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THESIS of Ph.D. DISSERTATION

Selection of transgressive mutants of *Zea mays* L.

by diallel analysis

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1. Introduction

Currently, the most important crop of national crop production is maize. Maize production gives the largest contribution to the gross production value of crop production. Therefore, it is very important to concern with the problems of production, use and at last but not least the difficulties in plant breeding as well as the future tasks of maize production.

Regarding the goals of plant breeding, further increase of yields - currently 12-13t/ha - can not be expected. It seems to be the limit of maize production.

Droughty years occur more and more often, causing great damage to field- and horticultures. So more and more significant yield decrease can be counted on in agricultural production also in the future.

It is very difficult to obtain reliable information on the drought resistance of plants, merely by conducting field trials. A study of drought resistance and adaptability to water-shortage can be made only in well-checked, reproducible and artificial circumstances, compared with field trial results.

Improving the nutritive values, the protein content, the rate of essential amino acids and increasing the tolerance to drought in plants generate important breeding projects in the future.

This process is strongly supported by cell-, tissue- and protoplast-culture techniques, as well as the integration of molecular-genetics methods with conventional methods. Their applications significantly increase the efficiency of breeding.

Somaclonal variability of cells and tissues provides a great opportunity to select such cell-lines of maize lines and hybrids, which have outstanding nutritive values and drought resistance. This is proved by the successful selection – with the help of tissue-culture techniques – of the cell-lines consisting of genes (XA 17, XI 12) resistant to the herbicide agent called imidasolinon. These genes were taken into the maize lines and hybrids by the application of back-crossing. At present, the hybrids containing these genes (Dekalb 471, Furio Sumo, Occitan Sumo, Horus) are applied in general maize production. Thus, it is a good example of the practical application of the results of in vivo and in vitro methods.

The correct or incorrect choice of hybrid maize seed to be sown, as biological base, determines the successfulness of maize production, the quantity of yield, as goods, and consequently, the efficiency of production.

In Hungary, theoretically 203 grain and 50 silage maize hybrids can be chosen. The data of the trials with nationally registered varieties prove that the base for the successful choice of hybrids is available. However, the actual hybrid offer is about 150. These hybrid seeds can be purchased on the market.

The current hybrid supply enables us to sow the most favourable hybrids on the areas of ca. 1-1,2 million ha for grain and 100-150 thousand ha for silage maize, using the maximum production capacity of these production areas.

However, due to specific breeding aims and gene erosion the genetic base of maize breeding decreased significantly in the last decades in Hungary, of which the unfavourable effects are well-known. Many foreign hybrids are used in maize production and their genetic background is provided by only a few inbred lines. In order to decrease the genetic vulnerability, it is very important to widen the base for maize breeding by the application of various methods.

A particular way of increasing genetic variability is the treatment with various mutagens. Besides, there are numerous stocks and populations which can not be used unlimitedly in the breeding programmes because of their unfavourable characteristics. Nowadays, these negative

characteristics can be improved not only by traditional breeding procedures, but also by biotechnological methods. However, the application of mutation methods can be useful, too (MARÁZ et al., 1993; PEPÓ et al., 1994).

Inbred maize lines with the highest combining ability which provide F₁ hybrid generation having the most valuable economic features can be selected by the application of diallel analysis.

The aim of the research work was to select inbred parent lines treated by radiation which provided F₁ hybrid generation – having long-term proved general and specific combining ability values (GCA and SCA) in quality parameters - by the application of diallel analysis.

F₁ hybrids must have the following features:

- high productivity, compared to standard hybrids,
- good adaptability,
- appropriate resistance to cold, drought and heat-stress,
- appropriate HARVEST index,
- and good quality.

With the help of diallel systems created by inbred maize lines as parents the general combining ability (GCA) and the specific combining ability (SCA) of each line can be determined.

Further objectives of the research work can be mentioned as follows: complete phenotypic and genotypic description and investigation on the parent lines and hybrids of diallel systems, including: examinations on flower biology; examinations on phenometry, according to UPOV standard; examinations on production biology; growth analysis; examination on combining ability and diallel analysis of yield components. All these investigations provide useful information for further breeding projects.

By means of the application of growth analysis in diallel systems more information can be obtained on the reaction of maize to the environmental factors (meteorological and soil conditions) and their effects on the phenotype.

The aim of the study on dry matter increase by the application of climatic stress function was to characterise the increasing dry matter of each partner of the diallel system during the vegetation period, and to provide information about conditions favourable or unfavourable for the plants.

On the basis of the examinations on the effect of the successive years the yield stability of each line and their hybrids can be estimated.

2. Materials and methods

2.1. Plant material of trials

Within the programme started in 1979-80 F₁ maize hybrid seeds were treated by radiation of Co⁶⁰ isotope. Later, in 1985 the trial was expanded and the seeds were treated by radiation of fast neutrons at the Atomic Research Institute of the Hungarian Academy of Sciences in Debrecen.

After the selection of the mutation lines developed in this way they have been self-pollinated

for several years. These homogeneous lines gave the material of the trials. During the selection of the inbred lines of diallel analysis, it was an important fact that their fertility and the stability of their seed production were extremely high, compared with the means of the previous years and despite of the deterioration caused by inbreeding. The ears became steadily fertile (up to the peak) and uniform. The plant stands of these lines were homogeneous, morphologically uniformed and the alleles became homozygous. They have constant plant height and leaf number. Their silk produces high number of pollens. They are not susceptible to offshoots growing. The latter is particularly an important feature because the lines that are susceptible to offshoot growing and applied as parent lines make seed production (isolated, directed poly-crossing) and complete silk removal more difficult in the field. Two complete diallel systems ("A" and "B") were created in 1993-95 by using 4-4 separate inbred lines as crossing partners, with favourable characteristics in respect of the improvement. Inbreeding, crosses and back-crosses were carried out during the creation of the diallel systems, which is shown in Table 1.

Table 1. The creation of inbred lines in the diallel system

Lines	Hybrid	Types of rad.	Dose Gy	Lines	Hybrid	Types of rad.	Dose Gy
A13	<i>F1(Pi3950MSC)M₂</i>	Co ⁶⁰ -fn	20-5	B13	<i>F1(Pi3709MSC)M₃</i>	fn	7,5
A14	<i>F1(Pi3978SC)M₃</i>	fn	5	B14	<i>F1(Pi3747SC)M₂</i>	Co ⁶⁰ -fn	20-7,5
A15	<i>F1(Pi3780MSC)M₂</i>	Co ⁶⁰ -fn	12,5-7,5	B15	<i>F1(Pi3764MTC)M₃</i>	fn	12,5
A16	<i>F1(Pi3901SC)M₃</i>	fn	7,5	B16	<i>F1(Pi3901SC)M₂</i>	fn	12,5

F1 = first generation after crossing

Mn = mutation generation **n**

Co⁶⁰ = gamma-radiation (produced with neutron generator)

fn = fast neutron (produced in cyclotron)

2.2. Setting method of trials

2.2.1. Soil type, surface soil and water management

The trials were settled at Látókép Research Station of the Department of Crop Production and Land Use of the Centre for Agricultural Sciences (University of Debrecen). The soil of the trial was calcareous chernozem (in textural classification: loam [clay content: 47%]) with neutrality. It has got average P-supply, average-good K-supply and average humus content. The depth of the humus layer was ca. 80 cm. Regarding water management characteristics the area can be classified into category IV., which means average water capacity and good water holding ability.

2.2.2. Preparatory work of diallel analysis; plant materials

The seeds of 12 F₁ hybrids and 4 inbred lines in each diallel system were sown in randomised block system at 12°C soil temperature, with 5 replications. Each plot consisted of 5m long rows. FAO 200-500 standard F₁ hybrids were also planted to carry out comparative examinations. Every time the same F₁ hybrid was planted on the edge of the particular plot in two rows. The role of the edge-plot is to eliminate the so-called “edge-effect”, which means that the plants of the edge plots are in more favourable position in respect of nutrient- and water-uptake as well as lighting, and providing wrong data about the development and productivity of the plants to be examined.

2.2.3. Production area and population density

Row width of 70 cm and plant spacing of 20 cm were applied. Thus, 50 plants per plot could be planted on the production area. There was one-meter space left to separate each replication and to make evaluations easier.

2.2.4. Inflorescence isolation

Depending on the specific flowering date of each inbred line, at the very beginning of ear formation – when the peak slightly bends from the stem – the isolation of the female flower and silk was carried out in the parent lines of the diallel systems, complying with the strict rules of cross-pollination.

In the case of female flowers, the first step of isolation was to incise the cut surface between the ear and the stem with a sharp knife, then to cut off the peak of the ear. Cutting off the peak made us possible to turn out the styles, which increased the efficiency of artificial pollination.

Then very strong isolating nylon bags sized 100x200 mm were fixed between the ears and the stems, down the emergence point of the ears. The isolating nylon bags were checked more times until pollination and they were set right if it was necessary. In case of damaged styles emerged from under the bags, they were removed and the young ears were eliminated from further breeding.

Before inbreeding and crossing the isolating nylon bags were removed from the ears. After pollination a parchment bag sized 210x400 mm was pulled on each ear to avoid fertilisation by pollens from other plants. Each parchment bag - having a weather-proof card-label describing the certain inbreeding or cross combination on it exactly, with the help of a registration code - was fastened to the ear and the stem. Besides perfect isolation, the parchment bag was permeable to air and light, so it made further development of the ear possible. The labelled parchment bag remained on the ear from artificial pollination until harvest.

2.2.5. Harvest

The harvest was implemented by two different methods, depending on that inbred lines and newly developed cross-combinations of the given year (diallel or random crossing) or hybrids were harvested.

2.2.5.1. Harvest of inbred lines and cross-combinations

In case of harvesting inbred lines and F₁ hybrids the labels were removed definitely, the fixing string was cut between the ear and the stem with a sharp knife. Then the parchment bags were also removed, the ears were husked and broken down at the base (meeting point of the ear and the ear-stem (pedunculus)). Then, finally the label containing the code of inbreeding or crossing was fixed on the ear with a rubber ring. Thus, the harvest of the materials could be carried out separately.

2.2.5.2. Harvest in the case of hybrid testing and diallel analysis

In order to carry out hybrid testing and diallel analysis the following materials were harvested from the plots in 4 replications:

- single cross (SC) hybrids (6 in each diallel system) and reciprocal F₁ hybrids (6 in each diallel system),
- inbred lines (4 in each diallel system),
- FAO 200-500 standard F₁ hybrids with different maturity time in order to implement comparative trials.

The two examined complete diallel systems were harvested in three successive years (1994, 1995, 1996) in order to compare the effects of each year. During hybrid testing and diallel analysis the following factors have been determined:

- total ear yield per plot [kg],
- number of ears per plot [pc].

The average ear weight [g] was determined from the two factors above.

At harvest, samples were taken (6 average ears for slice processing and 6 ears in full for registering the yield parameters). The samples were still processed during the day of the harvest, otherwise wrong data could have been obtained about the moisture content of the kernels at the date of harvest.

In the case of slice processing, the base and the peak of the ear were cut off by a manual slice-cutter. The middle, almost cylindric part of the ear was cut in 3 to 4 slices then the wet weight [g] of these slices (kernel and cob) was measured in each replication.

The samples were dried in an exsiccator on the temperature of 80 °C until the weight [g] became constant. Then the slices were shelled and the weight [g] of the dry kernels and the cob was measured.

With the help of slice processing the following factors can be determined:

- moisture content of the kernels at the date of harvest [%],
- wet seed grain per plot [kg],
- rate of kernels [%].

2.3. Phenometry

2.3.1. Examinations on phenometry, according to UPOV standard

The examinations on phenometry were carried out according to TG/2/6 UPOV standard.

2.3.1.1. Leaves examination

During the leaves examination the angle made by the stem and the leaf above the main ear was determined.

2.3.1.2. Stem examinations

During the stem examination the rate of zigzags, the anthocyanin values of the leaf sheaths and the supporting roots were recorded.

2.3.1.3. Flower examinations

The date of flowering (from 26 June to 10 August), when 50% of the silks and ears flowered, was recorded.

The date of silking was recorded when 50% of the plants flowered in the middle part of the silk.

In the case of silks, the rate anthocyanin values of the rings (base) at the bottom of the ear husks, the ear husks (without base) and the ports as well as the compactness of the ears were examined. The angles enclosed by the primary axis and the lateral axes of the silk as well as the position of the lateral branches were examined in the lower part of the silk. The number of primary branches was recorded.

In the case of the ears the date of 50% flowering was recorded. The measure and intensity of anthocyanin values of the stigmas were determined.

2.3.1.4. Examinations on the phenometric features of the whole plant

The anthocyanin values in the supporting roots and the leaf sheaths were determined three weeks after flowering, since these characteristics were the most expressive in that time. The codes of the anthocyanin values of the sheaths and the rate of their expressivity are the same as the stigmas.

At the same, time the leaf angles enclosed by the stem, the rate of zigzags on the stem, the compactness of the ears in the silk, the angles of lateral branches enclosed by the primary axis in the silk and the position of the lateral branches in the silk were all recorded.

2.4. Examinations on production biology

2.4.1. Examinations on production biology, methods of growth analysis

Besides, measuring the results of plant breeding in the final product, there is an opportunity to evaluate the dynamics of photosynthetic production (during the whole period of plant growth) by means of production biological examinations.

The aim of the growth analysis is to obtain exact production biological results on the dynamics of growth and growth factors of field crops, which are influenced by different ecological and agronomic factors. As a result of the trials, further studying of the reactions of the field crops to the above mentioned factors and their dynamics is also possible.

To determine the growth analysis indexes the sampling were carried out in 2-week intervals during the vegetation period. When evaluating the samples, the plant parts above the ground level were observed (method by WESTLAKE, 1963).

The leaf area index (LAI) was determined according to the number of leaves and MONTGOMERY formula (Σ leaf length [cm] x leaf width [cm] x 0,75)

2.4.2. Examination on the increase of dry matter content by the application of climatic stress function

During the vegetation period, sampling was carried out on 17 occasions, altogether. The first 5 samples were taken from the plants of 5 replications, respectively. Further samples were taken

from the plants sown for this certain purpose. When evaluating the samples, the plant parts above the ground level were taken into consideration (WESTLAKE, 1963) in the case of parent lines and hybrid combinations with various genotypes.

As far as meteorological elements are concerned, the temperature, the global radiation, the relative moisture content and the soil moisture were determined. The time scale applied in calculations consisted of 5 days.

Considering a shorter time scale for observing the meteorological effects was not reasonable because in most cases, plants are generally able to survive extreme weather conditions for 1 or 2 days (depending on their genetic tolerance to water- and nutrient-supply), without suffering from any kind of metabolic disturbance.

The wet weight of the stem, leaf, silk and ear was measured respectively, then the samples were dried in an exsiccator on the temperature of 80 °C until the weight became constant. After that, the dry weight of the samples was measured.

To consider the stress effect on the plant in the examinations on weight increase, the crop water stress index (CWSI), according to Idso et al. (1981) was applied which can be expressed in the formula below:



where:



is the difference between the temperatures of plant surface and air



is the difference between the temperatures of plant surface and air if the plant is well supplied with water



is the difference between the temperatures of plant surface and air if transpiration stops.

The close correlation between water stress index calculated from the surface temperature of the plant population and net photosynthesis was determined by CHOUDHURY (1986). As plant surface temperature data were not available, such kind of a parameter needed to be introduced that can characterise the different dry matter decrease of each element of the diallel system.

To reach this, first we had to determine the line of the average weight growth curve in the vegetation period based on the daily weight growth values of each partner of the diallel system (arithmetical means). After that, a logistic covering curve characterising the maximum values of dry matter growth in time was adapted to it and time-derivated. This curve represents the optimum daily rate of development, i.e. the development rate in every 5-day (Figure 1.).




Figure 1. The line of average daily weight growth in each diallel system during the vegetation period

The result obtained is similar to the relative growth rate (RGR) value used in growth analysis examinations. After that, the difference between the actual and optimum RGR was calculated for the vegetation period.

Presumably, this difference is caused by the sum total of climatic effects, which is called stress function. To this difference-function, a multifactorial linear regression curve was adapted. As it

was referred to earlier, a great problem in regression examinations is to fulfil the requirements of independence and normal distribution at the same time. To avoid this problem, such variables or forms of variables were introduced, that promote to fulfil both conditions.

(RT-RG) represents thermic variable and  represents hydric variable in multifactorial linear regression function, where **RT** is the relative heat supply obtained by dividing the actual by the basic temperature ($T_b = 10^\circ \text{C}$), **RG** is the rate of real and astronomically potential global radiation, **RN_{air}** is the relative air humidity, **RVK_{soil}** is the relative water content of the examined area and **VK_{min} = 285 mm** in the soil layer of 100 cm.

In order to prove the fulfilment of normal distribution the GEARY-trial was executed, which is suitable also for small samples.

The parameters of the stress function were chosen in such a way that they could represent well both atmospheric dryness and soil dryness as well as transpiration depending on the temperature and radiation. In addition to these conditions, it was a special requirement for the calculated stress function to be non-dimensional.

Thus, every variable had relative values. However, the stress function based on the average values cannot characterise the weight decrease of each partner of the diallel system. Therefore, again a linear regression correlation with one variable had to be set up between the value estimated from the average (independent variable) and the actual value (dependent variable). The correlation indexes were 0.91-0.97, which means significant correlation in every case, if $p=1-5\%$.

During the calculation of the dry matter growth in every 5 day, it seemed that the applied covering curve of the variety function ($F(t)$) was the same as the climatic-ecological optimum curve defined by SCHIMPER (1903). This assumption is proved by the covering curve created from the dry matter data in three examined years (1994, 1995, 1996). This was the methodology of the dry matter growth.

The basic formula of the dry matter growth is the following:



where:

c and **d** are regression constants (depending on FAO numbers, primarily),

F(t) is variety function

and **KSF(t)** is stress function.

The applied variety function fairly represents the potential growth in dry matter in every 5 day in each partner of the diallel system. The creation of it is based on the time derivation of the ecological optimum curve (covering curve) in dry matter accumulation, according to the formula below:



The sum total of the stress effects, is called climatic stress function is to be deducted from the climatic optimum curve. The examination of the two complete diallel systems enabled us to introduce a variety function with 2x16 elements.

The stress function was determined according to the regression correlation with the average

weight decrease function. It was created from the difference between the average weight decrease and the variety function characterising each element of the diallel systems. In this correlation, the parameters **c** and **d** enable us to estimate the weight growth in each element of the diallel systems in the vegetation period.

The applied stress function below is a very important parameter of the model (Figure 2.):



where **KSF (t)** $\neq 0$, **RT** is the relative heat supply obtained by dividing the actual by the basic temperature (**Tb** = 10 ° C), **RG** is the rate of real and astronomically potential global radiation, **RN_{air}** is the relative air humidity, **RVK_{soil}** is the relative water content of the examined area and **VKmin** = 285 mm in the soil layer of 100 cm.



Figure 2. The line of climatic stress function [KSF (t)] during the vegetation period

2.5. Investigation method of combining ability and diallel analysis of the yield components

The examination of the two diallel systems were carried out by the application of the further developed DIALLEL Analysis and Simulation programme (BUROW-COORS, 1993), according to GRIFFING 1 method (1956).

This program uses the next model for the analysis:

$$X_{ijk} = m + g_i + g_j + s_{ij} + r_{ij} + bk + e_{ijk}$$

where:

m = population mean,

g_i = GCA effect of the parent i,

g_j = GCA effect of the parent j,

s_{ij} = SCA effect of parents i and j,

r_{ij} = reciprocal effect for parents i and j,

bk = rep. (block) effect,

e_{ijk} = error.

Regarding the various quality parameters, with the application of this programme GCA and SCA, the means of parents and hybrids as well as the heterosis effect were analysed. Thus, on the basis of the data obtained during the field inspection, harvest and ear processing, the examination of the combining ability of the yield quantity [kg/plot] and average ear weight [g] was also carried out.

By means of the applied programme a variation analysis was carried out and R^2 value was calculated. The applied model compared the combining ability of parents with the help of the F-trial and calculated the effects of combining abilities and the standard differences between them.

3. Results

3.1. Results of the examinations on phenometry, according to UPOV standard

3.1.1. Results of flower biology examinations

Flower biology examinations were carried out according to UPOV TG/2/6 method. The complete flower biology examinations of the lines and hybrids of both diallel systems (“A” and “B”) in all three years (1994, 1995, 1996) included the determination of the followings:

- the date of silking (in days, from the date of emergence), when 50% of the plants flowers in the middle part of the primary axis on the silk
- the date of ear flowering (in days, from the date of emergence), when the stigma flowers on 50% of the plants.
- the anthocyanin values of the stigma (1-9)
- the anthocyanin values of the ports (1-9)
- the anthocyanin values of the ear husks (without base) in the silk (1-9)
- the anthocyanin values of the rings (base) of the ear husks (1-9).

The anthocyanin values representing each characteristic were the same in the different years examined, which shows high stability of the parent lines and hybrids. This proves the reliability of the conducted trials.

3.2. Production biology examinations

3.2.1. Results of the leaf area index (LAI) examinations and growth analysis

The maximum LAI was measured in the middle of July in both diallel systems.

The highest LAI was reached by the hybrids **A4** ($5,72 \text{ m}^2 \cdot \text{m}^{-2}$), **A10** ($5,41 \text{ m}^2 \cdot \text{m}^{-2}$), **A11** ($5,19 \text{ m}^2 \cdot \text{m}^{-2}$) and **B3** ($7,514 \text{ m}^2 \cdot \text{m}^{-2}$), **B10** ($5,84 \text{ m}^2 \cdot \text{m}^{-2}$) **B9** ($5,83 \text{ m}^2 \cdot \text{m}^{-2}$) in diallel systems “A” and “B”, respectively. The reason of the high LAI of the hybrids **A4** and **B3** was the high number of their offshoots. In respect of LAI, only hybrids **A10**, **A11**, **B9** and **B10** seemed to be valuable combinations because they could reach high values without offshoots. Besides, these hybrids had got more favourable plant height and more constant population than hybrids **A4** and **B3**.

By the end of August 1994, the LAI of most hybrids and lines significantly decreased or ceased. Those lines and hybrids can be considered to have good drought resistance, that could keep up their assimilation surface until the end of August or the middle of September. On the contrary, in 1995 and 1996 most lines and hybrids of both diallel systems had measurable assimilation surface even at the end of September.

3.2.2. Results of the examination on the dry matter growth by the application of climatic stress function

It is important to investigate diallel systems in order to get a general overview about the reactions of hybrids with different genetic background. The inheritance of quantitative characters, grades of tolerance and environmental adaptability can be estimated via this system.

The aim of this examination was to characterise the dry matter growth of each partner of the diallel system in the vegetation period and the decrease of it caused by unfavourable conditions based upon genetic, meteorological and soil conditions. These data provided information about

the conditions that are favourable or unfavourable for the plants.

On the basis of weight growth curves meteorological factors having a crucial role in plant development can be chosen. Furthermore, the critical values of them can be determined at/above which different types of stresses (heat and water) are formed. After stresses the decrease in dry matter growth was observed in each case. The basic formula of the established model is:



in which **c** and **d** are regression constants, $F(t)$ is variety curve, $ST(t)$ is stress function. The applied variety function fairly represents the potential growth of dry matter in each partner of the diallel system. The creation of it is based on the time derivation of the optimum curve (covering curve) in dry matter accumulation. The following stress function is an important parameter of the model:



in which RT is the relative heat temperature which comes from the division of real and basic ($T = 10\text{ }^{\circ}\text{C}$) temperature. RG is the rate of real and the astronomically potential global radiation, RN_{air} is the relative air humidity, RVK_{soil} is the relative water content of the examined area, minimum water capacity of the soil, $WK_{min} = 285\text{ mm}$. The results obtained were favourable. In the extremely dry year 1994, when 60-90 g weight decrease was observed in each maize hybrid, the differences between the estimated and measured data did not exceed 20-30 g/plant.

3.3. Results of the investigations on productivity, combining ability and the diallel analysis of yield components

3.3.1. Results of the examination on the productivity

In 1995, due to intensive drought, all hybrids of both diallel systems produced lower yields by 30-40 % than in the preceding and the following years. This was expressed both by a significant decrease both in productivity [kg/plot] and average ear weight [g].

In the case of diallel system "A", compared to standard and other hybrids, line A13 and its hybrids (A3 and A10) having the highest combining abilities produced outstanding yields in all the three examined years. In the droughty year 1995, in spite of their heterogeneous genetic composition, the differences between the yields of each hybrid decreased. Despite drought, this year the yields of hybrids A1 and A11 slightly decreased. In the parent lines of these hybrids, A13 and A16 – having excellent GCA – are included.

During three years, the productivity of the hybrids in diallel system "B" was lower and less constant on average than in the case of the hybrids in diallel system "A". Considering GCA and SCA, some hybrids (B1 and B4) and their parent lines proved to be very weak. Their plant height and productivity values often remained under the productivity level of the lines.

In the parent lines of the hybrids B3, B6, B9 and B10 with outstanding productivity we could always find the lines B15 and B16 which had got excellent GCA. This was proved by the results of all the three years. Due to the low productivity that was observed in all the three years, the hybrids B1 and B4 and their parent lines with low GCA values were eliminated from further breeding projects. The others could reach or exceed the productivity of the standard hybrid in all the three years, therefore the best of them can be applied in further testing and – in case of suitable results – in the development of new hybrids in the future.

As a result of the trials, we found that in spite of the different yield levels, the productivity of the newly developed hybrids above is satisfactory. Some of them can be characterised of outstanding productivity.

3.3.2. Results of the examinations on GCA and SCA in the case of productivity and average ear weight

As a result of the trials, one line (A13) – having the best GCA and improving effect on each hybrid and the examined characteristics (yield [kg/plot] and average ear weight [g]) in all three years (1994, 1995, 1996) - was selected in diallel system “A”. As the reliability of the results was good ($R^2 > 0,8$) this line could be applied as improving parent in further crossing projects. The GCA values of the lines A15 and A16 were low but still satisfactory, while line A14 became eliminated, due to low GCA value in each year of the trial. This line proved to be destructive in cross-combinations.

Regarding both examined characteristics, hybrid combination **A13x A16** in diallel system “A” reached the highest SCA value in each year.

In the case of diallel system “B”, the lines B15 and B16 had got the best GCA values. Considering the examined quality parameters, these two inbred lines proved to be improving in each year. Later, together with the selected lines with the best combining ability in diallel system “A”, they can be the plant material of another crossing project in which the most valuable ones of the 8 lines used so far can be selected safely. On the basis of GCA values, lines B13 and B14 had got destructive effect on the examined characteristics, so they were eliminated from further breeding projects. The hybrids **B13xB16** and **B14xB16** could reach the highest SCA values. These combinations can be used for further testing.

The result of the variation analysis proves to be a significant correlation between the values of SCA and GCA and the values of yields [kg/plot] and average ear weight [g] in each diallel system, respectively. As the value of the regression coefficient is always higher than 0.8 ($R^2 > 0.8$), the correlation seems to be significant.

Regarding GCA values, the analyses enabled us to select one line with excellent combining ability and two lines with improving effect in diallel system “A” and one with excellent combining ability and one with improving effect in diallel system “B”. On the basis of SCA values, one hybrid combination in diallel system “A” and two hybrid combinations in diallel system “B” were selected. Those inbred maize lines having unfavourable, low combining abilities (A14, B13 and B14) were eliminated from further crossings and hybrid development projects.

4. New scientific results

- Creation of two complete diallel systems from eight previously developed inbred and irradiated lines for conducting complex examinations.
- Examination of all the lines and hybrids of two complete examined diallel systems, demonstration of the effects of the different years.
- Development of 12 new SC maize hybrids in each diallel system (24 hybrids, altogether); description of the parent lines (8) and their hybrids according to UPOV standard; determination of their differentiation.
- Selection of the hybrids having the highest leaf area index (3-3 in diallel system “A” and “B”, respectively) according to complex investigations on production biology.

- Analysis of dry matter growth by means of climatic stress function. The model is suitable to estimate the dynamics of dry matter accumulation of each hybrid and line and to calculate yield decrease due to climatic conditions. Verification of the reliability of the results by statistical analysis.
- Exact determination of productivity and yield safety in three different years; comparison to standard hybrid; selection of the most productive hybrids (2 and 4 in diallel system “A” and “B”, respectively); elimination of weakly productive hybrids from the breeding project; verification of the reliability of the results by statistical analysis.
- Determination of general (GCA) of lines and specific combining ability (SCA) of SC hybrid combinations in two complete diallel systems; selection of the lines with the best GCA (1 and 2 lines in diallel system “A” and “B”, respectively) and selection of hybrids with the best SCA (1 and 2 hybrids in diallel system “A” and “B”, respectively) on the basis of two examined characteristics and three years; elimination of the lines and hybrids with low GCA and SCA from the breeding projects; verification of the reliability of the results by statistical analysis.

5. Suggestions for practical use

- The application of mutation breeding method is of great help in widening the range of usable genotypes in maize breeding. By means of induced mutants the gene pool of the population will improve, which is very important nowadays, when there is a decrease in the forms of varieties. With the help of mutations increasing the variability, such lines could be selected that resulted in new hybrid combinations, meeting the crop production requirements. The diversification of the breeding material could be executed by neutron radiation.
- Due to high genetic affinity, the more frequent application of neutron radiation in maize breeding programmes is reasonable in the future. After treating the seeds with fast neutrons, the strict inbreeding (genetic homogenisation) of the populations showing segregation then the selection of the lines having the most favourable agronomic characteristics were needed.
- According to our trials, we were led to the conclusion that cyclothron can be used successfully in the increase of genetic variability.
- We were able to produce so many homogeneous inbred stocks having high genetic variability by the application of mutation breeding. With the help of diallel analysis, the lines having the highest general and specific combining abilities (GCA and SCA) could be selected. By using these lines, such F1 hybrids can be produced, which have the best quality parameters.
- In order to implement this task, a wide-ranging diallel trial has to be set in with more replications in more vegetation periods. During the trial, more factors are needed to be examined then summing up the obtained results complex conclusions can be drawn to assist further selection. These investigations must be started just after sowing, recording the weight growth and the LAI of the various plant parts; and describing the lines and hybrids of the diallel system in details in every phenophase, according to UPOV standard. At the end of the vegetation period after harvesting each plot and each replication, we determined the productivity and carried out the diallel analysis of the yield components to compare the combining ability values of each line. The examinations mentioned above were found suitable to select the best lines and SC hybrid combinations.

- After summing up the data, comparisons were made with the standard hybrids of the given year, and the effect of the different years was investigated, too. We found that only those lines and hybrids can form the basis of further breeding projects which produced outstanding results and could meet the demands of each examined element during the examinations above.
- To increase the productivity of maize and the efficiency of the production are important tasks. Our suggestion is to take into account not only the yield or the components of it but also their physiological processes; that is, we have to select for their optimum values. One important method is the increase of the intensity of photosynthesis. This can be achieved in several ways. The performance of the photosynthesis can be increased by producing such hybrids that have very quick early growth. These plants cover the soil soon and utilise more photoenergy. This purpose is also served by the selection for the increase of the leaf area index (LAI) of the population. We suggest that attention should be paid to increasing the LAI of maize hybrids from 3.0-3.5 to 4.5-6.0.
- Neglecting the declines in the yearly dynamics of weight growth can cause great inaccuracy in the estimation of the total yield. In many cases, intensive growth in dry matter content can be experienced again, right after weight declines. It can increase significantly the neglected, non-decreased dry matter amount. Therefore, we suggest that the precision of the internationally used models – like CERES – should be increased during their adaptation in Hungary. Though, the model used during our examinations contains some determinants – e.g.: variety function ($F(t)$) –, it is able to work with stochastic weather conditions as well, by means of climatic stress function ($KSF(t)$). There is a reasonable fact on its side that it needs only 4 input data (temperature, global radiation, relative moisture content and soil moisture) and only linear factors are included in its calculation.
- We think important to increase the number of production years in order to keep up validity.
- The developed model is suitable for describing the growth features of the different hybrids and for meteorological characterisation of stress periods.
- We found that applying this model, we can select those favourable hybrid combinations, which are the least sensitive to drought that can be experienced more and more frequently in Hungary.

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