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INVESTIGATION OF GENETIC POLYMORPHISM, TASSEL COMPONENTS AND SOME QUANTITAVE FEATURES IN MAIZE GENOTYPES

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1. Introduction

With rice and wheat, maize is one of the most important crop plants of the world. In Hungary, its production area generally varies between 1 and 1.2 million hectares. It is used mainly as fodder corn, but it is predictable that in the future it will be utilised as basic material for food and other industries (starch, bioethanol, etc.) too.

Considering the large production area, the amount of crop influences the complete domestic agricultural sector. Growers with good reason can expect breeders to develop lower cost varieties with stable high productivity. Due to genetic evolution of corn, productivity of new hybrids increases with 1% per year.

Selection takes long years from the production of a line to the authorisation of a hybrid. This period (production, preparation and examination) can be shortened with complex studying methods suitable for classification of genotypes into relationship groups. Their genetic potential can be optimised for further hybrid breeding with selection of parental lines and decrease of crosses.

Drop of genetic diversity of field varieties leads to development of fewer hybrids in classical way and the decrease of variability of parental lines.

Future's maize selection in addition to classical criteria (enhancement of productivity, adaptability, disease resistance) will work to inhibit the decline of genetic material (gene erosion), maintenance and more accurate description - on DNA level - of breeding base material.

Important aims in hybrid maize breeding are to choose crossing directions precisely and know their effects. Production and selection of hybrids with different genetic composition is a significant yield influencing factor.

The male inflorescence of maize is a tassel, components of which are frequently studied. In maize breeding, increased attention is being paid to the selection of features that can help reach maximum yield with regulation of energy conversion. Such phenomena are plant height, ear height, leaf number, leaf area. In addition, tassel characteristics can influence plant performance and productivity significantly.

Maize is monoecious, heteroecious, so its reproductive success largely depends on the development of male flower. Morphology of tassel components primarily influencing pollen amount can be a significant factor of successful seed production and selection.

Interest on health preserving effects of cereals is increasing; however, there is only little information on such features of maize. Cereals contain large amount of biologically active substances that inhibit the production of free radicals or lower their levels in the organism. According to studies dealing with this topic, the role of maize should be increased in human diet in addition to rice and wheat and more attention should be paid on the selection of cultivars suitable for functional nutrients.

My Ph.D. work is the continuation of the noticeable maize breeding research Prof. Károly Pásztor began a few decades ago and is still going on with the supervision of Prof. Pál Pepó.

My research consisted of:

- Determination of relationship among four lines from the gene bank of our department on phenotypic, protein and DNA level.
- Determination of combining abilities of lines with complete diallel system.
- Investigation of productivities, test weight, crossing directions of hybrid combinations produced by complete diallel system, examination of maternal and reciprocal effects with involvement of some morphological features.
- Study of tassel elements of lines and hybrids, relations between them and applicability of tassel area index.
- Determination of water-soluble antioxidant contents of yellow, red and blue kernel corn genotypes.

My research was laid on a broad base to emphasize the common role of disciplines (molecular genetics, food science, plant breeding) in future's plant selection.

2. Materials and Methods

2. 1. Breeding base material

Field trials were elaborated on the experimental area of the Department of Genetics and Breeding later on Department of Horticulture and Plant Biotechnology (Centre of Agricultural Sciences, University of Debrecen) from 2004 to 2006. Descriptions of base material production can be found in the following papers: MARÁZ *et al.*, 1993; PEPÓ and TÓTH, 2004;). Plant materials - parental lines - of the study were positive transgressive, selected mutant maize lines produced by the seeds of irradiated (fast neutron, doses 5 and 15 Gy) F_1 hybrids (*Table 1*). Each inbred lines were self-pollinated for more than ten years, so they could be considered as homozygotes. Selection of these inbred lines was based on morphological homogeneity.

Table 1. Origins of examined inbred lines

Line	Origin	Type of irradiation	Dose [Gy]
UDL1	F_1*(NK-PX14)M_2**	Fast neutron	15
UDL4	F_1*(Pi3978SC)M_3**	Fast neutron	5
UDL5	F_1*(Pi3764MTC)M_3**	Fast neutron	5
UDL6	F_1*(Pi3478)M_2**	Fast neutron	5

*: first generation after crossing
**: mutation generation

2.2. Soil of the experimental area

The soil of the area was leached chernozem with a limeless upper layer. The ground water level was 7–9 m. Humus layer was medium category, 50-70 cm in depth. Organic material content was 2.57%. Results of soil studies are listed in *Table 2*.

Table 2. Characteristics of the soil at the experimental area

pH		$CaCO_3$	Y_1	Y_2	Al soluble		Humus %	Soil plasticity K_A
H_2O	KCl				P_2O_5 mg/kg	K_2O mg/kg		
7.0	6.5	traces	5.0	8.0	100	165	2.57	42

Because of the lack of lime in the upper layer, soil can become cracking during dry, droughty periods.

2.2. Weather in the experimental years

Weather in experimental years was more or less the same. In 2004 and 2005 during cultivation period, fall was above the average of 30 years (+76.9 and +152.2 mm, respectively), in 2006 it was close to the average. In view of maize production - except some extremes as temperature fall in May, heat-wave in June - the cropyear was favourable.

2.3. Setting method of field experiments

The trial was arranged as a randomized block design with 4 replicates in the experimental area of the Centre of Agriculture of Debrecen University from 2004 to 2006. Each plot consisted of 5 m long rows. Distance between rows was 70 cm, plant-to-plant distance was 20 cm, altogether 50 plants/plot could be examined. Plant material of the complete diallel system was consisted of 4 inbred lines and the seeds of 12 F_1 hybrid lines. In parallel, standard lines of DUS studies and standard F_1 hybrids of FAO 200-500 groups were sown as controls for the experiments. Line and hybrid productions were elaborated with a standard method. Genotypic composition of the complete diallel system can be seen in *Table 3*.

Table 3. Genotypic composition of the complete diallel system

Parental lines	UDL1	UDL4	UDL5	UDL6
UDL1	UDL1	UDH1	UDH2	UDH3
UDL4	UDH4	UDL4	UDH5	UDH6
UDL5	UDH7	UDH8	UDL5	UDH9
UDL6	UDH10	UDH11	UDH12	UDL6

2.4. The main features of the agrotechnology applied

In each year autumn ploughings were performed until the end of November, in the depth of 35 cm. Spring tillage and seed-beds with early spring harrowing were done with combination before sowing. Sowing was elaborated with hand with double numbers of seeds. Numbers of plants were set at the stage when 3-4 leaves were on the plants. To avoid edge-effect, 2-2 line wide edges were placed on both sides of the experimental material.

Whole amounts of P and K were applied in autumn, while 30% of N in autumn before ploughing and the remaining part in spring before planting. We applied N, P_2O_5 and K_2O as fertilizers in doses of 100, 90 and 90 kg ha^{-1}, respectively.

Chemical weed control was conducted with Primextra 500 FW (6 l/ha), in addition manual weed control (hoeing) was also done every year, mainly in the plots of inbred lines. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) appeared in 2004 without causing any damages. Against the insect, in 2005 and 2006 Counter 5G soil disinfectant was used at the dose of 20 kg/ha on the whole area.

2.5. Harvest

Yield was harvested by hand, separately on each plot. Processing was done on the same day. Wet grain yield/plot (kg) was determined. Samples were taken at harvest (12 average size ears, 6 for slice processing, 6 for the study of yield producing parameters). During slice sample processing lower part of the ear and its top were removed. The central, closely roller-shaped part was cut into 3-4 slices and wet weights (g) of whole discs (grain, cob) were measured (per material, per replicate).

Samples were dried at 40 °C for 2 days, then at 60 °C until weight did not decrease any more. After drying slices were shelled, dry grains and cobs were measured (g). Yield was calculated to 14.5% yield each year.

2.6. Description of phenotypic features according to CPVO-TP2/2 guidelines

Phenotypic features of 2x15 plants (per lines and per hybrids) were examined following the guidelines of CPVO (Community Plant Variety Office) TP/2/2. Evaluations were done in each experimental years (2004 to 2006).

Features were labelled in time and with mark - developmental stage - according to CPVO-TP2/2 during the whole generation time of maize, at harvest and sample evaluation. Data of these studies formed the base of polymorphism investigations (based on morphological features).

2.7. Zein analysis

We examined 4 parental lines of the diallel system. Alcohol-soluble proteins were extracted from individual grains and separated on ultra-thin polyacrylamide gel by isoelectric focusing. Protein pattern is characteristic to each inbred lines.

2.8. AFLP analysis

Examinations were conducted in the laboratory of the Institute of Food Processing, Quality Control and Microbiology, Centre of Agricultural Sciences, University of Debrecen.

For DNA isolation we used leaves of 10-12 day-old plants. Germination was done after sterilization of grains on sterile MS medium.

DNA isolation

Genomic DNA of lines were elaborated with QIAGEN DNeasy Plant Mini Kit. Leaves were destructed with liquid nitrogen. Quality of DNA was checked by agarose gel electrophoresis on a 0.8% gel. Isolated DNA was stored at -20 ºC.

AFLP analysis

Isolated DNA was digested with 10 U *Eco*RI (1 hour, 37 °C) and afterwards with 1 U *Tru*1I (*Mse*I) on 67 °C. AFLP adaptors were ligated to genomic DNA fragments.

Ligation mix was as follows: 10 µl double digested DNA, 1-1 µl (100 pmol) *Eco*RI and *Tru*1I adaptors, 2 µl ligation buffer, 1 µl T4 ligase, 8 µl H_2O. The mix was incubated for 2 hours at 20 °C.

Components of AFLP PCR mix were: 2 µl digested and ligated DNA as template, 1 µl (10 pmol) *Eco*RI primer expanded with AAC selective bases, labelled with NED laser induction stain, 1µl (100 pmol) *Mse*I primer expanded with CAG, CAC, CAT selective bases, 12.5 µl PCR MasterMix, 8.5 µl H_2O.

Cycles of PCR:

initial denaturation: 94 °C for 120 s,

at the beginning of each cycles: 94 °C for 30 s,

during 12 cycles: 65 °C for 30 s, 0.7 °C temperature decrease/cycle,

during 23 cycles: 56 °C for 30 s,

at the end of each cycles: 72 °C for 60 s,

last cycle: 72 °C for 120 s.

Amplified and selected DNA fragments were detected with capillary electrophoresis at the Agricultural Biotechnological Centre in Gödöllő.

2.9. Other morphological studies - leaf area index (LAI), tassel components

Leaf area (LA) was determined with Montgomery's formula following the guidelines of ANDA and TÓBIÁS (1999). Leaf area index (LAI) was calculated considering plant density. Lengths and widths of leaves were measured on 5-5 plants in four replicates.

LA (m^2/piece) = leaf length x max. leaf width x 0.75,

LAI = LA (m^2/piece) x plant density (piece/m^2).

Tassel components were examined according to CPVO TP2/2 guidelines, tassel area index was calculated by FONSECA *et al.* (2003) with the following formula:

TAI = π × diameter of main tassel stalk (mm) × length of main tassel stalk (cm) + π × 0,5 × diameter of main tassel stalk (mm) × total length of branches (cm).

2.10. Investigation of heterosis effect

We examined how traits of produced hybrid combinations ($\overline{F}$) exceeded the average of those in parents ($\overline{P}$):

- midparent heterosis: $\overline{F} - \overline{P}$,
- level of heterosis effect ($(\overline{F} - \overline{P}) / \overline{P}$) comparing to average of parents.
-

2.11. Determination of test weight and thousand kernel weight

For calculation of test weight and thousand kernel weight samples were taken after determining yield in four replicates. Processing was done by hand. After shelling and clearing samples were dried until no weight decrease was detected. Beginning temperature was 40 ºC, then it was gradually increased to 60 ºC. Determination of test weight was done by the descriptions of FVM - Ministry of Agriculture and Rural Development - (2006), while thousand kernel weight was calculated by measuring 3x200 kernels.

2.12. Water-soluble antioxidant activity determination of corn genotypes with different kernel colour using the FRAP method

Plant material

Yellow kernel corn: study was based on twelve hybrid lines (UDH1-UDH12 direct and reciprocal crosses) deriving from four inbred lines produced by induced mutation (UDL1, 4, 5, 6).

Blue and red kernel corns: twelve cultivars, origins and parameters are in *Table 4.* Samples were from an organic production area (Hajdúböszörmény, (47º 32’ North, 21º 36’ West)) - cultivated without fertilizer and herbicides.

Table 4. Origins and kernel colours of corn cultivars

Genotype	Kernel colour
Rdeci*	Red
Rotmais*	Red
Japonica**	Red
Hopi Blue*	Blue
Blaumais*	Blue
Hopi Turquoise***	Blue
Alamo Navajo Blue***	Blue
Taos Pueblo Blue***	Blue
Santo Domingo Blue***	Blue
Black Mexican***	Blue
Santo Pueblo Black***	Blue
Purple Red Flour***	Blue

Origin:

*Austrian Agency for Health and Food Safety (AGES) Gene bank, Linz, Ausztria

** Tápiószele Institut of Agrobotany, Hungary

***IPK Gene bank, Gatersleben, Germany

Extraction

Studies were conducted in the laboratory of the Institute of Food Processing, Quality Control and Microbiology, Centre of Agricultural Sciences, University of Debrecen. Three parallel samples were prepared from each genotypes. First step of preparation was grinding (Retsch SM100, HAAN, Germany, 1 mm screen). From every grinded sample 0.2 g was used for extraction. 10 ml 0.1 N HCl was given to samples and mix was incubated for 4 hours in a 90 ºC water bath; filtered with Filtrak, Grade388, QTL100 and filtrate (extract) was used for measurements.

Water-soluble antioxidant activity measurement using the FRAP method

Antioxidant content was measured with FRAP (Ferric Reducing Ability of Plasma) (BENZIE and STRAIN, 1996). The method is based on the reducing ability of biologically water-soluble materials and in addition, it gives information on the scavenging potential as well. The measurement: under buffered acidic conditions (pH=3.6) antioxidants reduce Fe(III) to Fe(II). Bivalent Fe ions form colour complexes with tripyridyl-triazine (TPTZ). The concentration of the produced colour compound is proportional with the concentration of antioxidants can be measured photometrically.

Solutions needed for the determination of the total antioxidant content:

- acetate buffer: pH 3.6; 300mM (3.1g Na-acetate*3 H_2O+16ml acetic acid to 1000ml with distilled water
- 54mg 20mM $FeCl_3$*6 H_2O+10ml distilled water
- triazine solution (TPTZ): 10mM, 40mM HCl → 31.23mg+10ml DV+33.6µl HCl
- FRAP reagent: 25ml acetate buffer, 2.5ml $FeCl_3$ solution, 2.5ml TPTZ solution

For automated measurement TECATOR FIASTAR 5000 was used. Measuring wavelength was 590 nm, reference wavelength was 750 nm. Reagent: FRAP reagent. Sample amount: 40 µl. The reported values are means of triplicate measurements, and on a dry weight basis. Data were processed with mono- and bifactorial variance analysis using SPSS 11.5 for Windows.

2.13. Evaluation of results with mathematical-statistical methods

For studying polymporhisms of morphological features, SPSS 11.5 for Windows statistical software was used. Comparing pairs of cultivars, Euclid's square distances were calculated between lines. Data of molecular genetic studies as well as standardized morphological data and capillary electrophoretic patterns were processed with the hierarchic cluster analysis software of SPSS 11.5 for Windows. Pattern was coded binary (1=presence of fragment, 0=lack of fragment). According to Jaccard index, we determined the genetic similarity coefficients and genetic distances of lines. Yield results were evaluated with one factor variance analysis.

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

The examination of the diallel system was carried out by the application of the further developed DIALLEL Analysis and Simulation programme (BUROW-COORS, 1994), according to GRIFFING 1 method (1956).

This program uses the next model for the analysis:

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

where:

μ = 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.

Directions of crosses and tassel components were examined with one- and two factors variance analysis and Pearson's correlation coefficient calculation method (SVÁB, 1981 and SPSS for Windows statistic program).

3. Results

3.1. Features of lines and study of their polymorphisms on phenotypic, protein and DNA level

Morphological descriptions of four fast neutron irradiated maize inbred lines (*UDL1, 4, 5, 6*) were elaborated. Studies were supplemented with the DUS descriptions of 12 hybrid lines deriving from direct and reciprocal crosses of above-mentioned lines. General (GCA) and specific (SCA) combining abilities of individual crossing partners can be determined with diallel systems made of inbred maize lines. Morphological (based on CPVO TP2/2 guidelines), biochemical (zein pattern) and AFLP markers of inbred lines are suitable for prediction of genetic similarities/distances between lines. It is possible to select inbred lines with the less genetic similarities even before production of diallel systems, creating opportunity for a preliminary selection, saving time and costs.

Conclusions of polymorphism studies on phenotypic, protein and DNA level:

► DUS description based on 30 traits and cluster analysis divided the four inbred lines into two groups. One consisted of *UDL5* and *6* (tightly connected on dendrogram), the other one consisted of *UDL1* and *4* (higher distance between them) (*Figure 1*).

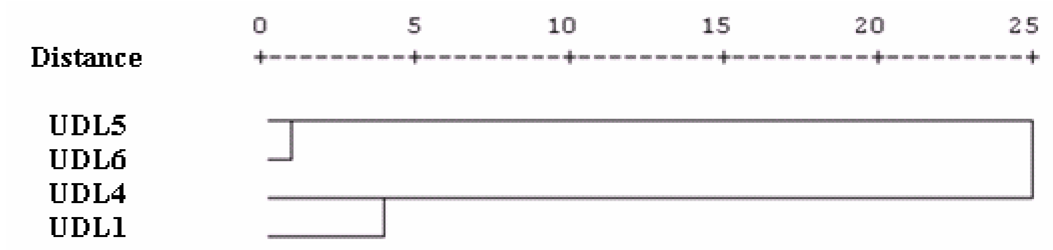

Figure 1. Dendogram obtained by cluster analysis based on the 30 morphological characteristics of four maize inbred line

► Results of Jaccard index and cluster analysis based on 14 polymorphic zein patterns modified results listed above: *UDL4* was categorized into the group made of *UDL5* and *6. (Figure 2).*

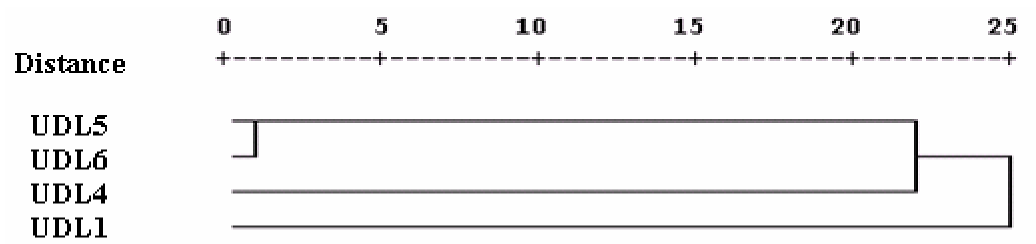

Figure 2. Dendrogram obtained by cluster analysis based on the zein patterns of four maize inbred lines

► AFLP analysis performed with three primer pairs resulted in 208 amplified bands (70 polymorphic) (*Table 5*). These results were similar to those of zein studies: *UDL4* is connected to *UDL5* and *6*. Relationship of the three lines became clearer. Analysis suggested that *UDL5* and *6* were half-sister according to studies of genetic similarity/distance based on Jaccard index (*Table 6*). Application of AFLP made results of zein studies more precise (*Figure 3*).

Table 5. Results of AFLP analysis

Primer combinations	Total number of bands	Number of polymorphic bands (pc)	Partition of polymorph bands (%)
M48-E32*	128	44	34,3
M49-E32*	38	7	20,6
M50-E32*	42	19	45,2
Mean	69	23	33,3

*= According to Keygene[R]

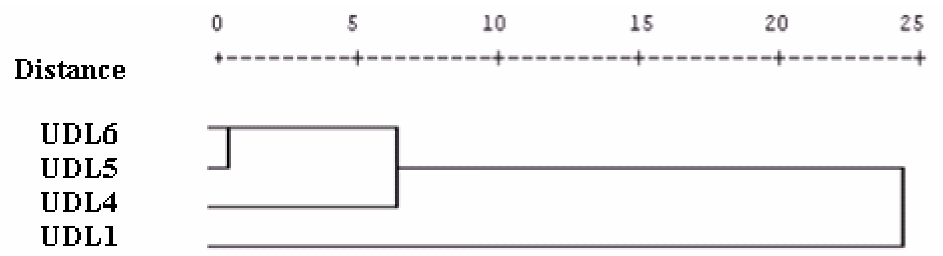

Figure 3. Dendrogram obtained from cluster analysis based on the AFLP amplification patterns of four maize inbred lines

Table 6. Genetic similarity among four maize inbred lines based on Jaccard index.

Line	Jaccard index			
	UDL1	UDL4	UDL5	UDL6
UDL1	1,000			
UDL4	0,430	1,000		
UDL5	0,455	0,648	1,000	
UDL6	0,385	0,676	0,740	1,000

► Relationship levels determined by morphological features were not reliable, application of biochemical-genetic markers would be needed during selection. Results of DNA studies different from DUS studies can be explained with lower number of primers. It would be practical to increase this condition for the more exact categorization of *UDL4*.

With the above-mentioned study, grouping of parental lines and insurance of genetic distance between them was effective, it is one of the criteria of heterosis breeding. The four inbred lines could be divided in a well-defined way into two groups (I. *UDL1*; II. *UDL4, UDL5, UDL6*). Highest genetic distance (GD) was predicted between *UDL1* and *UDL6* (GD=0.78), while *UDL5* and *UDL6* (GD=0.51) were the closest relatives.

3.2. Conclusions for the results of productivity, general and specific combining abilities

Studies showed that lines predicted to have the largest genetic distances performed highest productivity both in direct and reciprocal crosses (*UDH3* and *UDH10*) in all years.

After determining combining abilities, one excellent line was selected *(UDL1*). According to yield averages, the highest specific combining ability (SCA) was performed by *UDL1 x UDL6* (*UDH3*) hybrid combination in all years. These results confirm prediction of performance of hybrid combinations, based on complex methods, make possible the reduction of the number and time of field experiments, and make the selection of hybrid combinations with the highest heterosis effect more precise.

3.3. Investigation of crossing directions based on morphological features

Direct and reciprocal crosses were examined in case of four traits in average of three years. Reliable differences were observed only in a few instances, more frequent statistical differences were experienced in plant height and ear height. No statistical differences could be shown in case of leaf number with reversing cross directions.

Largest heteroses were experienced in case of plant height (32.9% and 31.8%) and ear height (33.9% and 33.1%) in the average of three years. Smallest hybrid dominances were in stalk diameter (16.4% and 14.1%) and leaf number (10.1% and 11.7%).

3.4. Changes of test weight and thousand kernel weight in different genotypes

A significant part of domestic maize products are sold on foreign markets. Among the ever-restricted quality requirements the demand for the measurement of test weight also appeared. This measurement is not unfamiliar in the case of other cereals such as wheat and barley, but it has not been applied widely in maize yet; likely since this we have a few information and research background in this topic.

Thousand kernel weights and test weights of twelve hybrids (6-6 dents and semidents) of the complete diallel system were compared. The aims of this study were to evaluate the cropyear effect on genotypes in view of these phenomena and the effects of kernel type on thousand kernel weight and test weight. Notable differences were observed. Genotype x cropyear effect was not significant in none of the years. Highest thousand kernel weight was observed in dent hybrids, while highest test weight in semidents (higher than in dents with a minimum of 2 kg/hl in both years). Negative, medium correlation was experienced between thousand kernel weight and test weight with high reliability level in the average of years (*Table 7*).

Table 7. Test weight and thousand kernel weight of maize hybrids of different kernel type in investigated years

Type of kernel	*Year*	*1000 kernel weight*	*Test weight*
Dent	2005	320,6^{+}	74,3*
	2006	322,5*	76,5*
Semident	2005	302,8^{+}	77,2*
	2006	274,3*	78,8*

Significant at level: *P=5 %, $^{+}$P=10 %

3.5. Study of maize tassel components

Except tassel stalk diameter, cropyear effect was the highest in all examined tassel components. There were significant differences among the components in studied hybrids. Highest tassel branch numbers were experienced in hybrids, which had *UDL6* as one of the parents. In the development of tassel branch numbers of hybrids, crossing partner with higher branch number was more influential. Heterosis in direct crosses was 116.7%, while in reciprocal ones 121.6%. In case of tassel length, *UDL5* and *UDL6* influenced this phenomenon.

Direct and reciprocal crosses in view of each tassel components were compared and reliable differences were found only in a few cases. Significant differences in tassel branch number reliably influenced main axis length and diameter of *UDH1* and *UDH4*, but these differences stayed below the limit of the distinction of the two hybrids.

Flowering-biological features and tassel components of lines and hybrids were also evaluated. Density of main tassel stalk and number of tassel branches were considered as vital important in view of selecting crossing directions. Lines with more dense main tassel stalk - more spikelets - and higher number of tassel branches are advised to be utilized as male crossing partners.

Roles of tassel components would be more and more important in the forthcoming years. More frequent draught periods related to climate changes during flowering would increase the importance of studies on reproductive organs of maize. Draught in flowering period could involve the shortening of shed period and decrease of pollen amount. As a consequence of proterandria and decreased pollen amount decrease or lack of fertilization could occur. Recent hybrids and parental lines have smaller tassel sizes as older ones. In seed and conventional production, more attention has to be attracted on such research.

Discovering the relationships among plant morphological and quantitative features is very important in maize breeding and production, particularly if they can be altered by selection or agro-technical methods.

Strength of connections between tassel components and some quantitative features were examined with Pearson’s correlation coefficient calculating method.

Traits can effect each other directly or indirectly. Medium or strong correlations on high reliability level can suggest indirect influence on features.

Tassel weight was positively correlated with total number of branches (0.59**), number of primary branches (0.53**) and tassel area index (0.63**), and negatively correlated with plant height (-0.45**) and yield (-0.39**). Tassel area index was negatively correlated with plant height (-0.63**) and yield (-0.55**), as were number of branches and Leaf Area Index (0.39**). Number of primary branches showed medium correlation with plant height (-0.43**). Test weight correlated negatively with plant height, ear height, yield, leaf area index (LAI) and 1000 kernel weight. Because a number of these traits are highly heritable, these correlations could be used as a basis for indirect selection of special features in maize breeding programs (*Tabel 8., Figure 4).*

Tassel area index represents effects of tassel components. It can be suitable for direct selection of traits with low inheritability values. Relationships of traits were presented on a dendrogram with hierarchic cluster analysis. Tassel components and yield with yield composing factors formed two different clusters.

Table 8. Pearson's correlation among investigated quantitative parameters

Traits	*Tassel weight*	*Tassel diam..*	*Tassel length l.*	*Tassel length u.*	*Total length br.*	*NPB.*	*TAI*	*Plant height*	*Ear. height*	*Yield*	*LAI*	*N. leaves*	*1000-kernel weight*	*Test weight*
Tassel weight														
Tassel diam.	0,24*													
Tassel length l.	0,36**	0,42**												
Tassel length u	0,20	0,36**	0,87**											
Total length br.	0,59**	0,12	0,35**	0,14										
NPB.	0,53**	0,05	-0,05	-0,22	0,75**									
TAI	0,65**	0,05	0,18	0,13	0,46**	0,43**								
Plant height	-0,45**	0,18	0,25*	0,21	-0,24*	-0,43**	-0,63**							
Ear height	-0,24*	0,20	0,23*	0,16	-0,13	-0,22	-0,33**	0,79**						
Yield	-0,39**	0,13	-0,04	-0,14	-0,10	-0,09	-0,55**	0,56**	0,51**					
LAI	-0,22	0,22	0,28*	0,23	-0,08	-0,39*	-0,27*	0,67**	0,77**	0,67**				
N. leaves	-0,07	0,08	0,14	0,15	0,01	-0,12	-0,07	0,41**	0,55**	0,17	0,48**			
1000 kernel w.	-0,45**	-0,01	-0,14	-0,14	-0,06	-0,11	-0,44**	0,52**	0,19	0,38**	0,31**	0,05		
Test weight	0,30**	-0,11	0,08	0,12	0,26*	0,26*	0,55**	-0,61**	-0,37**	-0,59**	-0,35**	-0,06	-0,46**	

Significant at the **P=0,01 %, *P=0,05 % level, ^{ns}: non significant

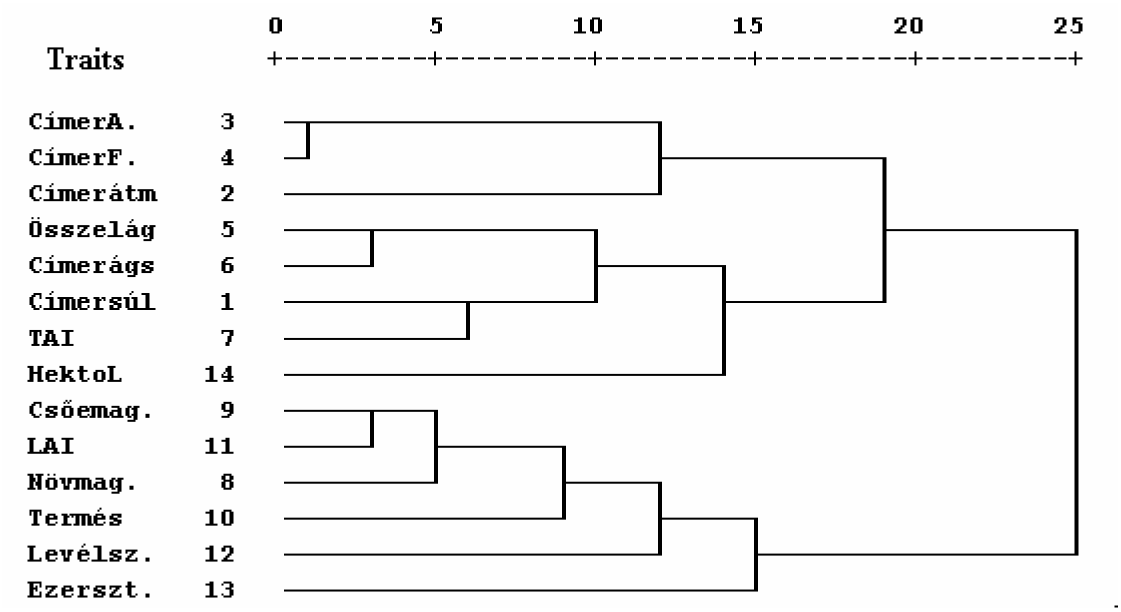

Figure 4. Dendrogram obtained from cluster analysis based on the features of hybrids

Abbreviation:
CímerA : Tassel: length of main axis above lowest side branch; CímerF.: Tassel: length of main axis above upper side branch; Címerátm.: Diameter of tassel; Összelág: Number of total primary branches; Címerágs: Tassel: number of primary branches (NPB); Címersúl: Tassel weight; TAI: Tassel Area Index; HektoL: Test weight; Csőemag.: Earheight; LAI: Lea Area Index; Növmag.: Plant height; Termés: Yield; Levélsz.: Number of leaf; Ezerszt.: 1000 kernel weight.

3.5. Water-soluble antioxidant activity determination of corn genotypes with different kernel colour using the FRAP method

Water-soluble antioxidant activities in yellow kernel corn lines and hybrids were examined supplemented with those of red and blue cultivars. Water-soluble antioxidant activities of yellow corns (four lines and twelve hybrids) depended on genotype and cropyear. Values were significantly lower (7.97-12.51 mg kg^{-1} vitamin C equivalent, yellow kernel type) than in blue and red ones (199-639 mg kg^{-1} vitamin C equivalent) (*Table 9*).

We found significant differences in antioxidant activities of different genotypes. The highest was in Santo Pueblo Black (639±19 vitamin C equivalent mg kg^{-1}), while the lowest in Black Mexixan Sweet (199±14.1 vitamin C equivalent mg kg^{-1}). Values of red varieties (Rdeci, Rotmais) and the two hybrids varied from 241±38.6 to 264±34.4 vitamin C equivalent mg kg^{-1}. Only Japonica was significantly different from them with 447±59.7 vitamin C equivalent mg kg^{-1}, which value is more than 1.5-times higher than those of other red corns. The results highlighted the great variability in antioxidant activities among cultivars. In the future, utilization of blue and red kernel corn genotypes has to be widened.

In future's plant selection, more attention has to be paid on breeding of cultivars suitable for functional nutrients. For this aim, new - possibly -, exotic landraces could be introduced into Hungary, and in addition, new technologies for processing are needed. Broad basic and applied research will be needed on this field. These would help widening of market, developing a new, domestic segment of it; and as a consequence, development of our agriculture and production of natural, health preserving products. More studies dealing with relations between corn genotypes, products and development of chronic diseases are necessary.

Table 9. Antioxidant activities of studied genotypes (red and blue corn)

Genotype	Mean (vitamin C equivalent mg kg^{-1}) ±standard deviation
Black Mexican	199±14.1
Hopi Blue	217±8.1
Rdeci	263±54.5
HopiTurquoise	263±7.2
Rotmais	264±34.4
Blaumais	331±5.5
Purple Red Flour	359±23.3
Santo Domingo Blue	403±30
Japonica	447±59.7
Taos Pueblo Black	450±84
Alamo Navajo Blue	206±22.5
Sandia Pueblo Black	639±19
$LSD_{5\%}$	52.9

4. New scientific results

1. Polymorphism studies based on morphology, protein and DNA levels were elaborated. Relationships of lines were determined, two lines were half-sister.

2. Determination of general (GCA) and specific (SCA) combining abilities of a complete diallel system. Selection of line with best GCA (*UDL1*) and hybrid with best SCA (*UDH3*) according to the results of three years.

3. Significant differences were found in thousand kernel weights and test weights of dent and semident hybrids. There was negative, medium correlation between the two traits with high reliability level.

4. In forming tassel branch number of hybrids, parent having the most branches had dominant effect.

5. Tassel components and some quantitative features were compared with Pearson's correlation coefficient determining method. Strength of relations between traits and directions of interactions were determined. Adapted tassel area index (TAI) showed negative, medium correlation with plant height, yield and thousand kernel weight on a high reliability level. With the knowledge of correlations between characteristics one can predict change of features related to each other.

6. In the average of three years in the selection of cross distances, it was concluded that direct and reciprocal hybrid pairs were closely related. Little maternal effect was observed only in plant height and earheight. These differences were smaller than needed for distinguishing two hybrids (based on CPVO TP2/2 guidelines).

7. Water-soluble antioxidant activities of sixteen yellow, three red and nine blue kernel corn genotypes were determined. There were significant differences between the values measured in different genotypes: red and blue cultivars significantly exceeded yellow ones in view of this phenomenon.

5. Results with practical application

1. Results of examinations on morphological, protein and DNA levels contributed to the complex evaluation of lines produced with induced mutation and heterosis studies.

2. Results of flowering-biological, phenological and quality studies on inbred maize lines can be used as components of new hybrids.

3. There were significant differences in test weights of hybrids with different kernel types. These differences has to be taken into consideration during hybrid selection.

4. Relationship studies of tassel components can contribute to breeding and seed production.

5. Density of main tassel stalk and tassel number were considered as most important features in selection of crossing directions. It would be advised to use Lines with more dense main tassel stalk - more spikelets - and higher number of tassel branches are advised to be utilized as male crossing partners.

6. Discovery of connections between tassel components and other quantitative traits can be used as indirect selection in maize breeding.

7. Selection of red and blue kernel colour genotypes and their utilization in human diet is strongly advised. FRAP (Ferric Reducing Ability of Plasma) method - based on Fe(III) reducing ability - is suitable for fast and precise measurement of water-soluble antioxidant capacities of different maize genotypes. Results of such studies can help breeders and growers in selecting good plant material and production of genotypes for special targets.

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