SZENT ISTVÁN UNIVERSITY

Analysis of correlations between the tissue structure of maize stalks and the *Fusarium* stalk rot infection of the genotypes, and the effect of these factors on lodging resistance

Main points of the PhD thesis

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Martonvásár
2011

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1. INTRODUCTION AND AIMS

1.1 The actuality of the topic

Nowadays maize production is one of the most important branches of crop production, both in Hungary and on a world scale. In Hungary it is sown on 1–1.2 million hectares a year. Throughout the world it is widely used in animal feeding, but in other continents it is also a major food cereal, and has recently gained importance as a raw material for bioethanol production. In addition, it has various uses for industrial processing. It is the most important fodder plant in Hungary, making up almost 90% of the grain feed. Some 8–10% is used for industrial purposes, and only a very small proportion of conventional maize is used for human consumption.

The most important criterion for new hybrids, in terms of both the market and the farmers, is a high yield per hectare. The average yield of maize has increased by around 600% compared to that achieved at the end of the 1800s. This can be attributed partly to technological developments, and partly to the outstanding genetic yield potential of hybrids with valuable genetic properties (Hallauer et al., 1988; Berzsenyi and Győrffy, 1995). The achievement of the potential yield depends not only on the hybrid, however, but also on the production technology and ecological factors. In recent years yield averages have exhibited great fluctuation, due to the increasing frequency of weather extremes, the drastic reduction in the fertiliser rates applied and the damage caused by various pests.

In a continental climate, the pathogens causing the most serious problems are species belonging to the *Fusarium* genus. These are able to infect all the organs of the maize plant, from germination right up to maturity. When the pathogen attacks the stalk, the plant dies earlier, reducing grain filling and resulting in small, light ears. In addition, the stalks break or lodge, resulting in further yield losses from ears that cannot be harvested. Data in the literature suggest that the yield losses due to fusarium stalk rot may amount to 6–35% (Manninger, 1967; Zuber and Kang, 1978; Lu et al., 1995; Logrieco et al., 2002).

The pathogens responsible for fusarium stalk rot have not caused serious damage in Hungary over the last 20 years, so breeders have paid little attention to this disease. The changing climate in recent years, however, has been favourable for the renewed spread of the disease. Nowadays intensive technologies are required to increase yields (Nagy, 2007), but this may also result in the increased frequency of fusarium stalk rot.

If defence against the pathogens causing stalk rot is to be successful despite the use of intensive technologies and major changes in environmental conditions, the breeding of maize lines and hybrids resistant to fusarium stalk rot will be increasingly important.

1.2 The objective of the research

The aim was to find answers to the following questions:

1. Are there any differences between the genotypes in their tolerance/resistance to fusarium stalk rot?
2. What breeding gains have been achieved in Martonvásár in the improvement of fusarium stalk rot resistance over the last few decades?
3. How could the use of an image analysis program help in identifying and evaluating the disease?
4. Does natural infection or artificial inoculation provide more reliable data on the stalk rot resistance of genotypes?
5. How are properties influencing the mechanical structure of the stalk (rind resistance, stalk diameter) correlated with stalk strength?
6. What role does the cellulase enzyme, which decomposes cell walls, play in fusarium stalk rot?
7. Is the determination of the cellulase enzyme activity of stalk tissue extracts a suitable method for identifying susceptible or resistant genotypes?
8. Can positive or negative correlations be revealed between the properties analysed, and how close are these?
9. How are the properties that influence stalk strength inherited?
10. What use can be made of the results in practical breeding?

2. MATERIALS AND METHODS

2.1 Genotypes

During the three years of the experiment, eight single-cross hybrids and their parental lines were examined. The hybrids were in general cultivation in various years (ranging from 1972 to 2006) and with two exceptions belonged to the FAO 300 group. The inbred lines had various genetic backgrounds: Iodent (Iod), Iowa Stiff Stalk Synthetic (ISSS), Iod/Lancaster, Iod/ISSS, ISSS/Minnesota13, Mindszentpusztai Yellow Dent (MYD), OH43, Minnesota Syn and Non-related.

2.2 Characteristics of the field location

2.2.1 Soil properties

The soil of the experimental field was chernozem with forest residues. Soil analysis indicated that the soil was a slightly calcareous crumbly loam, with good N supplies from the organic matter content. It had very good supplies of phosphorus and potassium, and had satisfactory quantities of the major microelements required for plant physiological processes, with the exception of Zn.

2.2.2 Meteorological data

The weather differed in the three years. In 2006 and 2007 the vegetation period was drier than the long-term mean. The total rainfall quantity in the growing season was lower in 2006, but the distribution was less favourable in 2007, with rainfall deficiency from emergence to flowering, but almost twice the average rainfall quantity in August and September. 2008 was a wet year, and the rainfall distribution over the growing period was also favourable. In all three years there were more very hot days than the average for 1999–2008. The greatest difference from the mean was observed in 2007. There was no significant difference between the years in terms of relative humidity and mean temperature data during the growing season.

2.3 Field experiments

2.3.1 Experimental design

The genotypes were sown in a two-factor split-plot design with four replications, with the genotypes in the main plots and four treatments in the subplots: two *Fusarium graminearum* isolates (1. FG36, 2. FGH4), 3. sterile kernels, 4. untreated control. The plant density was 52.600 plants/ha.

2.3.2 Preparation of the isolates used for artificial inoculation

Two *Fusarium graminearum* (Schwa.) isolates (FG36 and FGH4) were used for artificial inoculation. The oat kernels used for inoculation were surface sterilised in 70% ethanol for 2 min, then in 20% bleach solution containing detergent (Tween 20) for 20 min. This was followed by three rinses in distilled water. The oat kernels were then left to dry for 12 h, before being placed in 20 ml glass sample holders. Internal kernel infection was eliminated by sequential sterilisation for 2×5 min in a 60°C water bath, followed by 10 min in dry air at 121°C. The sterilised kernels were inoculated with a 2 ml quantity of a 10^6 conidia/ml suspension of the two isolates. To ensure the air exchange required for the development of the fungus, the sample holders were sealed using paper wadding and incubated for 14 days at 27°C. The sterile oat kernels used in the control were prepared in a similar manner, omitting inoculation.

2.3.3 Technique used for inoculation

The artificial inoculation was carried out using the method reported by Kohler (1960) and Szécsi (2005, personal communication). The plants were inoculated on the 12th day after flowering in the 2–3rd internode from the ground. After cleaning the stalk with alcohol, holes were made with a 2 mm Ø drill, and fungus-covered or sterile oat kernels were placed in the holes, after which the entrance was immediately sealed with sticking plaster. Six plants per plot were inoculated, and six plants were also evaluated on the fourth control plot in order to determine the rate of natural infection. Each year samples were collected starting in the first ten days of October, depending on the maturity group. The samples were stored at –18°C until required.

2.3.4 Determination of the extent of tissue rot

The extent to which the pith was rotted was determined using two methods: by calculating the F_{index} percentage and using a computerised image analysis program.

In the first case the frozen stalk samples were cut in half lengthwise and the state of the pith was scored on a 0–5 scale (Ikenberry and Foley, 1967; Kovács et al., 1988), after which the F_{index} percentage (F_i%) was calculated using the equation given by McKinney (1923). In the second case all the stalk samples were photographed using a digital camera, and the size of the infected area was determined as a % of the whole area of the internode using the Colim 4.0 image analysis software. The principle behind the measurements is that healthy and diseased tissues form patches with a different intensity range.

2.3.5 Evaluation of the mechanical structure of the stalk

The rind resistance was determined based on the method elaborated by Zuber (1973), using an improved rind resistance meter. Measurements were made in the middle third of the 2^{nd} internode, on the side of the stalk opposite the ear attachment site, on six selected plants for each genotype. The measurements were made on two occasions, during the last ten days of July and in October, immediately before the stalk samples were collected. The data are absolute values. The greatest diameter of the 2^{nd} internode was recorded in mm when the second rind resistance measurement was made. The extent of stalk lodging was also scored by determining the frequency (%) of plants lodged below the ear at an angle of over 30°.

2.4 Laboratory analyses

2.4.1 Preparation of stalk tissue samples

The material required for the determination of the cellulase enzyme activity in the stalk tissue was extracted from frozen stalk samples. The pith was first cut up with scissors, after which it was ground to the fineness of semolina and 1 g quantities were homogenised in 10 ml 0.1 M acetate buffer. The homogenate was filtered through paper wadding and centrifuged at 8000 rpm for 30 min at 4°C. The clear supernatant (approx 8 ml) was divided into two. One part was stored at –20°C until further use, while concentrated enzyme sources were prepared at two pH values from the second part. Twice 2 ml quantities of the remaining unconcentrated enzyme sources were measured into 10 ml plastic centrifuge tubes, and 6 ml ice-cold acetone was added to each. The mixture was kept at 4°C for 12 hours to ensure the complete precipitation of the proteins. The flaky protein precipitate was then centrifuged at 10,000 rpm for 10 min at 4°C. After evaporating to dryness, one was redissolved in 100 µl acetate buffer (pH=5) and the other in 100 µl 0.05 M TrisHCl (pH=8.5). The samples thus prepared were then stored at –20°C until further use.

2.4.2 Determination of the cellulase activity of the stalk tissue extracts

The cellulase activity was determined using the method of Dingle et al. (1953). The following components were used for a 100 ml gel plate: 1.74 g K_2HPO_4 (Reanal), 1.5 g agar-agar (Reanal), 0.84 g citric acid (Reanal), 0.1 g AZCL-HE-Cellulose (Megazyme) and 0.05 g Na-azide (Sigma), dissolved in distilled water. Each plastic petri dish was filled with 20 ml of this gel, and wells were formed in the plates using a 5 mm Ø cork drill to hold the enzyme solution. A 20 µl quantity of the above enzyme solution was pipetted into each well, and the gels were left to stand at 37°C for 24 h. The enzyme activity was expressed as the area of the activity ring (mm^2/37°C/24 h) with the help of the Colim 4.0 image analysis software.

2.5 Statistical analysis of the data

The data were statistically analysed by means of multifactorial analysis of variance and linear regression analysis, using the Agrobase 99® for Microsoft Windows® software (Agronomix Inc.) and the Microsoft Windows® Excel program. The results of the statistical tests were interpreted following the guidelines of Sváb (1981).

3. RESULTS

3.1 Results of stalk rot analysis

The genotype had a significant influence on the extent of stalk rot in the case of both hybrids and lines. The grand experimental mean was larger for the lines than for the hybrids using both evaluation methods, and the susceptibility of the lines to stalk rot exhibited greater variability.

On the basis of their susceptibility to stalk rot, the inbred lines could be divided into three groups, consisting of severely, average and weakly infected genotypes. The severely infected group included one ISSS/Minnesota13 (P03), one Iodent (P04), one Iodent/Lancaster (P05) and one MYD (P09) genotype. In the group with average infection there was one Minnesota Syn (P14), one Iodent (P13), one Iodent/ISSS (P08), one Iodent/Lancaster (P02) and two ISSS (P01, P12) lines, while the weakly infected group contained one OH43 (P10), one Non-related (P11) and two ISSS (P06, P07) inbred lines.

In the case of the hybrids, the greatest damage was suffered by P05×P03, in the FAO 300 maturity group, and the least by the two hybrids in the late maturity group. No significant difference in stalk rot infection could be detected between the two late hybrids, despite differences in their maturity dates. The order for the hybrids in the FAO 300 group, based on the F_{index} data was as follows: P07×P02 (2006) < P11×P02 (2003) = P01×P13 (2004) < P04×P12 (2000) < P05×P03 (1992) and P09×P14 (1972). The significantly least stalk rot damage was thus suffered by a hybrid bred in 2006 and the greatest by the hybrids bred the longest time ago, in 1972 and 1992.

Stalk rot in the field was determined using two methods: F_{index} calculation and the Colim image analysis software. In all three years the correlation between the two methods was examined, and it was found that the data determined with the two methods could be estimated from each other with a probability of 95–96%. A comparison was then made of the precision, sensitivity and usefulness of the disease information obtained with the two methods. The results showed that for three years (2006–2008) and four treatments (FG36, FGH4, sterile, control) more significant differences could be detected between the lines and hybrids in three cases using the F_{index} calculation and in eight cases using the image analysis software. In the other cases both methods detected differences between the same number of genotypes. An analysis of CV values revealed that, with the exception of the sterile kernel treatment in 2006 and the FGH4 isolate in 2007, the CV values for the image analysis were smaller in every case, i.e. the percentage deviation between the values was smaller in this case.

Correlations between the treatments were also analysed for each year separately. Averaged over the years the correlations obtained for the same treatment were the most balanced for the artificial inoculations (FG36, FGH4), with correlation coefficients between r = 0.52–0.60 with one exception; for four of the six correlation analyses the probability level was also the same (P=1%). In the sterile kernel treatment similar correlations could be detected, except that the three correlation analyses were significant at different levels of probability (r = 0.47–0.57; P=0.1–5%). The greatest difference between the pairs of years was found for the control treatment: the correlation coefficients ranged from r = 0.34–0.94 and the level of significance from non-significant to P=0.1%.

3.2 Results for properties influencing or characteristic of the mechanical structure of the stalk

In the case of both lines and hybrids, the smallest rind resistance values were recorded in 2006 and 2007 and the largest in 2008. The rind resistance of the hybrids was higher than that of the lines in both July and October in 2006 and 2008, but in 2007 that of the lines was higher at both measuring dates. Among the three years, 2006 and 2007 were dry, while 2008 was wet. The data thus suggest that rind resistance can be expected to be greater in wet years than in dry years, i.e. rainfall had a positive influence on rind resistance.

Averaged over the four treatments and the years, the greatest rind resistance values for both hybrids and lines were recorded in the control treatment, followed by the sterile kernel treatment, with the lowest values for stalks inoculated with the two *Fusarium* isolates. In the case of the hybrids there was no significant difference between the rind resistance values in the two artificially inoculated treatments, but the differences between the other treatments were significant. For the lines there were significant differences between the rind resistance values of all the treatments.

The mean rind resistance value of the hybrids in summer (32.95) was higher than the corresponding value for the lines (28.43). In autumn, however, the hybrids had lower rind resistance than the lines (24.07 vs 27.32). The better rind resistance values of the lines in autumn can be explained by the fact that the growing season of the lines was longer than that of the hybrids. This meant that the lines were in an earlier developmental stage in October than the hybrids, requiring greater force to break the rind.

The stalk diameter of both lines and hybrids was decisively influenced by the year. There were significant differences between the three years in the genotype groups. Among the hybrids, those with longer vegetation periods had thicker stalks. The smallest stalk diameter was observed for the two earliest bred hybrids (P05×P03, P09×P14), which represent the end of the FAO 300 group.

Based on the grand mean of the experiment, more than 1.5 times greater lodging was recorded for the inbred lines (3.20%) than for the hybrids (1.97%). Among the hybrids the lodging of the two hybrids developed in 1972 (P09×P14, P06×P09) was significantly the greatest, while no significant differences were observed for the other hybrids. In the case of the inbred lines, the genotypes exhibiting the highest percentage of lodged plants were P14 (Minnesota Syn), P09 (MYD) and P03 (ISSS/Minnesota13). The first two of these lines are old breeding materials, while P03 is several generations younger. The smallest lodging percentage was recorded for two ISSS lines (P07 and P12). These data suggest that lodging is determined to a great extent by the genotype.

The various treatments caused different extents of lodging in the inbred lines and hybrids. The greatest lodging in both groups was recorded on plots subjected to artificial inoculation. No significant difference was observed between the two *Fusarium graminearum* isolates for either the lines or the hybrids. In response to artificial inoculation there was an increase in the lodging of the genotypes, i.e. the pathogenic fungus played a role in the severity of lodging.

3.3 Results for the cellulase enzyme activity in stalk tissue extracts of the genotypes

Significant differences in cellulase enzyme activity were found between the genotypes, in both the inbred line and hybrid groups. The greatest cellulase enzyme activity was measured after artificial inoculation with the FGH4 isolate, followed by the FG36 isolate, and then the sterile kernel treatment. The activity recorded on the control plots was the lowest. The order was the same for both maize lines and hybrids. Higher enzyme activity was observed for tissue extracts concentrated in acetate buffer (pH=5) than for those concentrated in TrisHCl (pH=8.5), i.e. acidic pH was more favourable for enzyme activity. The enzyme activity was lowest in the unconcentrated tissue culture extract, but even in this case significant differences were obtained between the maize genotypes after artificial inoculation with FGH4 and FG36. In the case of unconcentrated tissue extracts the cellulase enzyme activity was lowest in the control treatment.

Correlations between the cellulase enzyme activities of unconcentrated and concentrated tissue extracts were also examined. In the case of unconcentrated tissue extracts, a close positive correlation was observed at the P=5% probability level between the cellulase enzyme activities recorded in the lines for the two artificially inoculated treatments (FG36, FGH4) and for the sterile kernel and control treatments. The correlations were not significant in the other cases. Very close positive correlations significant at the P=0.1% or P=1% levels were obtained for both the inbred lines and the hybrids between the unconcentrated and concentrated tissue extracts for the same treatments (FG36–FG36, FGH4–FGH4, etc.). This indicates that reliable information on the state of infection of a given population can be obtained from the cellulase enzyme activity of the unconcentrated tissue extract in the case of artificial inoculation and from that of the concentrated tissue extract for natural stalk fusarium infection.

3.4 Results for correlation analysis on traits influencing stalk strength

Correlations between traits characterising the stalk strength of inbred lines and hybrids were determined using the correlation coefficients calculated by means of linear regression based on the mean values of the three years and the treatments.

Very close positive correlations were observed between the stalk rot values determined using the two calculation methods in the case of both lines and hybrids.

A negative correlation was obtained between stalk rot and the rind resistance measured at two dates, for both inbred lines and hybrids. The correlation between summer rind resistance and stalk rot was moderate and significant at the P=10% level for the lines. The situation was similar for the hybrids, except that the correlation between stalk rot and rind resistance was not significant when the image analysis software was used. In the case of autumn rind resistance a closer correlation was observed for both inbred lines and hybrids.

The correlation between stalk rot and the stalk diameter was negative, and moderate for the lines but very close for the hybrids. Stalk rot had a positive effect on lodging in the case of the inbred lines, and this correlation was significant. No correlation was detected between these two factors for the hybrids.

The closest correlation was recorded between stalk rot and cellulase enzyme activity. The correlation between these two factors was very close, and positive at the P=0.1% and P=1% probability level for the lines and hybrids. Correlations between stalk rot and cellulase enzyme activity were determined for all four treatments for each of the 22 genotypes. A very close positive correlation was observed between the two parameters for all the treatments; genotypes exhibiting less stalk rot infection also had lower cellulase enzyme activity in all the treatments. Over the three years, no stalk rot was observed as the result of natural infection for a number of genotypes (in the control treatment). In these genotypes, no cellulase enzyme activity could be detected in the tissue extracts.

3.5 Results for the heritability of properties influencing stalk strength

The parent–progeny regression for stalk rot was significant (P=1%) for both measuring methods in the case of the parental mean–hybrid progeny correlation, and the h^2 values of this property were also high. This means that if breeding material with adequate variability is available, selection can be made relatively easily and reliably for positive variants. The female parent–hybrid progeny correlation coefficients for stalk rot, measured with both methods, revealed significant, very close correlations with stalk rot using both methods, while the male parent–hybrid progeny regression was not significant. According to the h^2 values, the maternal effect was decisive in the inheritance of stalk rot susceptibility, while the paternal effect was much less pronounced.

The parental mean–hybrid progeny regression for rind resistance was significant at both dates, but the correlation coefficient was higher for rind resistance measured in summer, and in this case the correlation was more significant (P=5%) and the h^2 value higher (0.91). The h^2 value was only 0.41 when rind resistance was measured in autumn, and at this date the probability level and the size of the correlation were also smaller. The female parent–hybrid progeny correlation for rind resistance was not significant at either date. On the other hand, the male parent–hybrid progeny correlation for rind resistance was close and significant in July and moderate in October.

The parental mean–hybrid regression for stalk diameter was significant and the estimated h^2 index (0.66) demonstrated the good heritability of this trait. The correlation coefficient calculated between the female parent and the hybrid progeny for stalk diameter (r=0.87) revealed an extremely close, significant correlation and the h^2 value was also high, while the male parent–hybrid progeny correlation was not significant and the h^2 value was also low, indicating that the female parent had a decisive role in the inheritance of stalk diameter.

The results of parent–progeny regression showed a close significant (P=1%) correlation between the parental mean and the hybrid progeny for the cellulase enzyme activity of all three tissue extracts. The h^2 values of this trait, measured in the unconcentrated and acidic concentrated extracts, indicated that the trait had good heritability, but in the case of the alkaline concentrate the data could not be interpreted. The heritability values of the trait suggest that the maternal effect is decisive for the inheritance of cellulase enzyme activity, while the paternal effect is much smaller.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions drawn from the results of stalk rot analysis

The results of the field experiments showed significant differences between the genotypes for resistance to fusarium stalk rot. The ISSS/Minnesota13 (P03), Iodent/Lancaster (P05) and MYD (P09) lines exhibited poor resistance to stalk rot. Inbred lines with a Minnesota Syn or Iodent background had far better stalk rot resistance than these, but were less resistant than the ISSS lines. Among the inbred lines the best resistance to fusarium stalk rot was exhibited by P06 and P07, both of which were related to ISSS. This is not surprising, as these Iowa Stiff Stalk Synthetic lines were bred for stalk rot resistance (Sprague, 1946). The ISSS lines and the lines derived from them are still extremely important and are widely used as initial sources in breeding for a number of important agronomic traits (drying down, root and stalk lodging, ear attachment height) (Holthaus and Lamkey, 1995).

It was reported by Marton (2002) that Martonvásár hybrids have satisfactory tolerance of stalk rot, as confirmed by data from official trials. The stalk rot resistance of hybrids bred in Martonvásár in various periods was tested in the experiment and it was found that the tolerance of stalk rot was always significantly better in each maturity group for more recently bred hybrids.

The measuring and evaluating methods used in plant pathology research often include subjective judgements and the exact quantification of visually obtained information is also difficult. Sherwood et al. (1983) reported that plant pathologists often overestimated the extent of the disease when evaluation was based on subjective judgements, especially at low rates of infection. The precision and sensitivity of disease evaluations carried out visually and using image analysis software were compared in the experiment, and with two exceptions the CV values were lower for the image analysis. As the CV for measurements can be considered as a relative error, it can be stated that image analysis is the more precise of the two methods, so this technique gives a more accurate picture of the extent of stalk rot. A number of authors have published similar conclusions on the efficiency of image analysis software (Todd and Kommedahl, 1994; Bock et al., 2008; Gergely, 2004).

An analysis was made of the effect of the year on correlations between the treatments. The data suggest that the greatest uncertainty is encountered if resistance to fusarium stalk rot is based purely on natural infection. The results proved that this form of evaluation depends to the greatest extent on the year effect. In relatively similar years the data correlate fairly well with each other (2006–2007), but when the years have very different weather, there may be no correlation between the data of the two years (2006–2008). The extent of stalk rot developing in response to natural infection is extremely environment-dependent, so the use of artificial inoculation is recommended for selection trials.

4.2 Conclusions drawn from results related to the mechanical structure of the stalk

In 2006 and 2008 the rind resistance measured for inbred lines in July was greater than that recorded in October, and this was true in all three years for the hybrids. This corresponds to the results published by Colbert and Zuber (1978). In 2007, however, the rind resistance of the inbred lines was smaller in July than in autumn. Among the three years, 2006 and 2007 were dry, while 2008 was wet. Marton (2002) also measured greater rind resistance for inbred lines in a dry year at two different plant densities. The two dry years differed in the rainfall distribution. In both years there was less rainfall than the long-term mean at flowering, but in 2007 there was above-average rainfall in autumn. These weather factors enhance stalk rot, which is in negative correlation with rind resistance. In addition, due to the longer vegetation period of inbred lines, they were in an earlier stage of development than the hybrids at both measuring dates. This probably explains the fact that the rind resistance was greater for the lines than for the hybrids in 2007.

The data presented by Mesterházy (1981) revealed no correlation between stalk rot and rind resistance. Kovács et al. (1988), however, found a correlation between the two factors, and in later studies Mesterházy (1983) also reported a correlation. In the present studies the greatest rind resistance was measured in the control treatment for both hybrids and inbred lines, followed by the sterile kernel treatment and inoculation with the two *Fusarium* isolates. The stalks of plants in artificially inoculated plots suffered greater stalk rot infection than those on sterile kernel and control plots, suggesting that the rind resistance depends on the extent of stalk rot.

The lodging data revealed differences between the genotypes for both inbred lines and hybrids. The least lodging was observed for two ISSS breeding materials (P07, P12), and lines related to Iodent also had good lodging resistance. The greatest extent of lodging was recorded for the two oldest lines (P09, P14). For both the lines and the hybrids the older breeding materials were found to lodge to the greatest extent.

4.3 Conclusions drawn on the basis of laboratory analyses

The unconcentrated tissue extract can be prepared more quickly and at less cost, so the experiments included a determination of whether satisfactory results could be obtained if the enzyme activity of the tissues was measured and evaluated purely on this basis. It was found that although differences could be detected between the genotypes, the enzyme activity was greater in the unconcentrated tissue extracts of artificially inoculated plants, and in the case of inbred lines measurements in extracts concentrated using acetate buffer (pH 5) revealed differences between a larger number of genotypes. Correlations between the cellulase enzyme activities recorded in unconcentrated tissue extracts and concentrated extracts were determined for inbred lines and hybrids. In both cases, very close positive correlations significant at the P=0.1% or P=1% probability level were found between the unconcentrated and concentrated tissue extracts for the same treatments. This suggests that the cellulase enzyme activity of a given genotype can be reliably determined even from unconcentrated tissue extracts. If cellulase enzyme activity is determined from the tissue extracts of naturally infected stalks, however, the method is more efficient if the tissue extract is concentrated using acidic buffer.

4.4 Conclusions drawn on the basis of correlation analysis

For the traits examined in the present work, the closest correlations were obtained between stalk rot and cellulase enzyme activity. For both lines and hybrids, very close positive correlations significant at the P=0.1% or P=1% probability level were found between these two factors. These results are in agreement with earlier data from the literature, which reported a positive correlation between the extent of stalk rot and cellulase enzyme activity (Szécsi, 1985; Chambers, 1987; Ahmad et al., 2006; Szőke et al., 2009). In all four treatments a very close positive correlation was found between stalk rot and cellulase enzyme activity: genotypes exhibiting less severe stalk rot levels had lower enzyme activity in all the treatments. Literary data reveal a positive correlation between the infectivity of *Fusarium* species and the quantity of enzyme they produce (Novo et al., 2006; Kikot et al., 2009). The present data suggest that the enzyme activity values of both inbred lines and hybrids can be used to characterise the susceptibility of the genotypes to stalk rot.

Correlations between the rind resistance values measured at two different dates were also analysed. Very close significant correlations were detected both for the inbred lines (r=0.97***) and for the hybrids (r=0.98***). Similar results were reported by Mesterházy (1981). This parameter is an important factor in stalk strength. The parent–progeny regression for rind resistance was significant at both dates. The correlation coefficient of rind resistance measured in summer was the higher of the two (r=0.76), and in this case the correlation was more significant (P=5%) and the h^2 value was higher (0.91). Plant variants with better rind resistance can thus be selected with greater certainty on the basis of rind resistance data recorded at flowering. In the case of male parent–hybrid progeny regression a close significant correlation was obtained when rind resistance was measured in July, while the correlation was only moderate in October. Georgiev (1977) and Marton (2002) reported closer, more significant correlations between the male parent and the hybrid progeny than between the female parent and the hybrid. The above data suggest that satisfactory information on this trait can be obtained at flowering, allowing breeders to use inbred lines with greater rind resistance for self-fertilisation.

Schertz et al. (1978) reported a very close negative correlation between the stalk diameter and lodging of sorghum. In maize Marton (2002) found a moderate negative correlation at a plant density of 70,000 plants/ha, while at 40,000 plants/ha a loose, negative, insignificant correlation was observed. A significant positive correlation was detected, however, between the stalk diameter and the rind resistance. The present results revealed a negative, moderately strong correlation between stalk diameter and the stalk rot determined by image analysis for the inbred lines, while the correlation was negative but considerably closer for the hybrids. In the case of the lines there was a positive moderate correlation between the stalk diameter and the rind resistance measured in autumn, while a close positive correlation was determined between these two parameters at both dates for the hybrids. The stalk diameter was in significant negative correlation with stalk rot and in positive correlation with rind resistance, so stalk diameter is an extremely important selection criterion in breeding for stalk strength.

According to Marton (2002) the correlation between the female parent and the progeny hybrid was closer for lodging and that between the male parent and the hybrid for rind resistance. A comparison of the regression analysis data for rind resistance and lodging revealed that for both parameters the male parent–hybrid progeny correlations were closer, and significant at a higher level of probability. All in all the data thus suggest that the paternal effect is decisive for both lodging and rind resistance. This is important because rind resistance is a major component in the complex evaluation of lodging resistance.

In all cases a negative correlation was observed between rind resistance and stalk rot. For the inbred lines the linear regression between the rind resistance measured in summer and autumn and the stalk rot infection was moderately strong and significant (P=10% and P=5%). Similar correlations were obtained for the hybrids. For both the inbred lines and the hybrids the values of the correlation coefficient indicated a closer, more significant correlation between rind resistance and stalk rot when measurements were made in autumn. This is probably due to the fact that the damage cause by fusarium stalk rot is greater in autumn, since the pathogen is a saprophyte. The data revealed a significant difference between the rind resistance values of infected and healthy plants. It can be concluded from the results that there is a clear correlation between fusarium stalk rot and rind resistance.

For both the inbred lines and the hybrids a significant negative, moderate correlation was found between rind resistance and lodging. These results confirm earlier findings that the use of a rind resistance meter provides satisfactory information on the resistance of a given genotype to lodging (Twumasi-Afriyie and Hunter, 1982; Anderson and White, 1994; Marton, 2002).

For both methods of measurement the parent–progeny regression for stalk rot was significant (P=1%) and the h^2 value of the trait was high. This suggests that, if the breeding material possesses sufficient variability, it should be relatively easy to select variants with reliable resistance to stalk rot. The h^2 value was greater for the female parent–hybrid progeny relationship than for the male parent–hybrid progeny. The regression was significant for the female parent–hybrid progeny relationship but not in the case of male parent–hybrid progeny. Similar results were reported by Kovács (1973), who also observed greater regression for the maternal parent–hybrid progeny. This author also analysed reciprocal effects and found substantial differences. The present data suggest that the role of the maternal effect is more decisive for the inheritance of stalk rot than that of the paternal effect. The present results and the findings of Kovács (1973) with respect to the reciprocal effect are worth taking into consideration when designing hybrids, particularly if this has no detrimental effect on seed production, which is an important question in the planning of new hybrids.

NEW SCIENTIFIC RESULTS

This work involved an analysis of fusarium stalk rot, the properties influencing the mechanical structure of maize stalks, and correlations between the two. The resistance of the genotypes to stalk rot was investigated in the case of both artificial inoculation and natural infection by recording the rind resistance, stalk diameter and stalk lodging of the plants. The field data also made it possible to study differences caused by the year effect.

Laboratory analyses were performed to measure the cellulase enzyme activity from pith samples taken from the stalks of plants exposed to artificial inoculation or natural infection in the field. The field and laboratory data, supplemented by correlation analysis on the traits, made it possible to quantify the abiotic and biotic factors influencing the stalk strength of maize and the correlations between these factors.

The following new results were obtained in the course of the work:

On the basis of field observations, differences were detected in the resistance of maize genotypes to fusarium stalk rot. Among the inbred lines tested, the genotypes with the best stalk rot resistance were those related to ISSS. Lines related to Iodent also had good resistance to stalk rot, but this was generally weaker than that of lines with an ISSS background. It was proved that more recently bred Martonvásár hybrids have better resistance to fusarium stalk rot than those bred in earlier years.

It was established that, of the two stalk rot evaluation methods tested, image analysis provided a more accurate picture of the extent of stalk rot than F_{index} calculations based on visual evaluation. Image analysis is a more sensitive technique than F_{index} calculations, making it more suitable for the detection of smaller differences between the genotypes.

The results showed that successful selection for stalk rot resistance cannot be achieved based on natural infection alone, since the latter is determined to a substantial degree by the weather in the given year. The use of artificial inoculation provides a more accurate picture of the resistance levels of the genotypes.

There was a very close correlation between the rind resistance values measured at two different dates (at flowering and prior to harvest), and the data revealed a high estimated heritability value on the basis of parent–progeny regression. This suggests that, if the breeding material has sufficient variability, adequate information can be obtained in the flowering phenophase on the susceptibility or tolerance of sublines to stalk lodging, allowing breeders to carry out self-fertilisation only on inbred lines with greater rind resistance.

Stalk diameter was found to be in significant negative correlation with stalk rot and in significant positive correlation with rind resistance, i.e. stalk diameter could be an important selection criterion in breeding for stalk strength.

All the measurements indicated a significant negative correlation between stalk lodging and rind resistance, while a significant correlation between lodging and stalk rot was only detected in one case, suggesting that lodging depends more on the mechanical parameters of the stalk than on stalk diseases. By breeding for better rind resistance, progress can also be achieved in lodging resistance.

A very close correlation was observed between the cellulase enzyme activity of the stalk tissue and the extent of stalk rot. The cellulase enzyme activity of the fungus was lower in more resistant maize genotypes and greater in susceptible genotypes. No cellulase enzyme activity could be detected in healthy tissues. It was proved that extracts concentrated using acidic buffer were more suitable than unconcentrated tissue extracts for the characterisation of enzyme activity after natural infection. The determination of cellulase enzyme activity could be an efficient tool in breeding for resistance to stalk rot.

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