were positioned on the phloem + side (strongly correlated with parameter dc3, relative to passive ingestion phase). Otherwise, position of B73 on axis 2 indicated that insect could spend less time making xylem ingestion in this line than in the other ones.

- Despite of a greater dispersion of the data within each genotype, experiment 2 (*Fig. 2*) confirmed results above: 37-2, and in a less extent A211, MP705, and Rev81, were positioned on the phloem – side of axis 1 (mainly correlated with non sequential parameters relative to non probing and stylet pathway), whereas susceptible lines B73 and Hi40 were positioned on the phloem + side (mainly correlated with non sequential parameters relative to passive ingestion and/or watery salivation). The biological significance of the axis 2, mainly contributed by phloem phase 3A and 3C parameters, remained unclear.

After all, both analyses showed that factors accounting most for resistance to transmission were : on one hand, much time spent by planthoppers making stylet pathway but also, to a less extent, non penetration time and xylem ingestion; on the other hand, less time spent ingesting and salivating in phloem tissues. It is noteworthy that non sequential parameters were much more discriminant than sequential parameters herein.

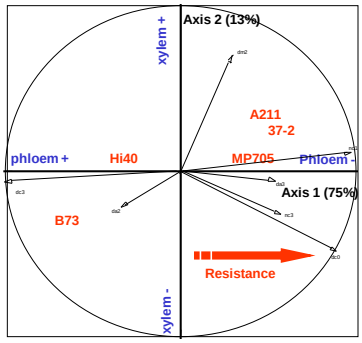

Fig. 1 : projection of recordings in the plane defined by axis 1 and 2, and correlation circle from principal component analysis performed on 7 parameters ***manually*** calculated from EPG experiment on maize lines B73, Hi40, A211, MP705, and 37-2 (each line positioned at the center of gravity of 10 individuals recordings).

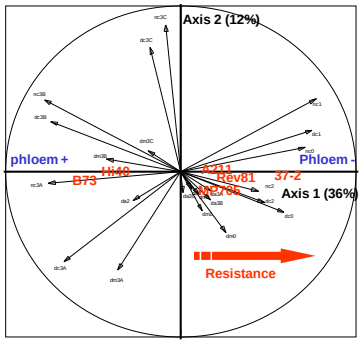

Fig. 2 : projection of recordings and correlation circle from principal component analysis performed on 23 parameters ***automatically*** calculated from EPG experiment on maize lines B73, Hi40, A211, MP705, Rev81, and 37-2 (each line positioned at the center of gravity of 40 individuals recordings).

CONCLUSION

• Resistance to transmission may be linked to problems encountered by the insect in reaching phloem vessels, and when found, in making sustained ingestion phases. This suggest that factors of resistance, located in the sieve elements as well as in the non-phloemian tissues, would reduce the planthopper's feeding rate which in turn could result in a resistance to the transmission of propagative phloem dependant MMV and MStV. Thus, the use of the EPG method followed by a multivariate analysis contributes to provide information helpful for (I) a better understanding of resistance mechanisms to *P. maidis* in maize, (ii) a differentiation between resistant and susceptible genotypes.

• Data obtained from automated and standardized analysis by the computer program 'EPG-SOFT' are characterized by a dramatic variability within each genotype. Nevertheless, this software has some decisive advantages over arbitrarily analysis: it may be used for a large number of EPG recordings (EPG-SOFT needs 10 min to analyze an 8 hours recording), it is more accurate by analyzing the waveforms by 5 seconds sequences, it should contribute to standardize EPG researches and to develop international collaborations. If this technique remains difficult to use in large plan screening or for resistance genetic studies, it may help the plant breeder to estimate the degree of resistance to transmission in elite parental lines and to accumulate this type of resistance with resistance to the virus.

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