# STUDY REGARDING THE REACTION OF SOME MAIZE HYBRIDS FROM ITALY TO THE ATTACK OF THE FUNGI *USTILAGO ZEAE* (BECKM) AND *HELMINTHOSPORIUM TURCICUM* PASS. IN THE CLIMATIC CONDITIONS FROM BANAT'S PLAIN

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***Abstract:*** *In the western side of Romania appear every year in the maize crops the fungi Ustilago zeae and Helminthosporium turcicum, but the obvious harvest loses appear in the years when are present the optimal limits, the trophic and climatic demands of the pathogen. The objective of this research was the testing of nine maize hybrids original from Italy from the point of view of the reaction to the attack of the pathogens specific in natural infection conditions. The experimental field was placed in the perimeter of the Didactic Station of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara. The hybrids were observed during the entire vegetation period in 2015. The nine hybrids with SIS provenance have been tested for the first time in the conditions of Banat's Plain. The response of the hybrids to the attack of the two above mentioned pathogens was set by analysing the field data on the resistance source scale and on the cultural scale. In the case of fungus Helminthosporium turcicum six hybrids from the experience were showed sensitivity to the pathogen (Kimbos 500, Kampius 480, Pico 450, Sunsis 420, Kabrios 380 and AGN 340) and three hybrids manifested tolerant reaction (Sistema 580, Cristina 600 şi Kalmus 600 – late hibrids). There was noticed in the late hybrids that the virulence is lower comparative with intermediate and ultra early hybrids. According with the cultural scale, less exigent, the hybrids Kimbos 600 and Kampius 480 are maintaining as sensitive and the others are medium resistant to the pathogen. In the climatic conditions of the year 2015 seven hybrids from experience have manifested resistance reaction to the attack of the pathogen Ustilago zeae, respectively Kalmus 600 (130 days), Sistema 580 (180 days), Cristina 600 and Kimbos 500 (125 days), Kampius 480 (123 days), Pico 450 (120 days), Sunsis 420 (120 days) and the hybrids Kabrios 380 and AGN 340 have manifested sensitivity reaction. In general the tested hybrids have tolerated well the pathogen, the obtained results being in accordance with the production company results. The good tolerance to the attack of the pathogens, even excellent in the case of some hybrids, the vegetative vigour and the tolerance to the water stress make possible their successful cultivation in non-irrigated systems and even on soils with lower soil fertility.*

***Key words:*** *Ustilago zeae, Helminthosporium turcicum, hybrids, fungi, reaction, common smut, maize.*

## INTRODUCTION

*Ustilago zeae* and *Helminthosporium turcicum* are pathogens that appear in the maize crops from Banat's Plain with different attack frequencies and intensities depending by the climatic conditions.

The fungus *Ustilago zeae* that produces the common smut of the maize is more frequent in the dry years [PAULIAN F. *et al*., 1957]. According with R. MARINHO DE MOURA (2001), the pathogen is similar from some points of view with wheat rust, vine bight and apple scab. In Romania the common smut appears anywhere the maize is cultivated, with greater frequency and intensity when the crop is repeated [POPESCU GH., 1989]. According with ANA HULEA *et al*. (1973), *Ustilago zeae* is very spread in Romania, mainly in the hill sub-carpathic areas, in the plots affected by hail and the hybridisation plots were are produced injuries by the breaking of the panicles.

It produces loses sometimes to 40% depending by localisation, number and dimension of the tumours. In 2003 VIORICA IACOB shows that the damages produced by this pathogen have comprised between 2.5 – 5% from harvest. The maize cobs can be totally or partially injured. The smut formations formed on cobs can reach big dimensions, respectively 10 cm length and 5 – 6 cm in diameter, and weight about 2 kg. The tumours with spores appear more frequently on the top or at the base of the cobs and are developing due to the bracts or ovaries that are hypertrophied [ELIADE EUGENIA, 1985]. In this situation the harvest loses can be great if the frequency of the attacked cobs is high. On leaves the tumours are forming on the base of the parenchimatic tissue aside the main nervure. They are forming usually on blade, more rarely on the sheath or ligulae. At the beginning these foliar excrescences are hard, reddish and then are becoming white. These formations rarely are opening, mainly are remaining as concretions that are producing the undulation of the leaves [POPESCU GH., 2005]. On stem the tumours are forming on the basal nodes or on the superior third of the plant above the cob. In the case that tumours appear above the cob, the disease effects are more severe.

Maize plants receptivity degree to the infection with *Ustilago zeae* varies depending by phenophase. The young plants are less attacked. Researches show that the strongest attacked after the plants have reached 50-70 cm height, but mainly when they have 120-140 cm since to the ripening. If they have passed this stage the plants receptivity decreases [BĂRBULESCU AL. *et al*., 2002; NAGY ELENA, 2004].

The researches show that the areas from Romania where this pathogen is often found are: Danube's Plain, Moldavia's north and Transylvania's east. In these areas the frequency of the attack varies from a year to other being highly correlated with the climatic factors [ALEXANDRI AL. *et al*., 1969; HATMAN M. *et al*., 1989**;** NAGY ELENA, 2004]**.** Being the most well known maize disease, this pathogen is found all around the world, in all the areas where is maize cultivated. In U.S.A. the registered loses are comprised between 2-6%, but in the favourable years loses can reach even 30/50% [NASIR M. *et al*., 1998]. In some countries (Mexic), the young fructifications of this pathogen are considered a delicacy, being prepared in expensive restaurants, both for locals and for tourists [MILLER S. A. *et al*., 1996]. They taste is similar with the mushroom taste.

*Helminthosporium turcicum* is an important pathogen of maize that appears in the maize crops in conditions of humid and warm climate, In the years with favourable climate it can produces loses that can reach to 40% from the harvest [CEBALLOS H. *et al*., 1991]. In Romania and other European countries the loses produced by this fungus are greater in the second crop in conditions of spraying irrigation and in the maize crops designated for forage [NAGY ELENA, 2004; POPESCU G., 2005]. According with other authors [A. D. RAYMUNDO and A. L. HOOKER, 1981] the harvest loses appear when the infection appears before the appearance of the floss. If the infection appears lately (6-7 weeks from the floss appearance) the losses are very low. During the time there was noticed that the early maize hybrids are more susceptible to infection comparative with the late hybrids [DEGEFU Y., 2003]. There can be mentioned that the economic importance of this disease is linked with the moment when are realised the infections and then with the number of the secondary infections. The maize plants are affected mostly when the infection appears before the emerging of the panicle. BĂRBULESCU AL. *et al* mention that spraying fertilisation favours the disease and the harvest loses that can appear in such conditions can be significant.

According with EUGENIA ELIADE (1985) and VIORICA IACOB *et al*., (1998) the pathogen can produce loses up to 30%, these loses varying depending by the climatic conditions, the applied technological measures and the sensitivity of the cultivated hybrids. The areas from Romania where the pathogen is frequently found are: Oltenia, Muntenia and Banat, with predilection for the sub-Carpathian area where the precipitations are more abundant in July and August. According with GHEORGHE POPESCU (2005) strong infections of *Helminthosporium turcicum* in maize were mentioned by many specialists in the Southern Romanian Plain in the second rotation irrigated by spraying, both in the forager maize. Following the injury of the foliar surface that results the limitation of the assimilation capacity, plants debilitation and wilted grains, that leads to the diminishing of the harvest. The infections are favoured by high temperatures during the day and lower during

the night, and by the high air humidity, there being necessary minimum 10 hours of moisturising of the leaves with dew [BAICU T., SEŞAN T. E., 1996].

## MATERIAL AND METHODS

In the framework of this research were observed nine Italian maize hybrids from the point of view of the reaction to the attack of the fungi *Ustilago zeae* and *Helminthosporium turcicum* in the climatic conditions from Banat's Plain. The experimental field was set in the area of Didactic Station of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara in the year 2015. The nine hybrids were cultivated on a surface of 3.5 hectares. The tested maize hybrids come from two Italian companies that are seed producers (maize, soybean, wheat *etc*.), respectively the companies S.I.S. (*Societa Italiana Sementi*) and *American Genetics* Italia. The experimental plot was set with the help of S.I.S. company that has offered the seeds ad consultancy. The nine hybrids are from different maturity groups.

The maturity groups according with the FAO classification of the hybrids from this research are the following:

- late hybrids FAO 600 - Kalmus 600 (130 days), Sistema 580 (128 days), Cristina 600 (130 days);
- intermediate hybrids FAO 500 - Kimbos 500 (125 days), Kampius 480 (123 days);
- intermediate hybrids FAO 400 - Pico 450 (120 days), Sunsis 420 (120 days), Kabrios 380 (118 days);
- early hybrids FAO 300 - AGN 340 (110 days).

*Aggressiveness* or frequency of the attack (F %) of the fungi *Ustilago zeae* and *Helminthosporium turcicum* was calculated using the classical plant pathology assessments methods. *Virulence* or *attack intensity* (I %) was estimated in scores using the 0 – 5 scoring scale, used often in plant pathology. The scores are transformed next in percent rates. Both incidence and virulence of the fungi were registered in the second decade of August. The aggressiveness of the fungi was determined on entire plant and on leaves (*Helminthoporium turcicum*) and on cobs in the case of *Ustilago zeae* attack. In this way there have been analysed 100 plants from every analysed hybrid.

The scores regarding the reaction of the maize hybrids to the attack of the monitored fungi were registered using two scales, respectively "***resistance source scale"*** used for the identification of the resistance sources for inbreeding or creation of new plant varieties or hybrids and the ***"cultural scale"*** used when are characterised the varieties or hybrids for cropping.

The scores that are recorded with "***resistance source scale"*** are the following: no infection (0) – immune form (I); infections between 0.1 – 2.0 (1 – 20%) – resistant form (R); infections between 2.1 – 4.0 (21 – 40%) – medium resistant or tolerant form (MR, T); infections between 4.1 – 9.0 (41 – 100%) – sensitive form (S).

The scores that are registered with the ***"cultural scale"*** are the following: no infection – immune variety or hybrid (I); infections between 0.1 – 2.0 (1 – 20%) – resistant (R); infections between 2.1 – 5.0 (21 – 50%) – medium resistant or tolerant variety or hybrid (MR, T); infections between 5.1 – 9.0 (51 – 100%) sensitive variety or hybrid. The cultural scale is wider, with lower accuracy and because of that some varieties or hybrids that have several percents over the inferior limit of the sensitivity can be classified as medium resistant or even tolerant, this reaction being desired by researchers and farmers in comparison with the sensitive reaction. This scale was adapted and proposed for use in the year 2007.

The climatic data from the analysed period (2015) have been collected at the Meteorological Station Timişoara. The monitored climatic factors that have been applied to the collected field data were precipitations and temperature.

## RESULTS AND DISCUSSION

In 2015 were tested nine S.I.S. hybrids for the first time in the field of Didactic Station of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara in

the climatic conditions of Banat's Plain. Those hybrids haven't been registered in the Official Catalogue of Varieties and Hybrids Cultivated in Romania at the moment of the experimental plot setting. The experimental field was set with the assistance of S.I.S. company that have offered the seeds and know-how. The nine analysed hybrids were Kalmus 600 (130 days), Sistema 580 (128 days), Cristina 600, Kimbos 500 (125 days), Kampius 480 (123 days), Pico 450 (120 days), Sunsis 420 (120 days), Kabrios 380 (118 days) and AGN 340 (110 days). Pico 450 and AGN 340 are produced by the company *American Genetic*s Italia. The other hybrids are produced by the company S.I.S. Italia.

The purpose of setting this experimental field was to analyse the reaction of the nine Italian maize hybrids to the attack of the pathogens specific to maize in the climatic conditions of Banat's Plain. The monitored pathogens in this research were *Ustilago zeae* and *Helminthosporium turcicum*, they appearing in every year in the maize crops from Romania. The testing was necessary because they are cultivated by numerous farmers from the area there being necessary their correct information regarding the qualities and the weaknesses of these hybrids.

The climatic conditions registered during this research haven't been totally favourable for the infection with *Helminthosporium turcicum.* The drought from summer has favoured the infection with *Ustilago zeae*, the temperatures from the analysed period (April – August), this being characterised by average montly temperatures higher than the multiannual average. In the other months of the year there have existed positive variations comparative with the multiannual average for the spring months, and negative averages for the autumn and winter months.

The precipitations from the spring of 2015 haven't passed over the multiannual average, there being registered deficit. In March the precipitation amount was 27.8 mm, the registered deficit being 13.8 mm; in April the deficit was 30.4 mm and in May it was 32.3 mm. in the months June and July wasn't rained at all, the registered deficit being equal with the multiannual average. The second half of August has rainfall excess, the multiannual average being passed over with 78.1 mm. The rainfall amount registered in 2015 was 465 mm, the deviation in relation with the multiannual average amount being 166 mm (figure 1). The distribution of the rainfalls wasn't even. The summer of this year was very dry, less the end of August when were 130 mm rainfall.

Corn smut cannot be controlled with chemical treatments but only by cultivating hybrids with resistance, tolerance or even immunity genes. There are many researches that show that he harvest loses are very low in case of cultivation of resistant hybrids. Other studies are highlighting the fact that the new hybrids are responding different to the attack of the fungus *Ustilago zeae*. This response is controlled by the quality of the genes. The sensitive hybrids have alpha-peptide proteins type produced by sensitive genes. The resistant hybrids have in their cells beta-peptide proteins produced by the resistant or toxic genes so called "killer toxin" [LI N., 1999; GAGE J. MATTHEW *et al*., 2001].

Analysing the nine hybrids from the point of view of the response to the attack of the fungus *Ustilago zeae* in the climatic conditions of the year 2015, the higher attack frequencies were registered in the hybrids Kimbos 500 (control, F=10%) and Sunsis (F = 12 %). In the other hybrids from experience the value of the attack incidence was between 1 % (Sistema 580, Cristina 600, Kalmus 600, Kampius 480, Pico 450 and Kabrios 380) and 2 % in AGN 340 (Table 1). The tumours were generally located on stem, with prevalence on basal part (figure 2a). In the hybrids Kabrios 380 and AGN 340, the tumours were located on cobs too (figure 2b). In a single case (AGN 340) the tumours appeared at the base of the leaf too. This position of the corn smut is more rarely observed.

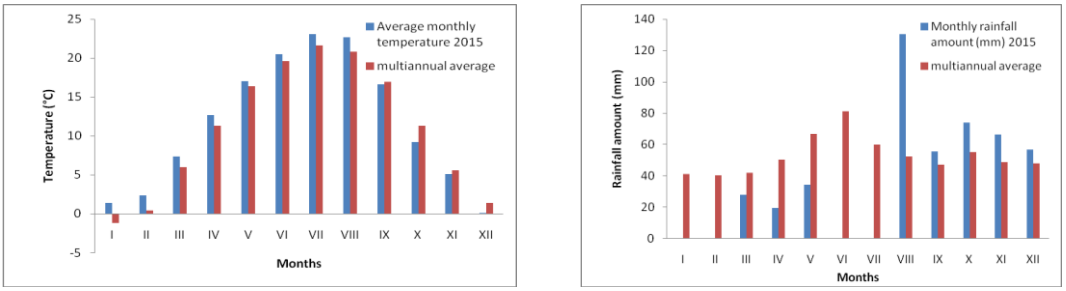


Figure 1. The average temperatures and rainfall amounts registered during 2015 year in comparison with the multiannual averages

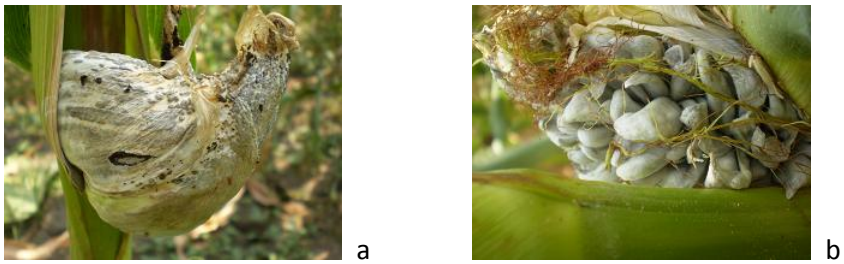

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Figure 2. a) tumour on stem; b) tumours on cob (photo original)

*Table 1*

Aggressiveness of the fungus *Ustilago zeae* on the nine maize hybrids tested in 2015

| Hybrid | F % attacked plants | F % tumours on stem | F % tumours on cob | F % tumours on leaves | Approximatted weigth (g) |
|---|---|---|---|---|---|
| Kalmus 600 | 1 | 1 | 0 | 0 | 20 |
| Sistema 580 | 1 | 1 | 0 | 0 | 15 |
| Cristina 600 F | 1 | 1 | 0 | 0 | 150 |
| Kimbos 500 F | 10 | 10 | 0 | 0 | 20-200 |
| Kampius 480 F | 1 | 1 | 0 | 0 | 20 |
| Pico 450 F | 1 | 1 | 0 | 0 | 40 |
| Sunsis 420 F | 12 | 12 | 0 | 0 | 5-20 |
| Kabrios 380 F | 1 | 0 | 1 | 0 | 200 |
| AGN 340 F | 2 | 0 | 1 | 1 | 30-100 |

*Table 2*

Attack frequency and intensity of the pathogen *Ustilago zeae* in the tested maize hybrids

| Hybrid | F % of attacked plants | I % attack | Tumour location |
|---|---|---|---|
| Kalmus 600 | 1 | 3% | stem |
| Sistema 580 | 1 | 2.5% | stem |
| Cristina 600 F | 1 | 4% | stem |
| Kimbos 500 F | 10 | 5.3% | stem |
| Kampius 480 F | 1 | 2% | stem |
| Pico 450 F | 1 | 1.5% | stem |
| Sunsis 420 F | 12 | 4.5% | stem |
| Kabrios 380 F | 1 | 45% | cob |
| AGN 340 F | 2 | 50% on cob<br>2% on stem | 1 on cob/1 on stem |

*Tabelul 3*

Statistical analysis of the obtained results regarding the attack frequency of the fungus *Ustilago zeae*

| Hybrid | Attack frequency F % | Difference with the control |
|---|---|---|
| Kimbos 500 F | 10 | - |
| Sistema 580 | 1 | -9** |
| Cristina 600 F | 1 | -9** |
| Kalmus 600 | 1 | -9** |
| Kampius 480 F | 1 | -9** |
| Pico 450 F | 1 | -9** |
| Sunsis 420 F | 12 | 2* |
| Kabrios 380 F | 1 | -9** |
| AGN 340 F | 2 | -8* |
| *DL 5% = 8.617; DL 1% = 11.869; DL 0,1% = 16.341; * - non-significant; ** negative significant* | | |

The virulence of the fungus was between 1.5 % (hybrid Pico 450) and 5.3 % in Kimbos 500, in the case of the attack of stems. The attack intensity on cobs was high, they being affected in a rate of 45 % in the hybrid Kabrios 380 and 50 % in AGN 340 (Table 2). The results obtained were statistically processed with variance analysis. Regarding the fungus aggressiveness in 2015, the differences with the control Kimbos 500 (F % = 10) were significant for five hybrids from the experience (Sistema 580, Cristina 600, Kalmus 600, Kampius 480, Pico 450 and Kabrios 380). The differences for the hybrids Sunsis 420 and AGN 340 were non-significant (Table 3). The interpreting of the results obtained in this research using the *resistance source scale* show that seven hybrids from experience have resistance response to the pathogen attack. Only two hybrids (Kabrios 380 and AGN 340) have sensitive response. According with the *cultural scale*, less exigent, the two sensitive hybrids mentioned above were classified as medium resistant to the attack of the fungus *Ustilago zeae*.

*Table 4*

Incidence and virulence of the fungus *Helminthosporium turcicum* on the maize hybrids tested in 2015

| Hybrid | F % attacked plants/leaves | I % attack on leaves | Observations |
|---|---|---|---|
| Kimbos 500 F | 100/42 | **60.30** | *3% cobs with leaves from husks* |
| Sistema 580 | 100/55 | 39.20 | |
| Cristina 600 F | 100/46 | **36.34** | *18% cobs with leaves from husks* |
| Kalmus 600 | 100/55 | 39.05 | |
| Kampius 480 F | 100/35 | 58.06 | *44% cobs with leaves from husks* |
| Pico 450 F | 100/30 | 47.34 | |
| Sunsis 420 F | 100/37 | 42.98 | *8% cobs with leaves from husks* |
| Kabrios 380 F | 100/45 | 49.66 | *29% cobs with leaves from husks* |
| AGN 340 F | 100/38 | 49.36 | *10% cobs with leaves from husks* |

*Table 5*

Variance analysis regarding the virulence of the fungus *Helminthosporium turcicum* in the monitored hybrids

| Hybrid | Attack frequency F % | Difference with the control |
|---|---|---|
| Kimbos 500 F (control) | **60.30** | - |
| Sistema 580 | 39.20 | -21.10* |
| Cristina 600 F | **36.34** | -23.96* |
| Kalmus 600 | 39.05 | -21.25* |
| Kampius 480 F | 58.06 | -2.24* |
| Pico 450 F | 47.34 | -12.96* |
| Sunsis 420 F | 42.98 | -17.32* |
| Kabrios 380 F | 49.66 | -10.64* |
| AGN 340 F | 49.36 | -10.94* |
| *DL 5% = 78.939; DL 1% = 108.728; DL 0.1% = 149.688; * = non-significant* | | |

*Table 6*

Response of the maize hybrids to the attack of the fungus *Helminthosporium turcicum* in 2015

| Hybrids | Virulence value I % | Response of the hybrids depending by: | |
|---|---|---|---|
| | | Resistance source scale | Cultural scale |
| Kimbos 500 F (control) | **60.30** | S | S |
| Sistema 580 | 39.20 | MR | MR |
| Cristina 600 F | **36.34** | MR | MR |
| Kalmus 600 | 39.05 | MR | MR |
| Kampius 480 F | 58.06 | S | S |
| Pico 450 F | 47.34 | S | MR |
| Sunsis 420 F | 42.98 | S | MR |
| Kabrios 380 F | 49.66 | S | MR |
| AGN 340 F | 49.36 | S | MR |

Legend: S – sensitive; MR – medium resistant.

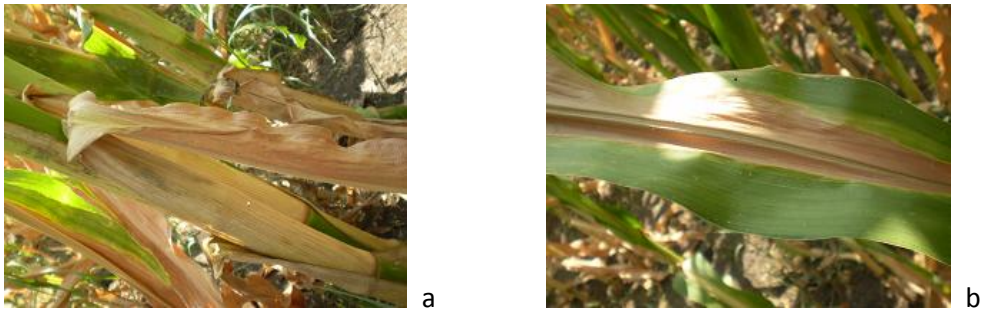

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Figure 3. a) and b) Symptoms produced by *Helminthosporium turcicum* in corn leaves (photo original)

The attack aggressiveness of the fungus *Helmithosporium turcicum* was registered on 100 plants from every analysed hybrid both on plants and on leaves. There was noticed that all the analysed plants have corn leaf blight located mainly on the basal area of the plants and less on the superior part, the attack frequency being 100 % in all analysed hybrids. In the case of leaves the situation was different. Following the analysis, the number of leaves with corn leaf blight symptoms varied between 3 and 7 leaves per plant (figure 3). The most affected leaves by disease were the basal leaves. The frequency of the attacked leaves was between 30 % (hybrid Pico 450) and 55 % in hybrids Sistema 580 and Kalmus 600. The analysis of the results regarding the fungus aggressiveness on leaves highlights the fact that in the late hybrids the attack frequency is greater in comparison with the intermediate and early hybrids, being between 42 % and 55 % (Table 4).

Attack intensities registered in 2015 in the analysed maize hybrids bring in front the hybrid Kimbos 500 (control), that has 60.30 % of the foliar surface attacked, followed by the hybrid Kampius 480 with 58 06 %. In the hybrids from the intermediate and early maturity groups the attack intensity was comprised between 42.98 % (Sunsis 420) and 49.66 % (Kabrios 380). The hybrid Sunsis 420 is characterised by the producing company as having a good tolerance to the fungus *Helminthosporium turcicum*. In the late hybrids the virulence has values of 36.34 % (Cristina 600), 39.20 % (Sistema 580) and 39.05 % in Kalmus 600.

Comparing the frequency of the leaves attacked by the pathogen with the attack intensity on leaves show that the hybrids with higher rate of infected leaves the virulence of the fungus is lower, below 40% (Table 4). In other hybrids (Pico 450, Sunsis 420 and Kampius 480) the incidence of the attacked leaves reach to 37 % and the virulence to 58 %, the leaves being practically compromised in these situations. The virulence registered

shows that a third of the leaf surface and more than a half in several situations is out of the functional circuit regarding the photosynthesis, respiration and transpiration.

The response of the hybrids to the pathogen was set by the analysis of the results using the source resistance scale and cultural scale. According with the resistance source scale there was noticed that six hybrids from the experience were manifested as sensitive for the pathogen (Kimbos 500, Kampius 480, Pico 450, Sunsis 420, Kabrios 380 and AGN 340). Only three hybrids manifested tolerance reaction to the fungus attack (Sistema 580, Cristina 600 and Kalmus 600 – late hybrids). There was noticed that in the late hybrids the registered virulence is lower in comparison with the intermediate and early hybrids. The cultural scale being less exigent shows that the hybrids Kimbos 600 and Kampius 480 are still maintaining as sensitive. The other hybrids are tolerant to the pathogen (Table 6).

The recommendations for farmers is good to be according to the cultural scale, because those hybrids can be successfully cultivated even if on the resistance source scale they are sensitive to the pathogen, due to their features of productivity, tolerance to drought stress and the attack of the pathogens specific for maize. The reports made by the producing companies regarding those hybrids show that they are highly valuable, have very good vigour, good and very good tolerance to diseases and the fact that they are highly tolerant to the water stress makes possible their cultivation in different climate and soil fertility conditions.

## CONCLUSIONS

The analysed hybrids were reacted different to the attack of the fungi *Ustilago zeae* and *Helminthosporium turcicum.* To the fungus *Ustilago zeae* seven hybrids from the experience manifested resistance reaction and only two hybrids (Kabrios 380 and AGN 340) have manifested sensitivity reaction (according to the resistance source scale). After the application of the cultural scale, the two above mentioned hybrids were reacted as medium resistant to the attack of the fungus *Ustilago zeae*.

In the case of the fungus *Helminthosporium turcicum*, six hybrids from the experience have reacted as sensitive to the pathogen (Kimbos 500, Kampius 480, Pico 450, Sunsis 420, Kabrios 380 and AGN 340) and three hybrids have manifested tolerance reaction (Sistema 580, Cristina 600 şi Kalmus 600 – late hybrids) – according with the resistance source scale. Analysis of the results with the cultural scale, less sensitive shows that the hybrids Kimbos 600 and Kampius 480 are still maintaining as sensitive and the others are tolerant to the pathogen.

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