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Evaluation of fusarium resistance tests on maize applied in the national variety registration in Hungary

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1. INTRODUCTION

Maize (Zea mays L.) is one of the most important arable crops in our country and in the world. Its starch-rich grain and the whole maize crop (green, ensiled) are important fodder, as well as being suitable for industrial use and direct human consumption. In 2020, the total harvested area in Hungary was 981 006 ha with an average yield of 8.58 t/ha. 2021). The most important pathogens are *Fusarium* species. As a direct pest, it reduces the harvestable yield, as an indirect pest it produces mycotoxins, which pose a significant human and animal health risk. Several control methods can be used with varying degrees of effectiveness to prevent the onset of the disease or to reduce its levels below economic losses. Effective chemical control of *Fusarium* spp. is limited and not widely used in (commodity) maize crop protection practices, among which the choice of the hybrid is crucial. There are significant differences between the fusarium resistance of commercially available hybrids, and the widespread, effective chemical control against fusarium in commodity corn crop protection practices is limited. Genetic protection through choice of variety (varietal selection) (use of resistant or at least less susceptible varieties) is a fundamental and indispensable element of integrated crop protection and one of the most effective pillars of food safety (Békési 2019).Sustainability is a key part of the Green Deal and the EU Biodiversity and Farm to Fork Strategy these days. Their objectives include reducing the use of fertilizers and pesticides and increasing the share of organic land. In order to meet these objectives without reducing yields, varieties with good nutrient utilization and adequate disease resistance are needed. During breeding, new and new hybrids are continuously produced in order to achieve higher and higher yields. According to Békési (1999), the most important value indicators of the variety are: productivity, quality, agronomic value and crop safety.. In addition to high yields, the role of crop safety is becoming particularly important due to changing climatic factors (Széles and Huzsvai, 2020). New hybrids are applicated to the Directorate of Agricultural and Genetic Resources of the National Food Chain Safety Office of Hungary (NFCSO) for national variety registration. Of the agricultural plant species applicated for national registration, maize is one of the species with the largest number of varieties, with over 100 candidate varieties to be tested each year. Registration may be based on a 3-year testing period, but 2-year consistent positive trial result.The National List

(Catalogue) of Varieties contains 320 (Csapó, 2021), while the European Common Catalogue has a total of 6288 (European Commission, 2021) registered maize hybrids. Unfortunately, there are very limited reliable data on the *Fusarium*-resistance of registered hybrids which are available for domestic maize growers. *Fusarium* ear and stalk rot are diseases that must be examined even today. A disqualifying factor during variety recognition is if a hybrid is classified as very susceptible (5) to fusarium wilt or tube mold, or more than moderately susceptible (4) to both forms of the disease (Ruga-Kovács 2016).In recent years, the testing methodology used for variety registration (recognition) has been modified several times: it has been introduced in the registration (recognition) system that if a candidate variety does not reach the productivity of standards (of production) but has a stable field (and adequate) resistance to *Fusarium* diseases for 3 years, the hybrid can be granted state registration (recognition). The procedure for calculating the resistance category has been clarified and the two-year *Fusarium* (disease) resistance tests (trials) have been extended to three years in case of inconsistent testing results. With the prospect of further tightening - a resistance category of more than intermediate susceptibility (4) should be a reason for exclusion - it became timely to examine the reliability of the current variety trial methodology and to discuss the possibilities for updating it.

The objective of our research was to evaluate the results of *Fusarium (*disease) resistance tests based on the current methodology in the state variety registration of maize. We sought to answer whether the methodology currently used is suitable to effectively and reliably determine the field resistance to *Fusarium* diseases in maize hybrids applicated for state registration in order to prevent the introduction of highly susceptible and susceptible hybrids into public cultivation.

Our main goals were the following:

1. To determine the effect of years, trial site and growing season on the development of ear and stalk rot using data from 2014-2016.

2. From five years of data (2014-2016, 2017 and 2020), to identify the environmental factors that are most likely to determine the occurrence of ear and stalk rot, and to estimate the probability of *Fusarium* diseases in a given year and trial site. 3. Identification of the *Fusarium* species responsible for stalk rot from naturally infected stalk samples.

4. To investigate the relationship between *Fusarium* ear infection and toxin level in naturally infected ear samples.

5. To determine the relationship between resistance to *Fusarium* ear and stalk rot and to argue that the two disease forms should be assessed separately in the state registration process

6. To justify the advantage of artificial stalk inoculation over natural stalk infection in state variety registration and to develop a quick, simple and reliable evaluation method.

7. Based on the results of the above studies, offer to upgrade the current variety trial methodology

2. Materials and methods

2.1. Trial Sites

Hybrids under test

During the evaluation we used the results of hybrids and standards applicated for national registration in 2014-2016. Statistical analyses were carried out based on the infection rates of all the hybrids tested to study the effect of year, trial site, and maturity group. The number of hybrids in the maize variety trial differs in years under tests, because new reports, registrations and withdrawals took place year by year The number of samples tested is therefore different. Statistical analyses were performed to investigate the effect of year, location and maturity group on the rates of infection of all tested hybrids. Data from the forecasting model analysing the impact of environmental factors were also supplemented with results for the years 2017 and 2020

Location and agrotechnology of the trial (experiment)

The Variety Testing Stations are located in different climatic areas of the country, including regions important for maize production in Hungary. The experimental plots were 1 row. The row length is 920 cm, the row spacing is 70 cm, while the row spacing is 19 cm for the super-early maturity group, 21 cm for the very early - early maturity groups, and 24 cm for the medium and late maturity groups. The experiment was sown at all locations and in all years using a seed drill from 14 April to 9 May. Soil preparation was the same at all sites: deep ploughing in autumn followed by disc-type seedbed preparation in spring. The pre-crop (sowing) was different at each location and in each year (vintage), except for Szarvas (Deer) and Röjtökmuzsaj. Fertilization was based on a soil test, using a N:P:K ratio of (120:60:120) per 10 t/ha of crop, corrected for the pre-crop effect. Chemical weed control and pest control were applied according to the list of authorised maize herbicides and insecticides.

Meteorological data

In 2014, spring was warm at a national average. Summer was initially dry, but there was a large amount of precipitation during the period of fertilization and incorporation kernel filling. The vegetation period in 2015 was characterized by extreme heat and drought. The summer in 2015 was the 4th hottest summer since 1901. A severe drought developed due to heat and lack of precipitation. The amount of summer precipitation was only two-thirds of long-term average, with frequent heat waves. 41 heat days (t_{max}> 30 ° C) and 13 hot days (t_{max}> 35 ° C) were registered in the country. Heat during silking resulted in fertility defects. In addition, we experienced kernel filling problems due to drought. Due to the warm, droughty weather, the drying of maize started early. In 2016, a warmer and drier spring than average was followed by a cooler and wetter summer than usual. Summer was followed by warm and rainy weather.In 2015, precipitation in July was lower at each trial site than in 2014 and 2016

Assesment methods

Assessment of ear rot was carried out per maturity group when the grain moisture of the hybrids in the group at harvest was below 20%. The tests were performed in 2 replicates in the 4th row of the 4-row plots. In the 4th row, cobs were evaluated on a scale of 0-4 (0=no cob infection, 1=cob end has an infection of roughly 1-10%, or 1-5 grains on the cob, 2=cob infection of 4-25 %, 3=cob infection of 26-50 %, 4=cob infection of 51-100 %). With respect to all the cobs and infected cobs, we determined the frequency of infection, which is given in infected plant % according to Hinfner and Békési (1969). Based on the estimated rate of infection, an infection index was calculated with the formula by McKinney (1923), which indicates the severity of diseases as well.

$$I = \Sigma \frac{(n * k)}{N} * K$$

K – all the cobs in the plot (parcel)

k – cobs for each infection category

n – infection category (0-4)

N – maximum value of the scale (4)

Hybrids were classified into resistance categories based on their infection index. Assessment is performed in each set of performance trials, however, classification into the resistance category is performed only on the basis of data from trials with adequate infection pressure (at least one hybrid tested has a minimum infection of 20 %). in each maturity group. If the trial mean is less than 50 %, classification into resistance category is based on the deviation between the infection rate of each genotype and the actual trial mean which considered to be 100% (Hinfner and Homonnay, 1966) as follows:

If the actual trial mean is less than 50 %, a given candidate variety is:

resistant (1) if its rate of infection does not exceed 25 % of the trial mean,

moderately resistant (2) if its rate of infection is 26 - 75 % of the trial mean,

mid-susceptible (3) if its rate of infection is 76 - 125 % of the trial mean,

susceptible (4) if its rate of infection is 126 - 175 % of the trial mean, and

highly susceptible (5) if its rate of infection exceeds 175 % of the trial mean.

If the trial mean of infection is over 50 %, classification is based on the percentage of the actual infection: resistant (1): 0-20 %, moderately resistant (2): 21-40 %, mid-susceptible (3): 41-60 %, susceptible (4): 61-80 %, highly susceptible (5): 81-100 %.

The evaluation of stalk rot was determined by the pressure test of the 2^{nd} internode of the stalk (of the plants) in the first rows. Soft stalks were visually evaluated and the number of plants showing *Fusarium* disease was recorded. Stalk rot is expressed as a percentage of the total number of plants. Subsequently, *Fusarium* stalk rot resistance was classified into the appropriate resistance category, similar to ear rot.

2.2. Laboratory tests

Identification of *Fusarium* species causing high levels of stalk rot

In 2015, we isolated the pathogen from infected stem-base collected in Szombathely and Kaposvár. Species identification was performed in the laboratory of the Institute of Plant Protection of the ATK. By PCR (polymerase chain reaction) a DNA fragment of about 700 bases was amplified. PCR products were sequenced by LGC Genomics GmbH (Berlin, Germany). The resulting chromatograms were corrected using the Staden software package (Staden et al. 2000) and the sequences were compared with sequences from the NCBI database (BLASTn)

Determination of toxin content from trials with severe ear rot infection

The toxin content was determined in the accredited Analytical National Reference Laboratory of (Analytical of) the Food Chain Safety Laboratory Directorate of Hungary with With the Evidence Investigator Myco 7 test, which has Randox Biochip basic technology and contains antibodies specific for various fixed mycotoxins. It uses a competitive chemiluminescent immunoassay during screening, so it is suitable for the quantitative determination of several toxins from a single sample at the same time.

2.3. Artificial stalk inoculation (infection) in trial sites

Hybrids under tests

Artificial stalk inoculation trials were conducted in Martonvásár and Röjtökmuzsaj in 2015 and 2019. Stalk inoculation was performed on 3 different genotypes. All three are Martonvásár-bred two-line hybrids of the FAO 300 maturity group. They were differentiated in terms of susceptibility to *Fusarium* stalk rot: one susceptible, one with average resistance and one with good resistance genotypes were treated. For the artificial inoculation, isolates of *F. graminearum* FG36 (Iregszemcse, 2003; maize stalk/ Plant Protection Research Institute) and *F. verticillioides* FV95 (Bicsérd, 2004; maize stalk/ Plant Protection Research Institute) were used. The isolates were maintained in permanent cultures, the monoconidial culture was started on SNA (Spezieller Nährstoffarmer Agar, Leslie 2006) medium. Selection of the two isolates was based on a preliminary pathogenicity test in a phytotron (Szőke et al. 2009).

Trial site and agrotechnology

Artificial stalk inoculation trials were conducted in Martonvásár and Röjtökmuzsaj in 2015 and 2019. In both locations and both years, maize was the pre-crop. After harvesting and stalk crushing, a 15:15:15 NPK complex fertilizer based on soil analysis was applied to the soil. The area was deep ploughed in autumn (as a base crop). In the spring, 50 kg of ammonium nitrate starter fertiliser was applied with a digging drill. The seedbed was prepared with a combinator. Sowing was done with a hand seeder in Martonvásár and with a seed drill in Röjtökmuzsaj. The experimental area was treated with pre-emergent and post-emergent herbicides according to the weed flora, supplemented with mechanical weed control later.

Meteorological data

In 2015, Röjtökmuzsaj experienced significantly wetter weather than Martonvásárhely, with the exception of June and October. In 2019, the monthly precipitation distribution of the two experimental sites was almost identical, with exceptionally high precipitation in May.

Methods of infection

Soil inoculation was carried out at flowering (first half of July) in both locations in 2015 and 2019. The aim of the soil inoculation is to increase the infection pressure on the plants by applying an inoculum into the soil in such a way that the applied provocation is as close as possible to the natural infection. A 10-20 cm deep hole was drilled 10 cm apart between seedlings sown 20 cm apart, into which 70 g of infected rice grain was applied (Yang et al, 2010). Toothpick inoculation was carried out 1-2 weeks after soil infestation, on days 10-12 after flowering, adapting the method of Young (1943).

Evaluation of the experiment

The stalks were collected in October. The plants were cut above the soil surface with pruning shears at 3 internode lengths and stored at -20 °C until processing. The stalk samples were cut in two lengthwise and the extent of rotting tissue was estimated on a scale of 1 to 5 (according to the condition of the intestinal tissue), based on visual evaluation, and the area of the infected internal tissues was determined to the nearest millimetre and calculated as a percentage of the total area.

2.4. Statistical processing of data

The Kruskal-Wallis test, the Dunn-Bonferroni post-test and the predictive modelling were carried out with SPSS statistical program. Toxin measurement data were evaluated in Microsoft Windows® Excel with the calculation of Pearson correlation with linear regression analysis (Pearson and Hartley, 1962).

3. RESULTS AND DISCUSSION

3.1. Evaluation of the results of field trials under natural infection

3.1.1. Effect of year on the evaluation of stalk and ear rot

According to the results of the trial under natural infection, the year has a significant effect on ear and stalk rot of maize. The amount of ear rot is basically determined by the amount of precipitation during the flowering period, which is the same as De la Campa et al. (2005) and Cao et al. (2014), that is, the amount of precipitation during flowering is critical for *Fusarium* infection and mycotoxin accumulation. According to Dodd (1980) and Balázs (1990), drought stress is one of the most important risk factors that increases the risk of developing wilt. Examining the composition of the *Fusarium* species that caused large-scale dry rot in the warm and droughty 2015 year, we also confirmed the presence of the heat-demanding F. *temperatum* (Molnár et al. 2017). Czembor et al. (2015) also associated the occurrence of F. *temperatum* in Poland with an increase in the frequency of warm and dry year. The year significantly influences the composition of the *Fusarium* species involved under natural infection, therefore it is advisable to supplement the resistance tests of the state variety registration with species determination. The Fusarium species present in a given year are not only important in terms of resistance, but also determine the infection and amount of toxins. The trial with the artificial infection of several prevalent species ensures the significance of the dominant species in the study area every year.

3.1.2. Effect of trial site on the evaluation of stalk and ear rot

The location of the experiment has a statistically verifiable effect on the infection of ear rot and stalk rot within a given year. Variety Testing Stations are located in different climatic areas of the country, so the climatic characteristics of the trial sites in a given year were different. The most significant aspect in this respect is the amount of rainfall during the growing season. Infection of *Fusarium* species is determined by other local environmental factors such as the pre-crop. Irrigation is provided at the Szarvas Variety Testing Station, which allows the infection of the more rainfall-demanding species in dry weather. In addition to the monoculture at Röjtökmuzsaj, it is expected that higher infection pressure will be obtained from the monoculture at Szarvas. For resistance testing, it is important to be able to make decisions based on the species composition of *Fusarium* in several naturally infected sites. The influence of years on stem rot was greater than that of the trial site. As a consequence, it is necessary to perform stalk rot inoculation trial as well.

3.1.3. Effect of maturity group on the evaluation of stalk and ear rot

The effect of FAO maturity groups on ear rot and stalk rot infection was statistically proven in only one year. In 2016, the FAO groups 240-299 had a higher susceptibility to ear rot and in 2014 the FAO groups 180-240 had a higher susceptibility to stalk rot. Nagy and Cabulea (1996) also found that breeding of early maturing maize resistant to ear rot is difficult. Manninger (1972) also found that hybrids from later maturing groups typically have better resistance to stalk rot, because the longer growing season allows more time for the establishment of the firming tissues and longer plant turgor. There is also a risk that later maturing groups, if left out in the field for a long time, will have higher levels of infection due to rewetting and over-infection, but resistance tests are carried out in the field before harvest and so are not reported in variety registration. The difference in susceptibility between the maturity groups seems to show a trend, which may be influenced by the genotypes applicated in a given group and in a given year. But differences may also be due to different flowering times, in case of uneven precipitation.

3.1.4.Effect of the weather and environmental factors on the forcasting ear rot and stalk rot infection

Our results suggest that summer precipitation and the trial site had the greatest influence on the infection rate of ear rot, similar to the findings of De la Campa et al. (2005) and Pfordt et al. (2020) that average temperature and precipitation during flowering are determinants of ear rot disease. Our studies also showed that weather was the most determinant of stalk rot (54.8%), including rainfall in August (23.7%). In comparison, Pfordt et al. (2020) found that weather conditions in September were the most determinant of *Fusarium* stalk rot's severity. The effect of trial site on the infection rate of stalk rot is almost the same percentage (22.2%) as that of ear rot. For the purpose of state variety registration of maize, disease resistance testing is carried out every year at the Variety Testing Stations at least 9 locations in the country, and therefore large amounts of infection data for both disease forms are available every year. The agrotechnological parameters of the trial sites are also documented, providing the opportunity to carry out future monitoring for forcasting purposes. This will allow us to decide which trial sites should be used for resistance tests under natural infection. In the future, we expect to complement our results with toxin measurements, so that we will have the possibility not only to predict pathogen infestation but also to predict toxin accumulation.

3.1.5. Relationship between ear rot and stalk rot resistance

A weak negative relationship (r=-0.29) was demonstrated between the resistance of hybrids to ear rot and stalk rot, suggesting independent genetic regulation. We confirmed the need to evaluate resistance to both disease forms in our resistance tests for the state registration of candidate varieties. Similarly, Nagy and Cãbulea (1996) found that there was no relationship between ear rot and stalk rot infection rate. In contrast, Li et al. (2019) argue that maize stalk rot exacerbates the incidence of ear rot and raise the prospect of conducting research on this.

3.1.6. Assessment of the resistance of hybrids tested in 2014-2016 to *Fusarium* stalk and ear rot based on years and trial sites tested

The infection rate of *Fusarium* ear and stalk rot is influenced by weather conditions, agrotechnology, insect damage and the resistance of the selected hybrid, among other factors. Although the mechanism of resistance to *Fusarium stalk and ear rot* is not yet fully understood and there is no maize hybrid with high-level field resistance our studies suggest that there are hybrids carrying good resistance. The 19 hybrids tested are distinguishable in susceptibility based on 3 years and pooled results from different trial sites. The majority of the tested hybrids belong to the moderately resistant (2) and mid-susceptible (3) resistance categories to both disease forms.

However, in the evaluation process, the final resistance category of a hybrid is determined by the infection pressure at which it was recorded and, because of the evaluation methodology, the resistance characteristics of the other hybrids in the maturity group have a major influence. The results often suggest that the resistance ranking of a hybrid is not stable because the experimental material in a maturity group may change from one year to the next due to new applications and withdrawals, and thus the hybrids that give the actual trial mean have a variable composition. Also, according to Robertson-Hoyt et al. (2006), some inbred lines found to be resistant at a particular trial site but behaved differently at other trial sites due to their genotype x environment interaction.

In order to relate the susceptibility (trait) of hybrids to a fixed point from year to year, the overall effect of location, pre-crop and year, it is necessary to set *Fusarium* checks in each maturity group. Hybrids that consistently produce infestion rates that match the actual trial mean from year to year, under different environmental conditions are suitable as standards. This is the case for hybrid 11 of the 19 hybrids tested. In the present methodology, the trial mean is considered to be 100% and the deviation from this mean is used as the basis for the classification of hybrids into the resistance category as described in the material and methodology chapter. Thus, when using stable medium-susceptible standards, the calculation method would not need to be changed, only the reference value would be independent of the susceptibility characteristics of the other hybrids giving the trial mean. A stable benchmark would be the candidate varieties for determine their susceptibility. Further studies are needed to find already registered hybrids per FAO maturity group that are suitable as a pathological standard. The use of a standard is part of the currently accepted methodology to establish the additional economic traits of a given candidate variety.

3.1.7. Significance of toxin test in the evaluation of ear rot

The national registration of maize hybrids is currently based on the determination of infection rate of candidate varieties, but mycotoxins produced by the pathogens and their amounts are receiving increasing attention. Based on toxin measurements from grain samples, fumonisins B1 and B2 were the most abundant, followed by DON toxin levels. A weak correlation (r=0.17) between DON toxin content and rate of infection (db%) was demonstrated from natural ear rot infection in Jászboldogháza. However, Mesterházy et al. (2020) in their studies found a strong correlation (r= 0.5827) between DON toxin content and *F. graminearum* infection using artificial inoculation. When analyzing the relationship between *F. verticillioides* and fumonisin B1 and B2 toxin content, no strong relationship was observed for the artificial inoculation trial (r=0.21). In contrast, we found a strong relationship between fumonisin B1 and B2 toxin content and the infection index from a heavy ear rot infection in Jászboldogháza (r=0.71). We also observed a week relationship (r= 0.22) between the fumonisin B1 and B2 toxin content and the infection index in samples from the lower infection pressure experiment in Szarvas. Of the hybrids tested in the Jászboldogháza experiment, one hybrid had high toxin levels with medium rate of infection. Since we did not experience a strong correlation between the rate of infection and toxin content under lower natural infection, it is recommended to introduce the setting of resistance tests with artificial ear inoculation and to complement it with toxin measurements of the resulting samples for food safety. As a result of the method development, hybrids with symptomlessly high toxin levels should not be introduced into public cultivation.

3.2. Evaluation of the results of artificial stalk inoculation from field trials

Due to the impact of years on stalk rot, we propose to introduce artificial stalk inoculation in resistance tests. Both methods are suitable for detecting (susceptibility) differences in susceptibility between hybrids. The differences in susceptibility between genotypes were consistent based on the infection rates obtained with toothpick inoculation. *F. graminearum* caused significantly higher levels of infection than *F. verticillioides*. Also in artificial ear inoculation trials, *F. graminearum* was found to have a higher pathogenicity compared to *F. verticillioides* (Mesterházy et al., 2020). In the case of artificial inoculation with toothpick, a havy stalk infection was also observed in the control treatment (sterile toothpick). Therefore, it is advisable to avoid wounding provocation methods in resistance testing for state registration. Similar to the opinion of Yang et al. (2010), we do not find the results of toothpick and stem injection methods reliable when the inoculum is directly applied to the plant. In the natural infection pathway, the pathogen penetrates the roots and enters the stem through the root. The soil inoculation method we use follows the natural infection pathway. Soil treatments with both *Fusarium* species had a provoking effect on the oneset (development) of infection. In conclusion, we can provide the necessary discriminatory power to test stem rot resistance in variety trials by artificial soil inoculation. Since a strong correlation (r=0.98***) between the infection rates obtained by the two methods of evaluation (visual symptom assessment, calculation of the area ratio of healthy and diseased stem parts) was justified, it is advisable to use of the simpler and faster visual evaluation (dissection) to determine the degree of stalk rot in resistance tests with a high number of hybrids.

4. SUMMARY OF NEW SCIENTIFIC RESULTS

1. We have found that the current variety trial methodology is suitable for testing the resistance of varieties to ear rot and stalk rot, despite the effect of years. (vintage). We proposed to upgrade our methodology to provide adequate infection pressure to further eliminate the year effect and to include the testing of toxins of food safety importance.

- Recommendation to conduct an artificial ear inoculation experiment. There is a close relationship between infection rate and toxin content only in the case of high infection pressure, which is year and trial site dependent in the case of natural infection
- Recommendation to set up an artificial stalk inoculation experiment. We have demonstrated that soil inoculation following the natural infection process provides reliable data compared to the toothpick method. In relation to assess stalk rot resistance, we found that rates of infection obtained by visual assessment of symptoms and by assessment the area of the infected internal tissues was determined to the nearest millimetre and calculated as a percentage of the total area are closely correlated and is not been statistically differences. Of the two methods, due to the large number of hybrids tested and the simpler and faster feasibility, the use of visual evaluation is recommended for resistance testing of candidate varieties to *Fusarium* stem rot.
- Recommendation to complement the resistance testing of state variety registration with species identification, as the composition of *Fusarium* species involved in natural infection is significantly influenced by the year. In Hungary, in the year following the first description in 2014, the presence of *F. temperatum* was confirmed in additional growing areas (at the variety trial stations).

2. Five years of data (2014-2016, 2017 and 2020) were used to identify the environmental factors that are most likely to determine the occurrence of ear and stalk rot, and to estimate the probability of *Fusarium* diseases in a given year and trial site. The maize VCU trials declared for state registration has a large number of varieties tested each year, and the disease forecast will help to determine which

variety trial stations will provide sufficient infection pressure for the resistance tests with a larger number of replicates than currently specified in the methodology. This will provide statistically more reliable results.

3. Our results show no evidence of a relationship between resistance to *Fusarium* ear rot and stalk rot in maize, suggesting different genetic regulation. Based on our findings the two disease forms should be evaluated separately in the state registration process of maize.

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