ROLE OF ABIOTIC STRESS FACTORS IN THE CULTIVATION AND SEED PRODUCTION OF HYBRID MAIZE

Main points of the thesis

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1. SCIENTIFIC BACKGROUND AND AIMS

One of the most profitable branches of agriculture in Hungary is seed production, including the production of hybrid maize seed. The quantity of hybrid maize seed produced each year is almost three times the domestic requirements, thanks to the extremely favourable ecological conditions and, equally importantly, to the long experience of the growers. In the present situation, when competition is intensifying, it is thus of great importance to retain both our domestic and foreign markets by producing a satisfactory quantity of seed with high biological value.

One way of achieving this aim is to reduce to the minimum, or wherever possible completely eliminate, the factors which have a negative effect on quality during the vegetation period, at harvest and in the course of processing. It is thus of primary importance to identify and investigate these factors.

Numerous abiotic stress factors affect the maize plants in the course of seed production. These include:

1. unfavourable conditions during seedling development (cold, unaerated soil)
2. drought and heat stress during flowering
3. mechanical damage to the foliage, tassels and silks
4. incorrect temperature during seed drying.

The primary aim of the research was to elaborate and test an experimental system capable of providing a good simulation of the suboptimum and extreme conditions occurring during hybrid maize seed production, so that the effects on plants and seeds of stress factors experienced during various phases of phenological development could be predicted (simulation of hail to study the mechanical damage to foliage and inflorescence).

An attempt was made to determine the consequences of the destructive, surface-reducing effect of hail on the growth and development of maize plants in various phenophases during the vegetation period, and how mechanical damage to the tassels at the beginning of silking affected the yield and biological value of the seed in years with different weather conditions.

Investigations were also made on the responses of seeds of various sizes and shapes to stress conditions at emergence, which could be attributed to the different biological values of the seeds. Among other things, tests were made on the importance of seed dressing treatments (fungicide, insecticide) for seed biological value and yield components (emergence, plant density, duration of vegetation period, grain moisture at harvest, grain yield).

In the case of field maize seed production, a satisfactory quantity of seed with excellent biological value is only obtained if the tasselling date of the male parent rows is synchronised with the silking date of the female parent rows. Drought stress arising during the vegetation period can best be measured as the water potential of the plant organs. Changes in the water potential of the husks and silks (stigma) were thus studied, together with the pollen-producing ability of the tassel, which is of decisive importance for pollination. The effect of temperature on the date of flowering (tasselling and silking) cannot be ignored either.

Another important aspect is the extent to which seed harvested with satisfactory quality despite the present of various stress conditions can be protected from further damaging factors in the course of drying in seed plants. It is essential to know how the drying temperature, the most important factor, influences the viability of the germ in the genotypes used most frequently for breeding in Hungary. An attempt was made to rank the genotypes according to their sensitivity to seed processing factors.

2. MATERIAL AND METHODS

2.1. Seed-lot fractioning

A seed-lot fractioning experiment was set up in 1995 using seven maize hybrids in the laboratories and nurseries of the Seed Department of the Agricultural Research Institute of the Hungarian Academy of Sciences.

The hybrids examined (Norma, Mv 1355, Mv 1444, Mv 1454, Mv 1486, Mv 1484, Mv 1488) were shelled using a Wintersteiger laboratory sheller, after which the seeds were divided into four fractions using a Hart-Carter laboratory rotary sieve. The four fractions were as follows:

Large flat (LF)	O 8.5–10.0 mm (round-holed sieve)
	= 3.5–5.5 mm (long-holed sieve)
Small flat (SF)	O 6.5–8.5 mm
	= 3.5–5.5 mm
Large round (LR)	O 8.5–10.0 mm
	= 5.5–7.0 mm
Small round (SR)	O 6.5–8.5 mm
	= 5.5–7.0 mm

Germination ability

The cleansed, fractioned seeds were geminated according to the standard (MSZ 6354,3-82). The germination medium was crepe filter paper, 1 g of which contained 1.6–1.7 g water. The moisture content of the paper was adjusted using a domestic centrifuge. Fifty seeds were placed on each sheet of moistened paper, covered with another sheet of paper and rolled up. Four rolls were placed vertically in polythene bags. Germination was carried out for 7 days at 25°C in a climatic chamber (Conviron) with a relative humidity of 70 %.

Cold test

The tests were carried out in a 1:1 mixture of sterile sand and maize soil containing pathogens. This soil was moistened to approx. 70 % water capacity and spread to a depth of 0.5 cm on a 15 × 40 cm layer of double filter paper. A hundred maize seeds were distributed over the soil and covered with a third filter paper. The whole was then rolled up and placed in a polythene bag for ten days at 10°C, followed by four days at 25°C. Germination was evaluated as laid down in the standard (MSZ 6354,3-82; MSZ-080250) (BARLA-SZABÓ 1985).

Early vigour

In order to study seed vigour the length of the germs was measured on the 12^{th} day of the cold test. These data were used to characterise early vigour.

Field tests

The experiment was set up in a random block design with four replications in the nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences on a total of 112 plots (plot size 3.5 × 2.25 m) each containing 76 plants. The soil was moderately heavy with a pH_{H2O} value of 7.1, 0.5 % $CaCO_3$, 121 ppm P_2O_5 and 220 ppm K_2O. After the usual seedbed preparation, including the application of 600 kg NPK in autumn and 150 kg/ha N in spring, the hybrids were sown on May 15^{th} with a hand drill, two to a hill. The weaker of the plants was removed after emergence. Weeds were controlled by manual hoeing and irrigation was carried out with 2 × 30 mm water using Nerthus irrigation equipment. Emergence was scored on June 10^{th}.

Harvesting was carried out at a grain moisture content of 30 % on all the plots on October 18^{th}. In order to eliminate border effects, only ten plants were harvested from the centre of each row. The ears were processed after drying for a uniform period in warm air at 38°C. The grain moisture content was determined using a GRAINER manual instrument of Japanese make. The yield values were transformed to a grain moisture content of 14 % and the thousand kernel mass was recorded as laid down in the standard (by weighing 4×500 kernels and then calculating the weight of a thousand kernels). The experiments were evaluated by single factor analysis of variance (SVÁB, 1981).

In 1996 the fractioning experiment was continued with 13 hybrids in the nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, and at the Experimental Station of Pannon University of Agricultural Sciences in Keszthely. The experimental material was expanded to include the hybrids Maya, Mara, Mv 272, Mv 525, Mv 233 and Faria.

In place of the cold test, the complex stressing vigour test was chosen as a reliable way of testing chilling tolerance without soil.

Complex Stressing Vigour Test (CSVT)

On the first four days of testing the seeds were exposed to stress factors which occur on certain occasions in nature and which represent a complex strain on the seedlings (oxygen deficiency and 5°C chilling treatment; for further details, see BARLA-SZABÓ 1985). After stressing, germination was carried out for four days. According to the length of the germs, the seeds were divided into groups with high and low vigour, and into abnormal and non-germinating categories, as laid down in the standard.

2.2. Seed chilling tolerance

A chilling tolerance and productivity experiment was set up with two inbred maize lines and two hybrids. The lines were AMO 407, which belongs to the Lancaster group and is sensitive to drought and cold, and HMv Exp 03, which is more tolerant than ANO 407 but nevertheless gives very poor emergence percentages under suboptimum environmental conditions. The germination percentages of seed lots of these lines at the beginning of the experiment, after storage for five years, were 89 % and 58 %, respectively.

The hybrids were Mv 444 SC, one of the most promising Martonvásár hybrids in the FAO 400 group, and Mv Maxima TC, which has very high biomass production and is recommended chiefly for silage purposes. The germination percentage of both hybrids at the beginning of the experiment, after storage untreated for five years, was 90 %.

The seeds of the lines and hybrids were dressed using the traditional dressing agent **Buvisild K** [Captan 30 g/l: cis-N(trichloromethyl) thio-4-cyclohexane-1,2-dicarboximide] and an experimental fungicide produced by Novartis, **Maxim AP 045 FS** [fludioxonyl 25 g/l: 4-(2,2-difluor-1,3-benzodioxol-4-yl)-1H-pyrol-3-carbonitryl + metalaxyl 20 g/l]. This fungicide treatment provides effective protection against the pathogens *Aspergillus, Fusarium, Penicillium* and *Pythium*, which occur at high frequency in cold, moist soil. The seed treatments were carried out using the recommended doses: 2 l/t Buvisild K and 1 l/t Maxim AP 045 FS.

The stress tolerance and vigour of the seed lots were determined using the complex stressing vigour test (BARLA-SZABÓ 1985).

The treated and untreated control seeds were sown in the experimental nursery of Pannon University of Agricultural Sciences, Keszthely, on experimental plots of the size recommended by the National Institute for Agricultural Quality Control (9.2 × 2.8 m) at two sowing dates, April 11th and May 9th 1998. The experimental design was a random block with four replications, divided into two parts for the two sowing dates. In order to avoid border

effects, the sweetcorn hybrid Mv Aranyos was sown to surround the plots. The nutrient and soil analytical data of the plots were the same as those given for the seed fraction experiment. Weeds were controlled by manual hoeing and no irrigation was applied.
Emergence was scored on June 5th and records were made of 50 % tasselling and silking. Harvesting was carried out by hand on October 24–25th. The processing of the ears and the data evaluation agreed with those described in the seed fractioning experiment.

2.3. Insecticidal and fungicidal seed treatments

In spring 1999 a seed treatment and productivity experiment was set up in the experimental nursery of Pannon University of Agricultural Sciences, Keszthely, using the following hybrids: Mv 272, Mv 273 (FAO 200), Róna (FAO 300), Mv 434, Mv 444, Major, Maraton, Márta, Mv 473, Mv 483 (FAO 400).
The germination percentage and vigour of the seed lots, which had been stored for five years, were determined at the beginning of the experiment. The hybrids were treated with fungicidal and insecticidal dressing agents, separately and in combination. The insecticide provided protection chiefly against rose-grain aphid (*Metopolophium dirhodum*), frit fly (*Oscinella frit*) and *Tanymecus dilaticollis*.
Treatments and designations:

	Control
	M: Maxim AP 045 FS (25 g/l fludioxonyl + 20 g/l metalaxyl)
	G: Gaucho 350 FS (350 g/l imidacloprid)
	G+M: Gaucho + Maxim
Doses:	M: 1 ml active agent/kg seed
	G: 3 μl active agent/seed
	G+M: 1 ml fludioxonyl/kg seed + 3 μl imidacloprid/seed

The combined seed treatment was carried out by leaving the seed surface to dry for an hour after dressing with fludioxonyl/metalaxyl, after which the seeds were treated with imidacloprid. The treatment was carried out using a Gustaffson laboratory seed dresser.

I. Laboratory analyses
Germination ability was determined according to the Hungarian standard (MSZ 6354.3-82) and vigour by means of the complex stressing vigour test (BARLA-SZABÓ 1985).

II. Field experiment
The experiment was carried out in the experimental nursery of Pannon University of Agricultural Sciences, Keszthely, with the soil and agronomic parameters given above. The experiment was set up in a random block design with four replications on a total of 160 plots (plot size 6.0 × 0.75 m) each containing two rows, on April 24th. Weeds were controlled by manual hoeing and the experiment was not irrigated. Emergence was scored on May 12–14th. In the course of vegetation records were made of the number of days to emergence, and the date of 50 % tasselling and silking. The stand density was scored prior to harvest.
Harvesting was carried out by hand on October 6th, when the grain moisture content was also measured (GRAINER). The plot yields were calculated in terms of 14 % moisture content and the grain yield per plant was calculated from the plot yields and plant density data. The data were evaluated using single factor analysis of variance with the aid of the ANOVA statistical program package.

2.4. Drought and heat stress experiments in the phytotron during the vegetation period

An experiment was set up in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences in 1996 on the line HMv 5502, which is used in the female combinations of hybrids (FAO 500) and on line Mo 17/Mv, which has been grown commercially for a considerable period. The inbred lines were provided by the Institute’s Seed Department. The main criterion considered when choosing the genotypes was that their vegetation periods should be as long as possible to ensure that the critical flowering time in the field should occur during the driest and hottest days or weeks (late July, early August).

Pot experiment

Two hundred seeds of each genotype were placed to germinate on filter paper saturated to maximum water capacity at 25°C on February 9th 1996. The best-developed seedlings were planted in 2 × 76 pots containing a 3:1:1 mixture of garden soil, sand and Vegasca and placed in a climatic chamber (Conviron PGV-36) on February 15th. In the first two weeks the plants were grown according to the climatic programme shown in Table 1. From the third week onwards the following programme was employed: 30/20°C day/night temperature, 14 h illumination provided by G metal halide lamps with a photon flux density of 250 μmol m^{-2} s^{-1} (TISCHNER 1993).

Table 1

Temperature, humidity and illumination intensity programmed in the PGV phytotron chamber during the first two weeks after emergence

(Martonvásár, 1996)

1st week	Time	Temperature, °C	Humidity, %	No. of lamps	Light intensity (Q)
					220 μmol m^{-2} s^{-1}
2nd week	Time	Temperature, °C	Humidity, %	No. of lamps	Light intensity (Q)
					240 μmol m^{-2} s^{-1}

Up till tasselling the plants were all watered every second day with the same quantity of water. After tasselling (April 14–16th for the well-known genotype Mo 17/Mv and April 18–20th for the inbred line HMv 5502) the experiment was divided into two parts (38 pots) for each genotype. Half of the pots were used to determine the effect of drought stress after tasselling. For this purpose irrigation was discontinued for six days in 19 pots (Treatment I), while it was continued in the other 19 pots (Treatment II).

Pollen quantity measurements
The tassels on each plant were isolated using parchment bags. After the six-day drought stress treatment irrigation was continued every two days in all the pots. At the end of the experiment, on May 1st, the tassels were removed from the plants and the quantity of pollen produced per plant was recorded for each genotype. After breaking off the bags containing the isolated tassels from the top of the plants, the pollen grains were shaken into the bags and the tassel fragments were removed. This was done within 30 s of breaking off the tassels. The pollen was then passed through a pollen sieve to remove the anthers and other contaminants, and the quantity per plant was measured using an automatic OHAUS electronic instrument.

After the appearance of the silk the second half of the pots was also divided into two for drought tolerance studies. In this case too irrigation was discontinued for six days in 19 pots (Treatment III) and continued as a control in the other 19 pots (Treatment IV).

Water potential measurements
During this drought period samples were taken from the husks to measure water potential. The stigma was then excised and water potential measurements were made for each plant in both the irrigated and drought-stressed plants for six days. The measurements were carried out using a manual water potential meter of the PMS type (Oregon, USA). In order to avoid dehydration the ear primordia were removed from the plants, sections were cut from the stigma and these were placed in the water potential meter within 30 min.
Water potential measurements on the husks were also carried out for each ear primordium. In this case sections measuring 4×1 cm were placed in the instrument.

Fluorescence induction measurements
In parallel with the treatments, fluorescence induction measurements were also made on samples excised from the leaves and husks of the same plants. The chlorophyll fluorescence induction measurements were carried out using a PAM-2000 (Walz, Effeltrich, Germany) impulse amplitude modified fluorometer. Prior to the measurements the excised leaf segments were dark-adapted for 30 min. The saturation white light used to determine the various fluorescence induction parameters had a PPFD of over 3000 μmol m^{-2} s^{-1}, while that of the actinic illumination (red light) was 150 μmol m^{-2} s^{-1}. The extinction parameters were calculated after 15 min actinic illumination. Measurements were made on initial fluorescence, maximum fluorescence and photochemical and non-photochemical quenching in the control (irrigated) and drought-stressed (non-irrigated) plants of maize lines Mo 17/Mv and HMv 5502. The statistical analysis involved a two-sample t-test.

2.5. Mechanical damage to the foliage
The experiments were carried out in the field and in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences in 1990–1992.

The *phytotron experiment* was set up in two GB chambers in 1990. Pre-soaked kernels of the maize hybrid MvNK 1485 were germinated in a thermostat at 25°C for 48 h, after which two well-developed seedlings were placed at a depth of 5 cm in each 18 × 18 cm plastic pot containing a 3:1:1 mixture of Ramann's brown forest soil, sterile sand and sand containing 1.5 % humus, supplemented with 5 g Plantosan. After emergence the weaker of the plants was removed. The experiment was set up in four replications and the following treatments were carried out in the 4-, 6- and 8-leaf phenophases:

– 25–50–75–100 % of the leaves were removed symmetrically;

– 25–50–75 % of the leaves were removed asymmetrically from one side of the plants;
– the leaves were shortened by 25–50–75 %;
– the leaves were torn lengthwise in 3 or 4 places;
– the leaves were cut crosswise up to the main vein every 3–4 cm;
– untreated control.

In 1991 the following treatments were carried out:

– the leaves were torn crosswise and lengthwise in the 4–5-leaf stage;
- the leaves were shortened by 25–50–75 %.

The *field experiment* was set up in Martonvásár (soil and agronomic parameters agree with those of the previous experiments) on May 4th 1990 with a row and plant spacing of 80 × 25 cm and a plant density of 50,000 plants/ha in a random block design with four replications. The plot size was 4 m^2, with 20 plants per plot.

Leaf removal was carried out on four occasions to four extents on a total of 68 plots including the control. The first treatments were begun on June 4th in the 4–5-leaf phenophase. when the 25 % treatment involved the removal of the largest leaf, the 50 % treatment that of two leaves, the 75 % treatment that of three leaves and the 100 % treatment that of all the leaves. In the 8-leaf stage 2, 4, 6 and 8 leaves, respectively, were removed. Similar treatments were carried out at tasselling (July 17th) and on August 16th, when it could be clearly seen that the kernels had started to grow on the ears. Records were also made of plant height, the course of silking and the stalk diameter 40 cm above the ground.

The number of treatments was expanded in 1991. In the 50 % leaf removal treatment a distinction was made as to whether the leaves were removed from the top or the bottom of the plant. When they were removed from the top, the treatment was again divided into two, according to whether the leaf next to the ear was retained or not.

In 1992 the treatments included the removal of 3, 6, 9 or 12 leaves. In a further treatment the plants were cut back to the ground, so that only the roots, the growing tips and the underground parts of the leaf primordia remained. The other treatments were the same as in the previous year.

The experiments were evaluated using single factor analysis of variance. When the effect of the treatment was not significant according to the F test even at the 10 % level, LSD values are not given.

2.6. Mechanical damage to the maize inflorescence

The experiments were carried out in 1987–1989 on a moderately heavy meadow soil on the Aranyhomok Cooperative Farm in Szabadszállás. The seedbed was prepared after the distribution of 200 kg/ha PK fertiliser (ploughing, disking), weed control and the application of 300 kg/ha mineral fertiliser in spring. The female rows were sown on April 30th, while the first male rows were sown when the female plants were in the earliest stage of germination and the second rows a week later. The plant density was 60,000 plants/ha, with a row distance of 70 cm and a 6:2 ratio of female to male rows. The experiment was set up in six 8-row blocks on a field 300 m in length. The female partner was the combination F564 × Mo17 and the male partner the single cross HMv 76 × HMv 77 (the parents of the experimental hybrid Mv Exp 23).

Treatments in the male rows: complete removal of the tassel (100 % detasselling)
removal of the main tassel branch (inflorescence axis)
removal of the side tassel branches
breaking of the tassel branches
untreated control with undamaged tassel.

In order to ensure the suitability of the data for evaluation and to eliminate alien pollen, plots given the same treatment were placed in the same block and the blocks were arranged in order of treatment severity. The most severe treatment, 100 % detasselling, was followed by the breaking of the tassel branches, the removal of the side branches, the removal of the main tassel branches and the untreated control. The 100 % detasselling treatment involved the complete removal of the tassel (which was left on the ground) at the beginning of tasselling, prior to anthesis. Isolation distances of 20 m were left between the different treatments, while the male rows were planted at a distance of 6.4 m from each other. The tassels of the female plants, the isolation rows and the plots treated to 100 % detasselling were removed between July 15–25th as soon as they were big enough to remove. The other treatments were carried out continuously, after tasselling but before anthesis, by going through the plots each day. The aim of these experiments was to determine whether the removal of the side branches or main tassel branch caused any change in fertilisation due to changes in the flowering date or quantity of pollen.

The yield was harvested by hand and dried to a grain moisture content of 12 %. Processing was carried out after the ear parameters (used for the characterisation of the yield) had been recorded. The biological value of the seed was determined on the basis of thousand kernel mass, germination percentage and vigour (BARLA-SZABÓ 1985). The experiments were evaluated using single factor analysis of variance.

Since the weather in the three experimental years differed substantially, the climatic data of the vegetation period must also be given. In 1987 the warm early summer and the warmer than usual autumn were only interrupted by a colder spell in early August (Table 2). All in all the temperature conditions were favourable, but the rainfall distribution was very varied. The winter was wetter than usual and there was exceptionally heavy rain in May (110 cm), but the drought in July soon exhausted the soil water reserves.

In 1988 drier than average months followed each other from April onwards (33 mm rainfall in May, 37 mm in June, 41 mm in July, 20 mm in August). The 20 mm rainfall in August was the only rainfall received over a 55-day period from July to early September, so the plentiful rainfall in September was unable to help the maize to any great extent.

In 1989 it was pleasantly warm in late May, but the weather became cooler and wet in June. July was hot and dry, but all in all the weather was favourable for maize development.

Table 2

Meteorological data (rainfall, temperature) for the vegetation period

(Szabadszállás, 1987–1989)

	Rainfall mm	Temperature °C	Rainfall mm	Temperature °C	Rainfall mm	Temperature °C
April						
May						
June		Tízedes pont!				
July						
August						
September						

2.7. Effect of drying temperature on the biological value of maize seed

An experiment was set up between 1988 and 1998 to determine the joint effect of the harvesting date (grain moisture content) and the drying temperature on the quality of maize seed.

The following inbred lines were used in the experiments: AMO 407, AMO 406, HMv 5414, HMv 5502, HMv 5405, LDR 08, LH 82, H 8431, A 665, CM 174, HMv 217, MA 75 A 211.
The locations of the seed multiplications were as follows: Béke Cooperative Farm, Dunavecse; Aranyhomok Cooperative Farm, Szabadszállás; Aranykalász Cooperative Farm, Tabajd.
Sowing was carried out with row and plant distances of 70 × 20 cm. At each of the three harvesting dates 30 ears were harvested by hand. The harvesting dates represented grain moisture contents of 35, 25 and 20 %.
In lines A 665, CM 174, HMv 217 and MA 75A 211 the ears were harvested and dried from the tassel removal stress experiment (tassels removed in combination with 0, 2 or 4 leaves).
Each lot was divided into three parts, after which 10 ears of each were dried at 35, 45 and 55°C to a grain moisture content of 14 %.
After hand shelling and basic cleansing the kernels were stored for 30 days in the granary, after which germination and vigour tests (CSVT) were carried out. The germ and root organs of the seedlings were excised and the shoot and root weights of the seedlings were measured after drying for 72 h in a drying cabinet at 100°C. Mean values were calculated from the data of 50 seedlings and evaluation was carried out for each genotype by single factor analysis of variance.

3. NEW SCIENTIFIC RESULTS

Among the results achieved on the role of abiotic stress factors in the cultivation and seed production of hybrid maize, the following are new results:

1. It was experimentally proved (in different locations and years) that, depending on the genotype, biologically more valuable *seed fractions* retained a certain superiority even in the grain yield resulting from their greater vigour.
2. *Seed treatment* not only have a fungicidal effect but also improve chilling tolerance in the field, resulting in better field emergence and, due to the improved stress tolerance, in a significant grain yield increase.
3. After the tasselling stage, even a week of drought stress causes a substantial reduction in the *pollen production* of the tassels.
4. As the result of *drought stress* there was also a change in chlorophyll fluorescence induction parameters. Photochemical quenching (q_P) only changed to a slight extent, whereas non-photochemical quenching (q_N) increased in non-irrigated plants.
5. Maize plants were most sensitive to factors causing injuries to the *leaf area* (simulated hail) at tasselling and in later phenophases (losses in grain yield and stalk thickness)
6. The unfavourable fertilisation conditions caused by *damage to the tassel* (hail injuries to the main tassel branch or the side branches) reduce the biological value of the seed and lead to an increase in thousand kernel mass and the ratio of the large round fraction. Due to protracted, poor fertilisation there is wide variability in the state of maturity of the kernels on the ears and a decline in the germination ability and vigour of the seeds.
7. Among the lines used for seed production in Martonvásár the genotypes AMO 407 and AMO 406 were the most sensitive to *drying temperatures* in excess of 45°C. Drying at 55°C was absolutely lethal for both lines. The seed type (flint, dent) does not give any indication of the sensitivity of the genotype to the drying temperature.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Fraction formation

On the basis of two years of experimentation it was established that, judging by the ***yields achieved with dressed fractions,*** the yield of the flat fractions was generally greater than that of the round fractions for all the hybrids.
According to data on 50 % male and female flowering the difference between the flowering dates of the fractions was not great enough in any case to cause poorer fertilisation.
The yield data are confirmed by the results of the stressing vigour test, since seed with better biological value still retains a certain superiority in the yield, due to its better vigour. Better environmental conditions at sowing (soil and air temperature, moisture conditions) may, however, reduce the differences between the fractions (Pásztor, 1962). Special mention should be made of the small round fraction, which generally gave the lowest yields.
The formation of seed fractions may be important not only for the genotype but also from the point of view of the production site, the growing conditions and the environmental factors.
Data from experiments using dressed seed clearly showed the advantage of the flat fraction (Fiala 1977). Since undressed seeds are more vulnerable to infection, experiments using undressed seeds gave more widely deviating results, so that the significance of seed fraction formation was less clear.

4.2. Field chilling tolerance

Stress is caused not only by the ***cold*** (Bruggink et al. 1991) ***as a factor physiologically inhibiting initial germ development***, but also by the hypoxia arising when the seeds are sown in cold wet soil. The stress effect can be most reliably demonstrated not by the cold test (Loeffler et al. 1985), which uses soil, but by the complex stressing vigour test, which is carried out without soil, after preliminary sterilisation.
Among the seed treatments special mention should be made of dressing agents containing ***fludioxonyl and metalaxyl***. Fludioxonyl is a synthetically produced active agent identical with the phenylpyrol extracted from the *Pseudomonas pyrocina* bacterium. The fungicide has no phytotoxic effect and can be used in combinations.
Germination ability was slightly improved by fungicidal seed treatment. The effect on stress tolerance was considerably more pronounced. The stress sensitivity of the line AMO 407 and the hybrid Maxima was significantly reduced by seed treatment with fludioxonyl, as was that of the hybrid Mv 1444. This means that the stress sensitivity of the parental genotype (AMO 407) can be improved in the hybrid.
Seed treatment with fludioxonyl was the most successful, as demonstrated by the emergence of the hybrid Maxima when sown at an early date. Under optimum conditions too an outstanding rate of emergence was achieved for seed of the hybrid Mv 1444 treated with fludioxonyl. Fludioxonyl treatment of these hybrids also led to the production of maize ears with a 2-4 % lower grain moisture content.
The close correlation of grain yield data with emergence is proved by the fludioxonyl seed treatment of the hybrids Maxima (first sowing) and Mv 1444 (second sowing). The latter hybrid gave a grain yield surplus of 3.5 t/ha compared with treatment with captan. These results were confirmed by the fludioxonyl treatment of the line ANO 407, which resulted in a 110 % yield surplus compared with the captan treatment (second sowing).
Seed treatment with ***Captan*** was successful when Mv 1444 was sown early and in the case of the line HMv Exp 03, although the results achieved with the latter genotype suggest that it should not be used in field seed production.
Even under optimum environmental conditions seed treatment had a substantial effect, since the grain yield production of individual plants of stress-sensitive genotypes (AMO 407, HMv Exp 03) increased, especially as the result of seed treatment with fludioxonyl (the results were not significant).

In summary it can be stated that in addition to their fungicidal effect, seed treatments also improve chilling tolerance, which is manifested in better field emergence and, due to improved stress tolerance, to a significant increase in grain yield.

4.3. Insecticidal seed treatment

The biological value of the seed was improved in some cases by ***seed treatment with imidacloprid***, and in combined treatments chiefly due to the fungicide effect. In contrast to the literature (Kashyap et al. 1994) no dominant insecticide superiority was observed with respect to seed vigour.

On the basis of the results of field experiments it was found that in the early maturing group insecticidal seed treatment on the hybrid Mv 272 led to more reliable emergence, giving a more complete plant stand than the untreated control.

Among the mid-season hybrids imidacloprid and combined seed treatments on Major, Maraton and Mv 434 resulted not only in a denser plant stand by the end of the vegetation period, but also to higher grain yield. In itself fungicide seed treatment was not sufficient in the case of Maraton to cope with the pests occurring throughout the vegetation period. The sensitivity of the hybrid clearly indicates the importance of seed treatment with imidacloprid.

Hybrids Mv 444 and Mv 483 were more resistant to external stress conditions. The hybrid Márta, which had the highest yield (G+M treatment) in the experiment, proved the significant yield-increasing effect of the combined seed treatment.

Only in one case (Major) did the imidacloprid treatment lengthen the vegetation period (Bosák 1999), leading to higher grain moisture content at harvest. In two hybrids (Mv 272, Mv 483) fungicide treatment resulted in a lengthening of the vegetation period, resulting in high grain moisture content and later harvesting.

Although the 600–800 kg/ha differences in grain yield do not justify the profitability of insecticidal seed treatment (due to the seed prices), the treatment results in better emergence and ensures a complete plant stand right up to the end of the vegetation period even without chemical pest control, which is a particular advantage in years less favourable for maize production.

4.4. Heat and drought stress

In a ***model experiment on heat and drought stress*** in the phytotron the following conclusions were drawn:

- among the genotypes examined the time to 50 % tasselling in the line with the shorter vegetation period (Mo 17/Mv) was reduced by 10 days and the time to silking by 6 days. These reductions were even greater in the late-maturing line HMv 5501 (13 and 10 days, respectively). Temperature and light conditions were found to have a greater influence on the flowering phenophases in later maturing genotypes, in contrast to earlier studies (I'só and Szalainé 1969);
- the water potential of the husk declined more drastically as the result of drought stress than that of the female inflorescence. The sensitivity of the silk to drought and high temperature was somewhat more favourable due to the protection provided by the husks;
- the results suggest that drought stress had a more severe effect than high temperature. The plants were able to counteract above-optimum temperatures by increased water uptake, whereas drought may cause irreversible damage during flowering;
- in contrast to field hybrids the sensitive maize inbred lines exhibit stress symptoms even after less than ten days of water deficiency (Westgate and Boyer 1986);
- drought stress for six days after tasselling caused a substantial reduction in the pollen producing ability of the tassels. No difference was observed between the genotypes in this respect. Not only mechanical injuries, but also drought may thus reduce the quantity of

pollen produced per plant in the genotypes used as male partners in seed multiplications. For the majority of genotypes the reduction in the pollen quantity to a third will result in a considerable yield loss. This is of particular importance in the male rows of mass crosses (Berzy 1994);

- numerous stress factors have a direct or indirect influence on the functioning and efficiency of the photosynthetic apparatus. The method generally used to study these effects is the measurement of chlorophyll fluorescence induction. As the result of drought stress changes were recorded in several chlorophyll fluorescence induction parameters. The changes were greater in Mo 17/Mv than in HMv 5502. Photochemical quenching (q_P) only changed slightly as the result of drought stress, while, in contrast to the other parameters, non-photochemical quenching (q_N) increased in non-irrigated plants. The HMv 5502 line was much more tolerant to drought stress than Mo 17/Mv.

4.5. Mechanical leaf damage

4.5.1. Leaf injuries and plant development

The lacerating, surface-reducing effect of mechanical damage (hail injury) to maize plants in various phenophases during the vegetation period have the following consequences on ***plant development and flowering time***:

- the effect of various leaf injuries on plant height is not consistent, though the time when the injury is suffered is of decisive importance;
- the plants are most sensitive to the laceration or wounding of the leaf surface during the tasselling phenophase. In earlier phenophases (4- to 8-leaf stage) the complete destruction of the aboveground parts of the plant also reduced the plant height. Plant height is generally of significance in the case of male genotypes with lower growth habit, since it may limit their ability to supply pollen to the female flowers. The silking of plants defoliated at the beginning of tasselling was greatly retarded compared to that of undamaged plants. In this way pollen supplies were restricted not only in space but also in time, i.e. there was a lack of synchronisation in the flowering of the male and female parents in seed multiplications. Similar results to those published in the literature (Pintér et al. 1977) were obtained as regards the assimilation activity of the leaves;
- the role of the leaf next to the ear changes in various phenophases and no confirmation was obtained of its special role. This was underlined by grain yield data. All the treatments reduced the stalk diameter: the earlier the damage occurred, the greater the reduction.

4.5.2. Leaf injuries and fertilisation

The lacerating, surface-reducing effect of mechanical damage (hail injury) to maize plants had the following consequences on fertilisation, and thus on the ***grain yield and thousand kernel mass, and on the tissue structure of the stalk***:

- the date of injury was found to be of fundamental importance. The most serious consequences were observed when damage occurred during or after tasselling;
- a reduction in the leaf area led to a reduction in the dry matter content of the stalk. In agreement with the literature (Koehler 1960; Iwata 1973; Pintér and Kálmán 1979), during yield formation nutrients are extracted from the stalk by the grain yield after mechanical leaf damage, leading to a deterioration in the structure of the stalk. In over-ripe maize this leads to increased lodging and *Fusarium* infection;
- it was found that ear development was influenced substantially by a leaf area reduction of at least 50 % from the beginning of tasselling onwards;

- the plants may respond to mechanical stress prior to or during tasselling by reduced seed setting (reduction in number of kernels), while in phenophases after tasselling the same extent of damage will also lead to a decline in the thousand kernel mass;
- damage to 25 % of the leaf area did not reduce the yield even in the most sensitive phases;
- 50 % leaf damage may reduce the yield by 15 % and 75 % leaf damage by 30 % compared to undamaged plants, though the yield losses also varied for individual genotypes. Understandably, the lowest grain yield was associated with high thousand kernel mass (after damage during phenophases preceding tasselling);
- the date and extent of mechanical damage to the plant surface is thus of great importance.

4.6. Mechanical damage to the tassel

Mechanical damage to the tassel (simulation of the lacerating effect of hail), combined with heat or drought stress, led to changes in the following yield components over the course of 3 years in the seed production of the maize hybrid Mv Exp 23 DC:

- in years highly suited to seed production, when the air temperature and soil moisture conditions are optimum, partial tassel damage did not cause a substantial loss of yield, but this 10–20 % of a high yield could be the whole yield in poorer years;
- in a moderate year partial tassel damage may lead to yield losses of 25–50 %, i.e. 1.0–1.5 t/ha less seed of high biological value;
- in poor years the combined effect of heat and drought stress with mechanical injuries may be dramatic. Partial tassel injury may lead to a complete loss of yield and even the undamaged parts may produce no yield, or at most 300–500 kg/ha;
- among the partial tassel injuries the breaking of the tassel branches proved the mildest, causing hardly perceptible damage in good years, since the undamaged lower parts of the tassel branches and even the broken, but remaining upper parts also produced pollen;
- the removal of the main tassel branch may delay the beginning of pollen shedding, while the removal of the side branches shortens the duration of anthesis. The effect of these injuries on the yield depends on synchronisation and on the weather during flowering, and varies widely. The damage may range from 10–60 % depending on the year. On areas where the whole tassel was removed (100 % treatment) there was little chance of pollen adhering to the stigma due to the isolation distance, and yield losses of up to 90 % were recorded;
- the deterioration in fertilisation due to tassel injuries reduces the biological value of the seed and increases the thousand kernel mass and the ratio of the large round fraction. Due to the protracted, poor fertilisation the kernels on each ear are at various stages of maturity, causing problems during harvest and drying and eventually spoiling the germination ability and seed vigour;
- the results obtained with respect to hybrid maize seed production can only be considered to be valid for the given combination in the given location and year, since the compatibility of the parental pairs or lines may vary considerably due to the presence or absence of synchronisation and the weather during flowering.

4.7. Drying and germ (embryo) viability

The vigour values indicative of the viability of the seedlings and the weight data of dry seedlings demonstrate that ***drying with air at a temperature greater than 45°C*** causes substantial damage to the biological value of the seed.

The damage was less severe for lines A 665 and CM 174, while MA 75A 211 and especially HMv 217 were more sensitive to harvesting at higher grain moisture content (35 %) and high drying temperature (55 °C).

Genotypes AMO 407 and AMO 406 were the most sensitive to drying temperatures above 45°C. Drying at 55°C was absolutely lethal for both lines.
Lines LH 82 and LDR 08 were somewhat more tolerant, but still had a high degree of heat sensitivity.
In the case of HMv 5405, H 8431 and HMv 5414 the damage to seed quality was more moderate, while the most heat-tolerant line was definitely HMv 5502.
The grain moisture at harvest and the drying temperature had a considerable influence on the viability of the seedlings and thus on the biological value of seed lots. Seeds harvested with lower grain moisture content suffered less damage at drying temperatures of 45°C or more.
The stress tolerance of the embryo and seedling and their performance in an O_2-deficient, low temperature environment are best expressed by the complex stressing vigour test results (i.e. cold test without soil) and by seedling dry weights. The seedling dry weight data give the best indication of the degree of damage to the seed and embryo.
Since no differences were observed in germ length, the embryo and germ weights appear to be responsible for the vigour and stress tolerance of the seed (Burris and Navratil 1980).
The seed type (flint, dent) gave no clear indication of the sensitivity to drying temperature. The most sensitive lines (AMO 407, AMO 406, HMv 217) were all related to Mo 17.