AGRICULTURAL ACADEMY - SOFIA

MAIZE RESEARCH INSTITUTE - KNEZHA

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ABSTRACT

of a dissertation
on the subject of

STUDY OF MOISTURE RELEASE RATES OF INBRED MAIZE LINES FOR USE IN BREEDING PROGRAMMES

for obtaining a DOCTORAL degree in Education and Science

Doctoral program **"Breeding and seed production of crops"**

Scientific field 6**. Agricultural Sciences and Veterinary Medicine**

Professional field 6.1. **"Plant production"**

Scientific supervisor: **Assoc. Prof. Natalia Petrovska, PhD**
Scientific consultant: **Prof. Stefan Valchinkov, PhD**

Knezha
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The dissertation has been discussed and directed for defence at a meeting of the Department of Genetics, Breeding and Seed Production at Maize Research Institute - Knezha (Protocol No. 194 of 27th September 2023)

The dissertation research was conducted in the experimental field of Golden West Seed Bulgaria in the village of Brashlen in the period 2016-2018.

The dissertation is written in 144 pages and contains 24 tables and 41 figures. 193 literature sources were used - 45 in Cyrillic and 148 in Latin.

The defence of the dissertation will take place on ..... at..... hours in the meeting room of the Maize Research Institute - Knezha in an open meeting of the scientific jury appointed by order ..... of the President of the Agricultural Academy, composed of:

1. Introduction

One of the most important breeding traits in maize, along with yield, is the moisture content of the grain at harvest. This trait is determined primarily by the duration of the vegetation period of a given genotype and the rate of moisture release in the grain after the onset of physiological maturity (Misevic, et al., 1988; Petrov, 1999).

The rate of moisture release (the rate of water loss from the grain between reaching physiological maturity and harvest) has become a very important trait in the last few decades (Baute et al., 2002; Hellevang, 2004; Filipenco et al., 2013), which is why both recombination breeding and mutation breeding methods have been used to improve this trait (Pepo, 1990).

Many studies have revealed that maize genotypes differ in rates of moisture release and that the differences are heritable (Troyer and Ambrose, 1971; Mukhtanov, 1985; Cross and Kabir, 1989; Newton and Eagles, 1991; Angelov et al. 1995; Angelov and Valchinkov, 2009; Filipenco et al. 2013; Ghete et al. 2016). The rate of moisture release is defined as the percentage of moisture loss during the entire drying period, i.e., from the onset of physiological maturity to grain harvest or parts of this period (Crane et al., 1959; Wang et al., 2012; Shi et al., 2022). The rate of moisture release is a highly heritable trait.

2. Purpose and tasks of the study

The objective of this work was to study the grain moisture content and its rate of release after the 40th day from the silking of the cobs to the harvesting of mid-early crosses of Iodent and Lancaster genetic source lines. The objects of the research were grain water content on the 40th day after silking, the rate of moisture release from the grain after the onset of physiological maturity, and the grain moisture content at harvest of the studied hybrids and their parental components. To achieve our objective, we set the following tasks:

- Study of the grain moisture content of the parental forms and their hybrids, from the 40th day after silking until harvest.
- Study of the correlation between grain moisture content of the parental components and their hybrids.
- Study of the rate of moisture release from the grain of the 40 hybrids and their parental components in the research.
- Classification of hybrids and lines by grain moisture content at harvest.
- Carrying out the comparative assessment of the hybrids by moisture content and by average yield.
- Study of the factors on which the grain moisture content of the studied hybrids and their parents at harvest depends
- Breeding of lines that have a low grain moisture content and that transfer this trait consistently in hybrid combinations for recurrent breeding purposes.

3. Materials and methods

3.1 Cultivation methodology of the field experiment

The study was conducted in the period 2016-2018 on the experimental field of Golden West Seed Bulgaria in the village of Brashlen. The agricultural technical activities during the vegetation were carried out on time according to the agricultural technology for the area for non-irrigated conditions.

There are twelve corn lines of Golden West Seed Bulgaria with a close vegetation period (from FAO 300 to FAO 399) included in this study in order eliminate as much as possible the influence of the duration of the vegetation period on the moisture content at harvest. These lines are from various heterotic groups, but mainly Iodent and groups combining with it - mainly Lancaster. The research includes their hybrid combinations, implemented according to a full set crossing scheme, as well as four checks from selected FAO groups. Their crosses were obtained in 2015 at the Winter Nursery in Puerto Vallarta, Mexico.

All p^2 combinations consisting of parents and direct F1 crosses are included in the work (Petrov, 1999). This includes 36 hybrid combinations, four checks (standards) and twelve inbred lines. Hybrids from three maturity groups DKC4590 (350 FAO), P9528 (380 FAO), P8745 (280 FAO) and PR37F73 (430 FAO) are used as checks.

Over the next three years, the F1 hybrids as well as the parental lines previously bred in the breeding field were tested in field trials using the block method, in four replications in experimental and harvest plots of 7 m2 each and a seeding density of 5800 row/daa, under non-irrigated conditions. Samples for grain moisture content analysis were determined from an average sample of 3 cobs from each replicate of the experimental plot.

Table 1 shows the percentage of plasma content from the different groups and that is consistent with the theoretical model rather than the actual situation, which is difficult to determine by classic breeding methods. Six of the lines refer mainly to the Ioden heterotic group, and the other 6 belong mainly to the Lancaster heterotic group. Their hybrid combinations and experimental standards are listed in Table 2.

Table 1. Origin of the lines involved in the set crossing scheme

	Theoretical heterotic group					
Line	Iodent	BSSS	B37	Lancaster	OH43	Flint Europe
GW57010				80%		20%
GW59008	100%					
GW57091	90%	10%				
GW58005	70%		30%			
GW51150				90%		10%
GWC06021	80%	20%				
GW58012			50%	50%		
GW58008				100%		
GW59024	100%					
GW57095				50%	50%	
GW52090			50%	50%		
GW58268	100%					

The first analysis to determine the moisture content of the grain was carried out on the 40th day after the silking of the cobs, the aim being to do this around the time of the onset of physiological maturity of the variants in the trial, with subsequent analyses every five days until the trial was harvested. On six dates, 3 cobs (from the experimental plot) were collected from each variant of the four replications, in order to measure grain moisture content with a SAMAP-O-TEST H 40 electronic moisture meter and determine the rate of moisture release. The last sample was taken during combine harvesting of grain only and was determined electronically on a representative sample of each harvest plot variant.

Table 2. Hybrid combinations and checks included in the study

Hybrid 1	GW59024	x	GW58008
Hybrid 2	GW59024	x	GW57010
Hybrid 3	GW59024	x	GW57095
Hybrid 4	GW59024	x	GW51150
Check 5	P8745		
Hybrid 6	GW59024	x	GW58012
Hybrid 7	GW59024	x	GW52090
Hybrid 8	GW58005	x	GW58008
Hybrid 9	GW58005	x	GW57010
Hybrid 10	GW58005	x	GW57095
Hybrid 11	GW58005	x	GW51150
Hybrid 12	GW58005	x	GW58012
Hybrid 13	GW58005	x	GW52090
Hybrid 14	GW57091	x	GW58008
Hybrid 15	GW57091	x	GW57010
Hybrid 16	GW57091	x	GW57095
Hybrid 17	GW57091	x	GW51150
Hybrid 18	GW57091	x	GW58012
Hybrid 19	GW57091	x	GW52090
Hybrid 20	GWC06021	x	GW58008
Hybrid 21	GWC06021	x	GW57010
Hybrid 22	GWC06021	x	GW57095
Check 23	DKC4590		
Hybrid 24	GWC06021	x	GW51150
Hybrid 25	GWC06021	x	GW58012
Hybrid 26	GWC06021	x	GW52090
Hybrid 27	GW58268	x	GW58008
Hybrid 28	GW58268	x	GW57010
Hybrid 29	GW58268	x	GW57095
Hybrid 30	GW58268	x	GW51150
Hybrid 31	GW58268	x	GW58012
Hybrid 32	GW58268	x	GW52090
Check 33	P9528		
Hybrid 34	GW59008	x	GW58008
Hybrid 35	GW59008	x	GW57010
Hybrid 36	GW59008	x	GW57095
Hybrid 37	GW59008	x	GW51150
Hybrid 38	GW59008	x	GW58012
Check 39	PR37F73		
Hybrid 40	GW59008	x	GW52090

3.2 Mathematical treatment of the results obtained from the field experiment.

The comparative assessment of the hybrids in terms of moisture content and average yield was carried out by applying one-factor analysis of variance (ANOVA) and Duncan's test (when there is no check variety or LSD-test (when there is a check variety) (Laudau and Everitt, 2004). The statistical data satisfy the Levene's test (Laudau and Everitt, 2004) for equality of variances, which in turn means that the hybrids can be compared according to the relevant indicators.

The statistical processing of the data was performed using the IBM SPSS 22 software.

A classification of the crosses was made by hierarchical cluster analysis (Laudau and Everitt, 2004). This is a multivariate statistical method that enables the identification of relatively homogeneous groups of objects, called clusters, on the basis of similarity in certain indicators. The agglomerative method by which it was conducted was intergroup linkage, and the measure of similarity was the squared Euclidean distance. Dendrograms were constructed to graphically represent the phased clustering of individual objects.

Data were pre-standardized in order to avoid the different dimensions of the indicators.

Correlation analysis and regression analysis were also used in the analysis (Laudau and Everitt, 2004). Based on the latter, trend lines representing the variation of moisture content of the studied hybrids were constructed.

4. Soil and weather conditions

The experiments were conducted in land of the village of Brashlen, Province of Ruse. The village is located at 43°59 north latitude and 26°22 east longitude in the climatic region of the Hilly Danubian Plane. Its altitude is 26.5 m.

The main soil type in the area of the village of Brashlen is the highly leached, medium-strength, sandy loamy chernozem overlying loess sandy clay. It is characterized by a powerful humus-accumulative horizon /60-100 cm/, with dark brown colour, loose structure and crumbly-grainy structure. The transitional horizon has a thickness of 40-50 cm, it is compact and fine-textured. Carbonates are washed to a depth of 60 to 120 cm. The amount of humus varies from 3 to 5%, gradually decreasing in the depth of the profile. The reaction is slightly acidic in the humus-accumulative horizon and neutral in the upstream horizon. The maximum field capacity (MFC) of the soil layer is 20 to 30% and the humidity of permanent wilting (HPW) is from 10 to 15%. Depth filtration over the entire profile is good and ranges from 1.5 to 2.0 mm/min. (Lenkov and Atanasov, 1980).

Table 3 presents data on the average temperatures and the amount of precipitation during the test period. From the indicated data, it can be concluded that in all three years of testing, the observed rate of precipitation was close to or slightly above the average for the region during the vegetation period (341.2-461.4 mm), with 2018 proving to be the wettest on average due to its very large winter reserves of soil moisture, while the wettest maize vegetation period was in 2017. A dry and relatively warm spring was observed in 2018, which adversely affected the simultaneous germination of maize crops.

The highest amount of precipitation during the maize vegetation season was observed in 2017, when a total of 461.4 mm of rain per m2 was recorded in the March-September period. This has a beneficial effect on flowering, silking, the onset of physiological maturity, yields and grain moisture content at harvest.

Table 3. Average monthly temperatures, relative humidity and precipitation totals during the maize vegetation season for the 2016-2018 study period.

Month	Average monthly temperature, °C			Precipitation per ten-day period, mm/m²			Precipitation total per month	Precipitation per ten-day period, mm/m²			Precipitation total per month	Precipitation per ten-day period, mm/m²			Precipitation total per month
	2016	2017	2018	2016				2017				2018			
	t °C			I	II	III	mm/m²	I	II	III	mm/m²	I	II	III	mm/m²
October	11.90	10.90	12.10	7.60	31.00	40.40	79.00	16.60	32.00	8.60	57.20	88.40	0.00	66.40	154.80
November	9.20	5.80	7.00	0.00	0.00	54.40	54.40	64.60	7.00	5.00	76.60	8.80	38.80	45.80	93.40
December	4.60	-0.3	3.70	11.80	6.80	0.60	19.20	0.60	0.80	4.80	6.20	15.80	28.40	14.80	59.00
January	-3.10	-5.4	0.90	30.50	9.50	13.80	53.80	6.40	12.00	3.40	21.80	2.20	27.00	29.20	58.40
February	7.60	0.90	1.30	19.60	6.00	1.40	27.00	4.40	12.00	11.00	27.40	13.20	42.00	16.80	72.00
Total October-February							**233.40**				**189.20**				**437.60**
March	8.60	9.60	4.40	23.60	5.60	25.80	55.00	13.80	59.40	5.40	78.60	29.20	14.00	38.80	82.00
April	14.30	11.80	16.70	1.80	46.40	9.20	57.40	5.80	62.40	11.40	79.60	4.20	5.00	0.00	9.20
May	16.80	17.10	19.90	55.40	0.20	25.20	80.80	38.80	6.00	31.40	76.20	12.80	3.80	5.00	21.60
June	22.90	22.90	22.60	36.20	19.80	24.00	80.00	25.40	9.80	0.60	35.80	21.20	26.00	91.80	139.00
July	24.70	23.90	23.30	5.20	3.20	0.00	8.40	80.20	11.00	42.20	133.40	22.40	2.20	36.00	60.60
August	23.50	24.00	24.40	0.80	33.80	5.60	40.20	0.80	0.00	33.80	34.60	2.20	16.00	2.20	20.40
September	19.60	20.00	19.00	0.00	16.00	3.40	19.40	18.20	0.00	5.00	23.20	3.20	9.60	17.40	30.20
Total March-September							**341.20**				**461.40**				**363.00**
Total for the crop year							**574.60**				**650.60**				**800.60**

5. Results and discussion

5.1 Classification of maize crosses studied by hierarchical cluster analysis and Duncan's test.

When the studied maize hybrids were grouped by hierarchical cluster analysis, it was revealed that, on average, three clusters were formed during the study period (2016-2018), with the first cluster consisting of two subclusters (Figure 1). The second cluster is separated from the first cluster by a distance of ten Euclidean units, and the third cluster is separated from the first two clusters by a distance of 25 Euclidean units, demonstrating a significant degree of divergence between the hybrids of the different groups.

The first subcluster of the first cluster consists of maize hybrids with high to medium moisture content ranging from 23.3% (Hybrid 22) to 21.64% (Hybrid 13). The second subcluster contains hybrids with slightly lower moisture content, between 21.52% in Hybrid 32 and 20.75% in Check 9, and due to its values, which are still higher, it is clearly differentiated from the previous subcluster. As in two of the three years of the experiment, two of the checks fall in the first cluster, one in the second and one in the third, but they are among the variants of the experiment with the highest yields.

The second cluster contains hybrids with moisture contents ranging from 20.58% to 19.30%, which are statistically significantly different from those of the first cluster.

The third cluster includes maize hybrids with the lowest moisture content. It varies from 18.4% to 18.7%, with the lowest being Check 5, not only in this group, but also among all hybrids.

Considering the results of the comparative assessment conducted (Table 4) it cannot be claimed that yield has a significant effect on the distribution of hybrids in different clusters, it is mainly the

moisture content that influences the distribution. Within each cluster there are both high and lower yielding hybrids, with valid differences between them.

In the group of 40 maize hybrids studied, there are no significant differences in the standard deviation values in terms of moisture content, which proves that no strong dips or peaks of moisture content values were observed over the three-year time period of the experiment, i.e., moisture when harvesting maize can also be very strongly influenced by external conditions, but the influence of these conditions in different hybrids is approximately equal and unidirectional.

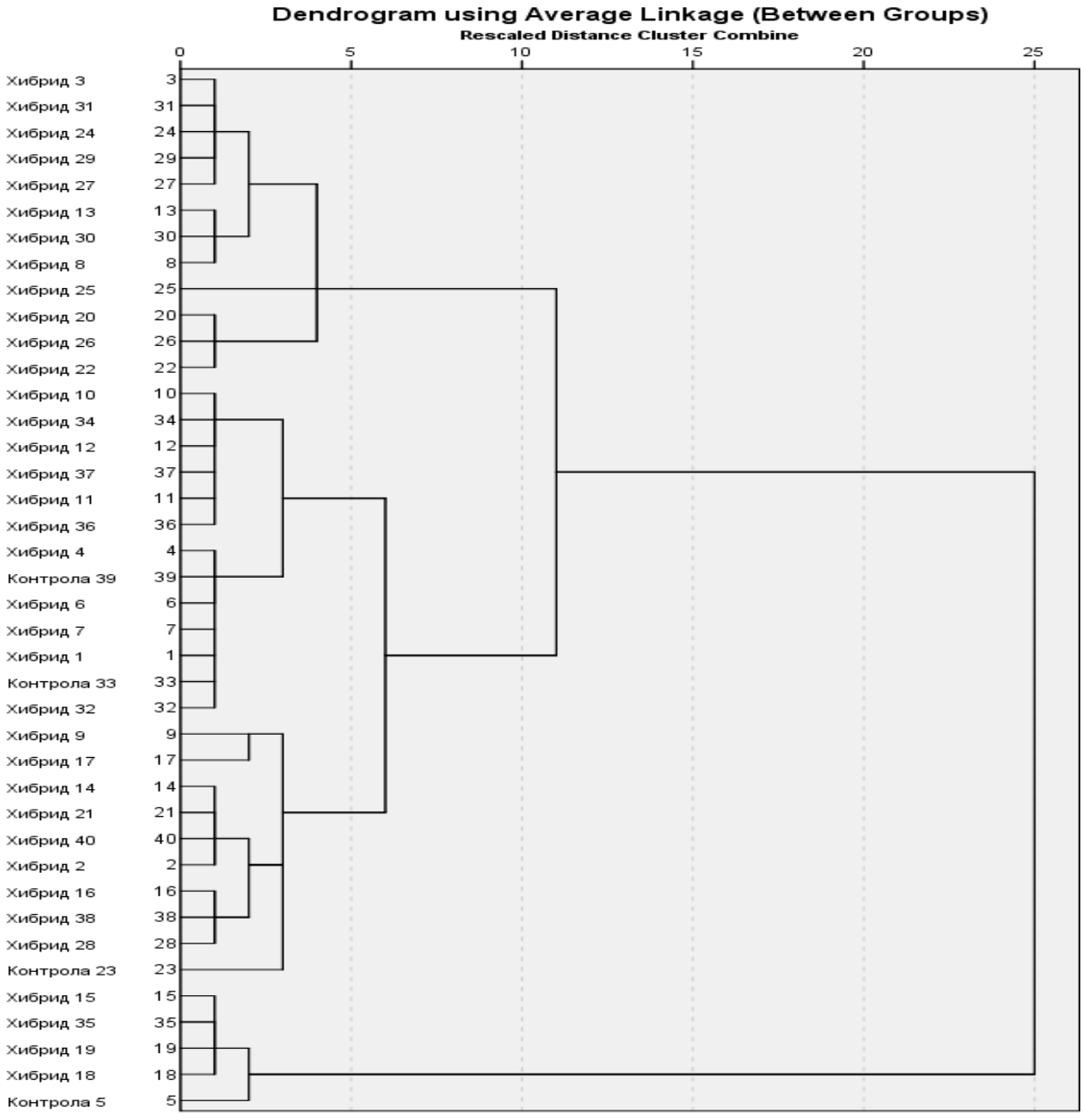


Figure 1. Dendrogram visualising the classification of maize hybrids according to moisture content /%/ and average yield /kg/da/ for the period 2016-2018 by hierarchical cluster analysis

Table 4. Comparative assessment of maize hybrids by moisture content /%/ and yield /kg/da/ for the period 2016-2018 by one-factor analysis of variance and Duncan's test to assess differences at a statistical significance level a= 0.05

Cluster №	Subcluster №	Hybrid	Moisture /%/	Standard deviation	Yield /kg/da/	Standard deviation
I	1	Hybrid 3	22,11^{abcdefg}	6,2417	855,05^{abcde}	242,7474
		Hybrid 31	22,10^{abcdefg}	5,7137	893,36^{abcd}	260,861
		Hybrid 24	22,31^{abcdef}	5,4633	889,76^{abcd}	293,5475
		Hybrid 29	22,39^{abcde}	5,2147	861,50^{abcde}	278,0811
		Hybrid 27	22,55^{abcd}	5,4770	923,07^{abc}	337,6586
		Hybrid 13	21,64^{abcdefgh}	5,6523	890,95^{abcd}	354,0106
		Hybrid 30	21,67^{abcdefgh}	5,6401	781,60^{abcde}	188,2272
		Hybrid 8	21,97^{abcdefgh}	5,3865	812,93^{abcde}	183,1388
		Hybrid 25	21,90^{abcdefgh}	5,0681	652,86^{cde}	234,217
		Hybrid 20	22,79^{abc}	5,6097	797,98^{abcde}	196,5471
		Hybrid 26	22,99^{ab}	5,6309	760,76^{abcde}	278,2985
		Hybrid 22	23,30^{a}	5,3617	816,76^{abcde}	239,1513
	2	Hybrid 10	21,10^{abcdefghi}	5,9335	748,29^{abcde}	206,4668
		Hybrid 34	21,18^{abcdefghi}	5,8307	710,71^{abcde}	211,5283
		Hybrid 12	20,77^{bcdefghij}	6,1289	812,57^{abcde}	256,7145
		Hybrid 37	20,82^{bcdefghij}	6,0125	755,83^{abcde}	279,4023
		Hybrid 11	21,03^{abcdefghi}	5,4561	821,29^{abcde}	146,3447
		Hybrid 36	21,06^{abcdefghi}	5,8844	770,26^{abcde}	197,7383
		Hybrid 4	20,8^{bcdefghij}	5,3661	955,64^{abc}	339,9777
		Контрола 39	20,75^{bcdefghij}	5,5265	984,48^{a}	223,5879
		Hybrid 6	21,25^{abcdefghi}	6,5335	855,40^{abcde}	146,5869
		Hybrid 7	21,15^{abcdefghi}	5,8594	894,40^{abcd}	181,507
		Hybrid 1	21,21^{abcdefghi}	5,0183	951,40^{abc}	278,1505
		Контрола 33	21,21^{abcdefghi}	5,7386	962,50^{ab}	164,9186
		Hybrid 32	21,52^{abcdefghi}	5,0832	989,57^{a}	191,9384
II	1	Hybrid 9	19,78^{hijk}	5,6008	527,81^{e}	181,9201
		Hybrid 17	19,30^{ijk}	6,1410	710,19^{abcde}	205,3361
		Hybrid 14	20,07^{fghijk}	5,7122	794,45^{abcde}	217,0946
		Hybrid 21	20,21^{efghijk}	5,9610	788,36^{abcde}	179,0933
		Hybrid 40	20,37^{defghijk}	5,4602	829,64^{abcde}	261,8931
		Hybrid 2	19,77^{hijk}	5,7692	750,55^{abcde}	250,5758
		Hybrid 16	20,38^{defghijk}	5,9850	719,07^{abcde}	186,429
		Hybrid 38	20,58^{cdefghijk}	4,9660	692,69^{abcde}	260,221
		Hybrid 28	20,02^{fghijk}	5,4306	638,69^{cde}	250,442
		Контрола 23	19,92^{ghijk}	5,4207	859,07^{abcde}	260,6324
III	1	Hybrid 15	18,42^{k}	4,8804	654,79^{cd}e	191,7813
		Hybrid 35	18,45^{k}	5,0810	627,62^{de}	246,9456
		Hybrid 19	18,42^{k}	5,1020	763,22^{abcde}	286,64
		Hybrid 18	18,70^{jk}	5,3873	776,52^{abcde}	220,416
		Контрола 5	18,40^{k}	5,2920	846,86^{abcde}	182,9523

$^{a,b.c.d}$… degree of evidence by Duncan's method with an error of a =0.05

5.2 Classification of parental components of studied maize crosses by hierarchical cluster analysis and Duncan's test

As a result of the conducted hierarchical cluster analysis, the studied maize lines, also as found for their hybrids, are grouped into three clusters according to the degree of similarity mainly by grain moisture content for the period 2016-2018 (Figure 2). Yield is again not significant for clustering as there are high and low grain yield lines in all clusters.

The first cluster includes the maize lines that have the highest moisture content: GW59024, GW58268, GWC06021, GW58008. It ranges from 21.36% in GW58268 to 22.12% in GW58008 (Table 5).

The second cluster is formed by lines with lower moisture content ranging from 19.54% in GW57095 to 20.43% in GW52090 and yields ranging from 455.38 kg/daa in GW52090 to 232.74 kg/daa in GW57095.

The third cluster consists only of the maize line GW57010 (moisture content on average for the period 17.06%), standing out with the lowest grain moisture content at harvest and average grain yield. Considering the minimum value of the standard deviation of this line on the moisture content indicator, it could be argued that its occurrence is the most stable on this trait over the three-year study period.

To the extent that the three clusters contain lines from different heterotic groups, it can be concluded that, at least for the breeding materials of Golden West Seed Bulgaria FAO 300 maturity group from the previous cycle of improvement work, the belonging of the line to one or another heterotic group does not affect its clustering with respect to grain moisture content. Lines have been developed that transmit low or high grain moisture content to hybrids from both of the major heterotic groups for mid-early maize breeding.

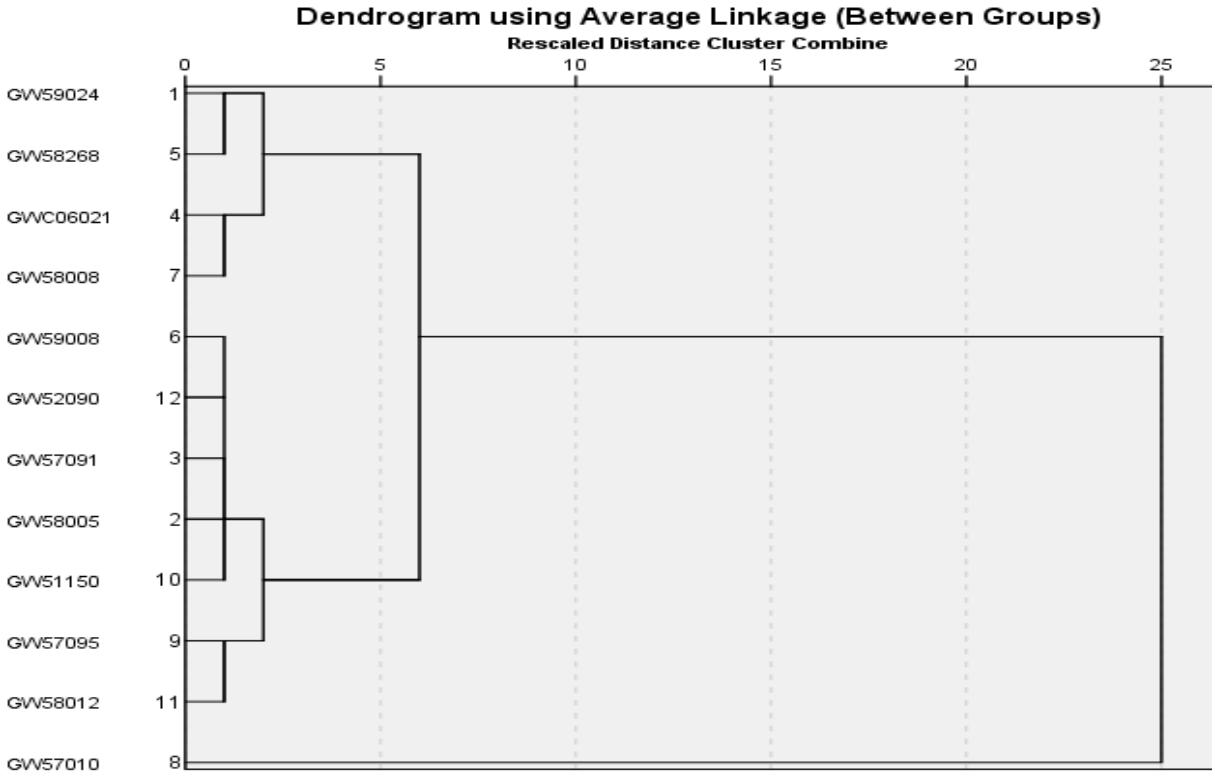


Figure 2. Dendrogram visualising the classification of maize lines according to moisture content /%/ and average yield /kg/daa/ for the period 2016-2018 by hierarchical cluster analysis

Table 5. Comparative assessment of parental lines according to moisture content /%/ and average yield /kg/daa/ by Duncan's test at significance level α=0,05 for the period 2016-2018.

Cluster №	Line	Moisture /%/	Standard deviation	Yield kg/da	Standard deviation
1	GW59024	21,55^{abc}	5,96	407,74^{ab}	247,74
	GW58268	21,36^{abc}	5,68	409,36^{ab}	151,63
	GWC06021	21,88^{ab}	5,45	315,52^{ab}	163,05
	GW58008	22,12^{a}	6,00	247,21^{b}	175,60
2	GW59008	20,26^{abc}	4,83	439,71^{ab}	210,72
	GW52090	20,43^{abc}	6,23	455,38^{a}	153,17
	GW57091	20,22^{abc}	6,44	429,14^{ab}	266,17
	GW58005	20,41^{abc}	6,00	368,50^{ab}	215,11
	GW51150	19,77^{bc}	5,11	414,74^{ab}	213,45
	GW57095	19,54^{c}	5,10	232,74^{b}	146,85
	GW58012	20,21^{abc}	4,81	237,95^{b}	97,14
3	GW57010	17,06^{d}	4,44	355,55^{ab}	266,94
$^{a,b.c.d}$…. degree of evidence by Duncan's method with an error of a =0.05					

5.3 Comparative assessment of yield and moisture of the studied maize hybrids with the corresponding checks

The results of the comparative assessment of the maize hybrids with their corresponding checks for 2016, 2017 and 2018 (Table 6) in order to identify differences and similarities between them in terms of moisture content, show that most of the experimental hybrids do not have significant differences with the checks, which was expected given their similar genetic makeup, insofar as the Iodent germplasm was close to 50% in the checks.

Hybrid 1, Hybrid 3, Hybrid 7, Hybrid 8, Hybrid 10, Hybrid 11, Hybrid 12, Hybrid 13, Hybrid 20, Hybrid 24, Hybrid 25, Hybrid 26, Hybrid 27, Hybrid 29, Hybrid 30, Hybrid 31, Hybrid 32, Hybrid 34, and Hybrid 38 are shown to have proven differences with Check 5, for the period of 2018, as it is characterized by the lowest moisture content in the trial, significantly below that of the mentioned hybrids.

In any case, significant differences are found between the hybrids in terms of moisture content at harvest, as also found in other studies (De-Jager et al., 2004; Valchinkov and Valchinkova, 2018b).

In 2017, when conditions were more favourable for the hybrids to show their productive potential compared to 2016 (Table 7) and the effect of their tolerance to drought stress decreased its importance for the formation of the yield, the number of proven yield differences between the checks on the one hand and the experimental maize hybrids on the other increased significantly. This shows that the breeding of maize should be guided by considering the productive potential first and drought resistance only second.

Table 6. Comparative assessment of maize hybrids with checks according to moisture content /%/ by LSD test at significance level α=0.05 for 2016, 2017 and 2018.

Name of Hybrid	Check 5			Check 39			Check 23			Check 33		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
Hybrid 1	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 3	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Hybrid 4	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 6	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Hybrid 7	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 8	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 9	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 10	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 11	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 12	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 13	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 14	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 15	n.s.	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Hybrid 16	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 17	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 18	n.s.	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Hybrid 19	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
Hybrid 20	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Hybrid 21	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 22	*	n.s.	n.s.	n.s.	*	n.s.	*	*	*	n.s.	n.s.	n.s.
Hybrid 24	*	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 25	*	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 26	*	*	*	*	n.s.	n.s.	*	n.s.	*	*	n.s.	n.s.
Hybrid 27	*	*	*	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.
Hybrid 28	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 29	*	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 30	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 31	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Hybrid 32	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 34	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 35	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Hybrid 36	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Hybrid 37	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 38	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 40	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
n.s.- no proven difference compared to the studied Check												
*- there is a proven difference compared to the studied Check												

Table 7. Comparative assessment of maize hybrids with checks according to average yield /kg/daa/ by LSD test at significance level α=0.05 for 2016, 2017 and 2018.

Name of Hybrid	Check 5			Check 39			Check 23			Check 33		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
Hybrid 1	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 2	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 4	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 9	n.s.	n.s.	*	*	*	*	*	*	n.s.	*	*	*
Hybrid 10	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	*	*	n.s.
Hybrid 11	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 12	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 13	n.s.	n.s.	*	n.s.	*	n.s.	n.s.	*	*	*	*	*
Hybrid 14	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 15	n.s.	*	n.s.	*	*	n.s.	n.s.	*	n.s.	*	*	n.s.
Hybrid 16	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	*	*	n.s.
Hybrid 17	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 18	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 19	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 20	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	*	*	n.s.
Hybrid 21	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 22	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*	*	*	n.s.
Hybrid 24	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	*	n.s.	*	n.s.
Hybrid 25	n.s.	*	n.s.	*	*	n.s.	*	*	n.s.	*	*	n.s.
Hybrid 26	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 27	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 28	n.s.	n.s.	*	n.s.	*	*	n.s.	*	n.s.	n.s.	*	*
Hybrid 29	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 30	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 31	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hybrid 32	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Hybrid 34	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	*	*	n.s.
Hybrid 35	n.s.	n.s.	*	*	*	*	n.s.	*	n.s.	*	*	n.s.
Hybrid 36	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Hybrid 37	n.s.	n.s.	*	n.s.	*	*	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 38	n.s.	*	*	n.s.	*	*	n.s.	*	n.s.	n.s.	*	n.s.
Hybrid 40	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	*	n.s.
n.s.- no proven difference compared to the studied Check												
*- there is a proven difference compared to the studied Check												

5.4 Correlation and Path-coefficients and regression equations representing the relationships between maize hybrid forms /y/ and maternal /x1/ and paternal /x2/ lines.

As a result of the conducted correlation analysis and the determined Pearson correlation coefficients, a very strong, positive relationship between the moisture content of the parental forms and the hybrid form was proven for all maize hybrids on average over the three years of the study (table 8). This corroborates the research of Li et al. (2012) who found a strong correlation between the rate of moisture release in the grain between parental lines and their hybrids.

In Hybrid 1, Hybrid 3, Hybrid 6, Hybrid 12, Hybrid 27, Hybrid 28, Hybrid 31, Hybrid 34, Hybrid 35, Hybrid 36, Hybrid 37 and Hybrid 40 there is a stronger direct, positive effect of the maternal line and a correspondingly stronger indirect effect of the paternal line.

In Hybrid 9, Hybrid 12, Hybrid 21, Hybrid 25 and Hybrid 29, the direct effect of parental lines on the moisture release of the hybrids is stronger compared to the indirect effect.

Hybrid 2, Hybrid 4, Hybrid 7, Hybrid 8, Hybrid 10, Hybrid 11, Hybrid 13, Hybrid 14, Hybrid 15, Hybrid 16, Hybrid 17, Hybrid 18, Hybrid 19, Hybrid 20, Hybrid 22, Hybrid 24, Hybrid 26, Hybrid 30, Hybrid 32, and Hybrid 38, demonstrate a more significant, positive direct effect of the paternal line and a stronger positive indirect effect of the maternal line.

The presence of high, statistically proven correlation coefficients between the relevant indicators is a reason for conducting a regression analysis. The constructed linear regression models represent the relationship between the rate of moisture release of the parental lines and hybrids in an analytical form. All models indicated are statistically significant at a confidence level less than or equal to 0.05. The presence of negative coefficients in some of them is determined by the negative effect of the respective parental component on the moisture release of the hybrid. Considering the statistical validity of the regression equations, each of them would be an appropriate tool for making future predictions about the moisture release of the respective hybrid while maintaining the objective conditions affecting the moisture content in maize. Thus, our research confirms the conclusions of Li et al. (2020) about the influence of conditions during the year on moisture content at the time of physiological maturity.

When grain moisture content varies, the direct positive maternal effect has the strongest influence in two of the three years of the study, while the direct positive paternal effect has a weaker influence on their hybrids. This is evidence that in the inheritance of grain moisture content in maize there are also many non-nuclear heritable factors involved.

For all the years of study, there was a much higher, positive correlation between the moisture content of the parent components on the one hand and their hybrids on the other than in most previous studies.

Table 8. Correlation and Path-coefficients and regression equations representing the relationships between hybrid forms /y/ and maternal /x1/ and paternal /x2/ lines for 2016-2018 at a statistical confidence level less than or equal to 0.05/**/

Hybrid	Parental component	Correlation coefficient	Regression equation	Statistical significance of the regression model	Beta-coeff.	Path-coeff.
Hybrid 1	GW59024	0,906**	y=3,970+0,516x1+0,277x2	0,000	0,613	0,293
	GW58008	0,873**			0,330	0,543
Hybrid 2	GW59024	0,819**	y=-1,419+0,324x1+0,833x2	0,000	0,335	0,484
	GW57010	0,894**			0,641	0,253
Hybrid 3	GW59024	0,907**	y=-0,638+0,603x1+0,499x2	0,000	0,575	0,331
	GW57095	0,876**			0,407	0,468
Hybrid 4	GW59024	0,860**	y=1,798+0,352x1+0,578x2	0,000	0,391	0,469
	GW51150	0,883**			0,550	0,333
Hybrid 6	GW59024	0,846**	y=-0,684+0,624x1+0,420x2	0,000	0,569	0,277
	GW58012	0,819**			0,309	0,510
Hybrid 7	GW59024	0,850**	y=2,772+0,389x1+0,489x2	0,000	0,396	0,453
	GW52090	0,865**			0,520	0,346
Hybrid 8	GW58005	0,850**	y=2,772+0,389x1+0,489x2	0,000	0,396	0,453
	GW58008	0,865**			0,520	0,346
Hybrid 9	GW58005	0,822**	y=0,185+0,418x1+0,650x2	0,000	0,448	0,374
	GW57010	0,840**			0,514	0,326
Hybrid 10	GW58005	0,867**	y=-0,086+0,421x1+0,645x2	0,000	0,426	0,442
	GW57095	0,894**			0,554	0,339
Hybrid 11	GW58005	0,845**	y=1,782+0,356x1+0,606x2	0,000	0,392	0,454
	GW51150	0,881**			0,568	0,313
Hybrid 12	GW58005	0,861**	y=-1,126+0,491x1+0,588x2	0,000	0,481	0,380
	GW58012	0,857**			0,461	0,397
Hybrid 13	GW58005	0,830**	y=4,912+0,326x1+0,494x2	0,000	0,346	0,484
	GW52090	0,852**			0,544	0,308
Hybrid 14	GW57091	0,814**	y=2,169+0,195x1+0,631x2	0,000	0,220	0,594
	GW58008	0,860**			0,662	0,197
Hybrid 15	GW57091	0,587*	y=2,542+0,127x1+0,780x2	0,000	0,168	0,419
	GW57010	0,809**			0,709	0,099
Hybrid 16	GW57091	0,743**	y=-0,011+0,256x1+0,778x2	0,000	0,276	0,468
	GW57095	0,858**			0,663	0,195
Hybrid 17	GW57091	0,639*	y=-0,373+0,052x1+0,942x2	0,000	0,055	0,584
	GW51150	0,825**			0,784	0,041
Hybrid 18	GW57091	0,745**	y=-0,488+0,129x1+0,820x2	0,000	0,154	0,591
	GW58012	0,857**			0,732	0,125
Hybrid 19	GW57091	0,728**	y=5,022+0,069x1+0,588x2	0,000	0,087	0,640
	GW52090	0,795**			0,717	0,078
Hybrid 20	GWC06021	0,741**	y=4,825+0,227x1+0,587x2	0,000	0,221	0,520
	GW58008	0,811**			0,628	0,183

Hybrid	Parental component	Correlation coefficient	Regression equation	Statistical significance of the regression model	Beta-coeff.	Path-coeff.
Hybrid 21	GWC06021	0,773**	$y=-3,773+0,498x_1+0,767x_2$	0,000	0,455	0,318
	GW57010	0,824**			0,571	0,253
Hybrid 22	GWC06021	0,777**	$y=2,337+0,376x_1+0,652x_2$	0,000	0,382	0,395
	GW57095	0,863 **			0,620	0,243
Hybrid 24	GWC06021	0,825**	$y=1,544+0,305x_1+0,713x_2$	0,000	0,304	0,521
	GW51150	0,904**			0,667	0,237
Hybrid 25	GWC06021	0,784**	$y=3,678+0,385x_1+0,485x_2$	0,000	0,414	0,370
	GW58012	0,793**			0,460	0,333
Hybrid 26	GWC06021	0,829**	$y=5,634+0,104x_1+0,739x_2$	0,000	0,101	0,728
	GW52090	0,906**			0,817	0,089
Hybrid 27	GW58268	0,827**	$y=4,505+0,547x_1+0,288x_2$	0,000	0,567	0,260
	GW58008	0,783**			0,315	0,468
Hybrid 28	GW58268	0,842**	$y=0,946+0,449x_1+0,556x_2$	0,000	0,470	0,372
	GW57010	0,840**			0,454	0,386
Hybrid 29	GW58268	0,853**	$y=3,663+0,414x_1+0,506x_2$	0,000	0,451	0,402
	GW57095	0,861**			0,495	0,366
Hybrid 30	GW58268	0,846**	$y=1,434+0,315x_1+0,684x_2$	0,000	0,317	0,529
	GW51150	0,890**			0,620	0,270
Hybrid 31	GW58268	0,852**	$y=2,084+0,558x_1+0,401x_2$	0,000	0,555	0,297
	GW58012	0,826**			0,337	0,489
Hybrid 32	GW58268	0,803**	$y=6,057+0,220x_1+0,527x_2$	0,000	0,245	0,558
	GW52090	0,858**			0,646	0,212
Hybrid 34	GW59008	0,898**	$y=-2,100+0,751x_1+0,365x_2$	0,000	0,621	0,277
	GW58008	0,833**			0,376	0,457
Hybrid 35	GW59008	0,887**	$y=-0,708+0,545x_1+0,476x_2$	0,000	0,518	0,369
	GW57010	0,876**			0,415	0,461
Hybrid 36	GW59008	0,896**	$y=-2,527+0,650x_1+0,533x_2$	0,000	0,533	0,363
	GW57095	0,881**			0,462	0,419
Hybrid 37	GW59008	0,912**	$y=-2,987+0,637x_1+0,551x_2$	0,000	0,511	0,401
	GW51150	0,906**			0,469	0,437
Hybrid 38	GW59008	0,783**	$y=1,752+0,200x_1+0,731x_2$	0,000	0,194	0,589
	GW58012	0,869**			0,708	0,161
Hybrid 40	GW59008	0,888**	$y=0,175+0,587x_1+0,406x_2$	0,000	0,519	0,369
	GW52090	0,877**			0,463	0,414

5.5 Tracking the trends in the rate of moisture release in the studied maize hybrids

The trend lines representing the change in moisture content over time are located almost parallel throughout the study period, indicating practically equal rates of moisture release from the grain. The differences in the starting moisture content (first sampling) reach up to nearly 25%.

Here it is appropriate to point out that the starting moisture content is obviously determined, in the first place, by the duration of the vegetation period of given genotypes and by the moisture content

of their grain at the time of reaching physiological maturity. Thus, our work corroborates the data from some studies that during the onset of physiological maturity, different genotypes can have different moisture content in the grain (Aldrich, 1986; Nielsen, 2013; Ghete et al., 2016; Derillo, 1979). As stated above, only maize lines from the mid-early group with a close vegetation period were included in the study to minimize the influence of the duration of the vegetation period in the study on grain moisture content at each sampling time for the included maize forms. To the extent that the differences in the duration of the vegetation period of the hybrid combinations in particular are small, up to 2-5 days, it can be assumed that the differences in their initial moisture content are largely due to differences in the moisture content of the grain when they reach physiological maturity. This predetermines that the moisture content of the grain at harvest will depend on the moisture content of the grain at the time of reaching physiological maturity of a given genotype, on the one hand, and its rate of grain drying in the period thereafter, on the other.

Figure 3 traces the trend in the rate of moisture release for maize Hybrids 1, 2, 3, 4, 6 and Check 5 over the period of 2016. The differences in starting moisture content (first sampling) reach up to nearly 5% i.e. to more than two hundreds according to FAO. At the beginning of the moisture recording period, Hybrid 3 stands out as having the highest moisture content, while Hybrid 1 has relatively the slowest drying rates. In Check 5, there is a retention of moisture release that can be observed at the end of reporting period. The values of the coefficients of determination /R2/ prove that the percentage of grain moisture content between 91% and 98% depends on the number of days between the initial and the specific sampling, as expected.

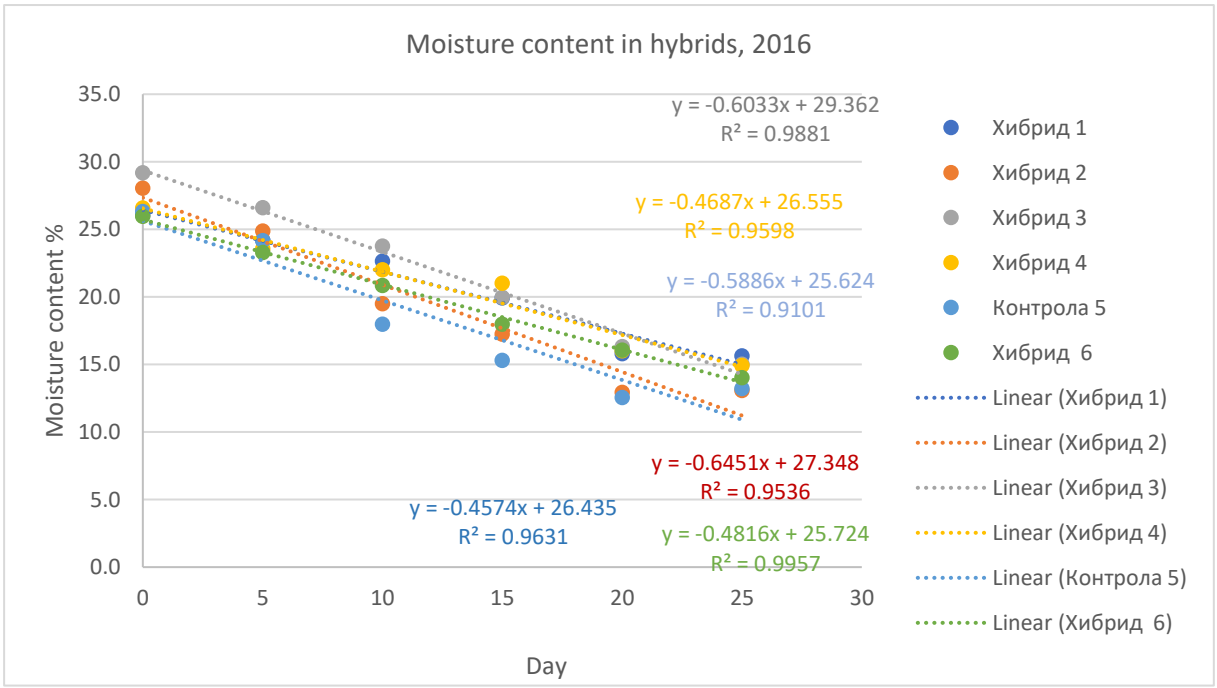


Figure 3. Moisture release in variants 1 to 6 in the 2016 trial

In 2017, among the aforementioned hybrids (1, 2, 3, 4, 6 and Check 5), Hybrid 4 exhibited slower moisture release throughout the 25-day study period (Figure 4). Check 5 stands out with faster moisture release at the beginning of the reporting period and moisture retention at the end of the period, as observed in the previous year 2016. In the other maize hybrids, the trend of moisture release does not differ much.

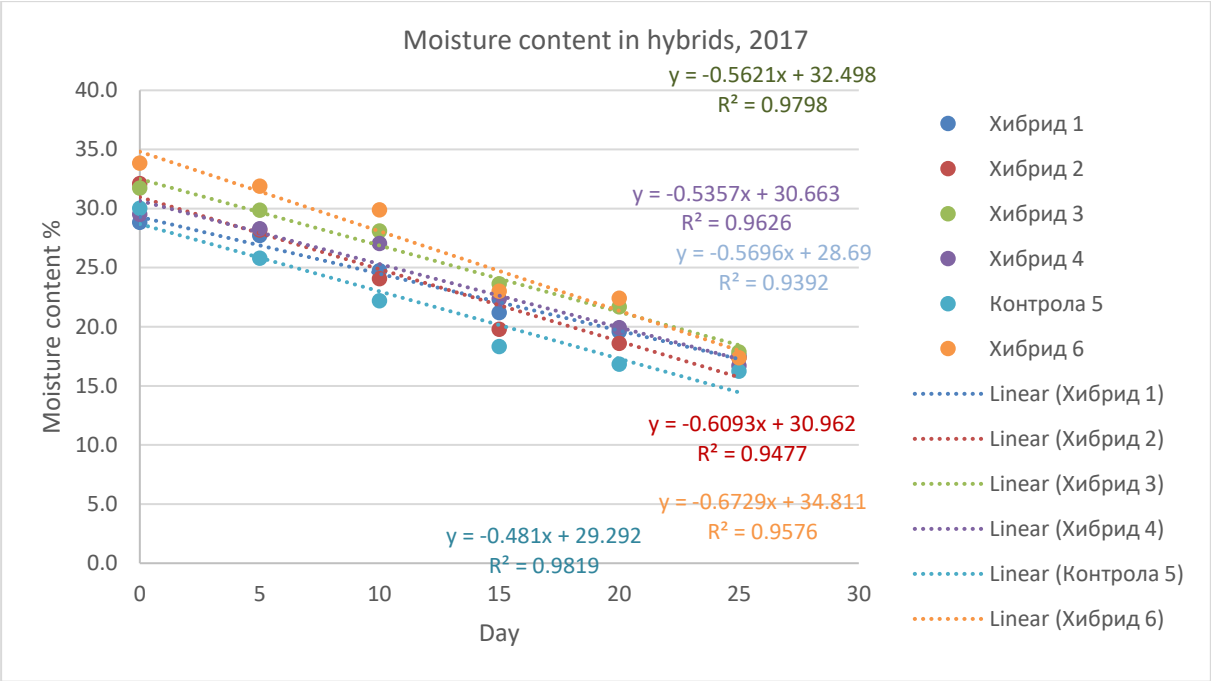


Figure 4. Moisture release in variants 1 to 6 in the 2017 trial

Figure 5 shows the regression equations for changes in moisture recorded in the studied Hybrids 1, 2, 3, 4, 6 and Check 5 in 2018. Check 5 has the lowest moisture content over the entire 25-day study period, but the trend line has a minimal slope, demonstrating the slowest rate of moisture release.

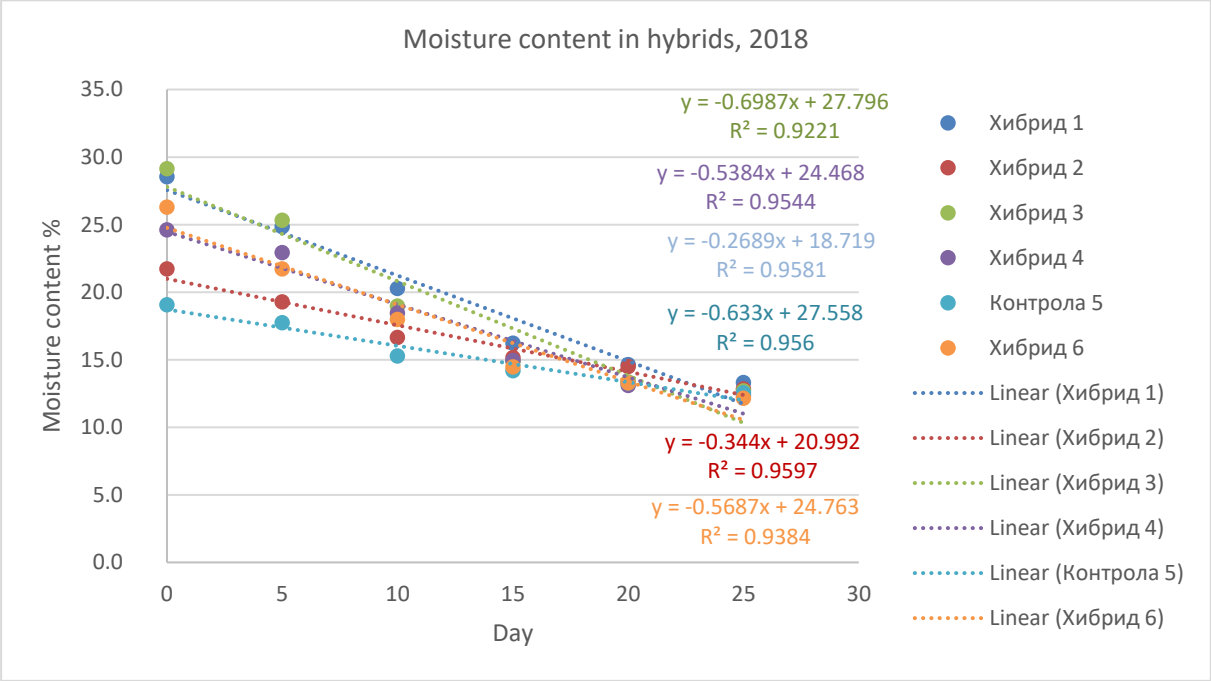


Figure 5. Moisture release in variants 1 to 6 in the 2018 trial

The next group of hybrids is represented by Hybrids 7, 8, 9, 10, 11, and 12. Among them, Hybrid 8, which has the lowest regression coefficient and, at the end of the reporting period, has the highest moisture content - about 17% (Figure 6), is characterized by the lowest rate of moisture release in 2016. The fastest moisture release is observed in Hybrid 12. The differences in the starting moisture content at the first sampling are smaller than the respective differences in the hybrids of the first group.

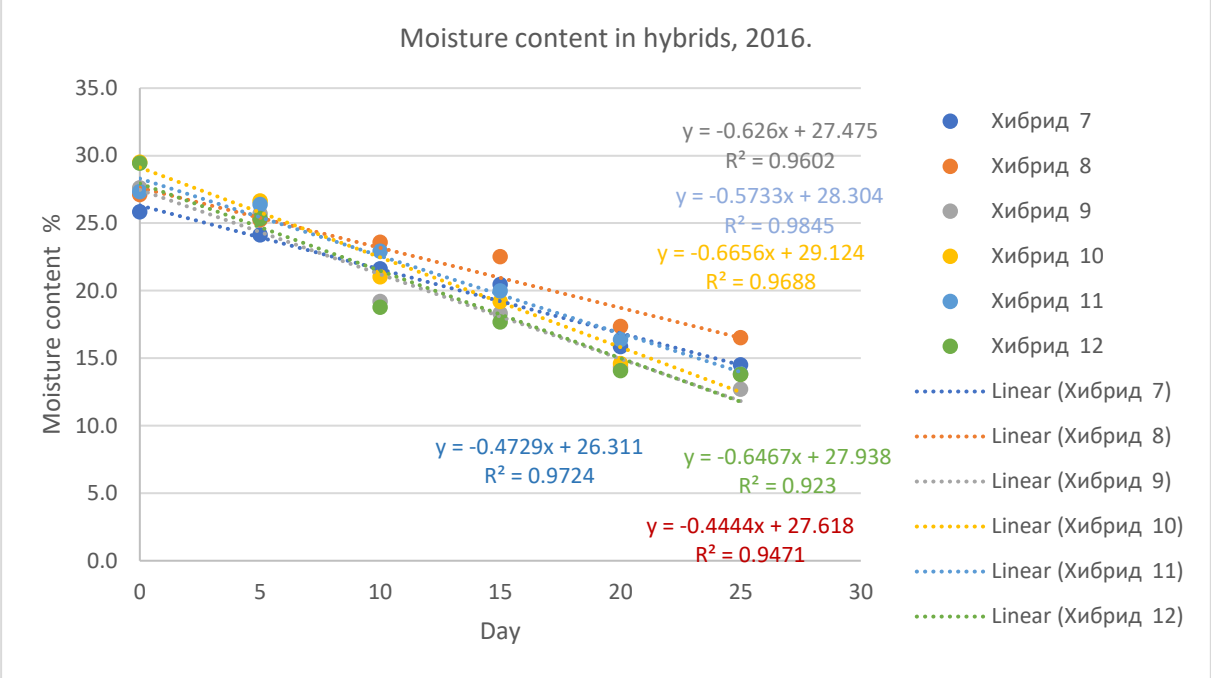


Figure 6. Moisture release in variants 7 to 12 in the 2016 trial

It can be seen from Figure 7 that the trend of moisture release of maize Hybrids 7, 8, 9, 10, 11 and 12 is practically the same for the studied year - 2017. The trend lines visualising the change in moisture content over time are located almost parallel. Hybrid 12 is demonstrated to have the highest moisture content at the beginning of the period and Hybrid 10 has a higher moisture content at the end of the 25-day period. There are differences between the moisture contents of the hybrids studied at the time of the first sampling, although they are less than in the first group of hybrids.

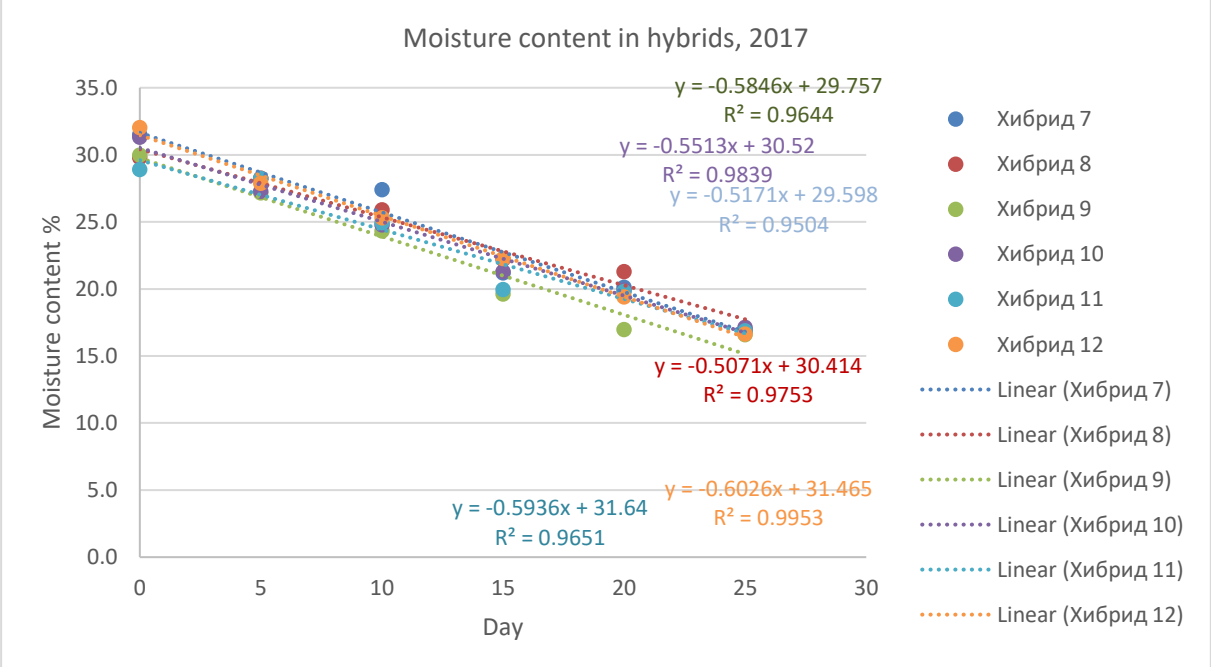


Figure 7. Moisture release in variants 7 to 12 in the 2017 trial

Figure 8 shows the trend of the moisture release rate of the second group of hybrids (Hybrids 7, 8, 9, 10, 11 and 12) for 2018. At the beginning of the moisture reporting period, Hybrid 8 stands out with the highest moisture content, as was also reported in 2016. The values of the coefficients of determination prove that between 90% and 97% of the moisture content of the grain is determined by the duration of the period after the date of the first sampling. In all hybrids of this group, there is a clear tendency towards a slowing down of moisture release (the regression ceases to be linear) at moisture levels below 15%.

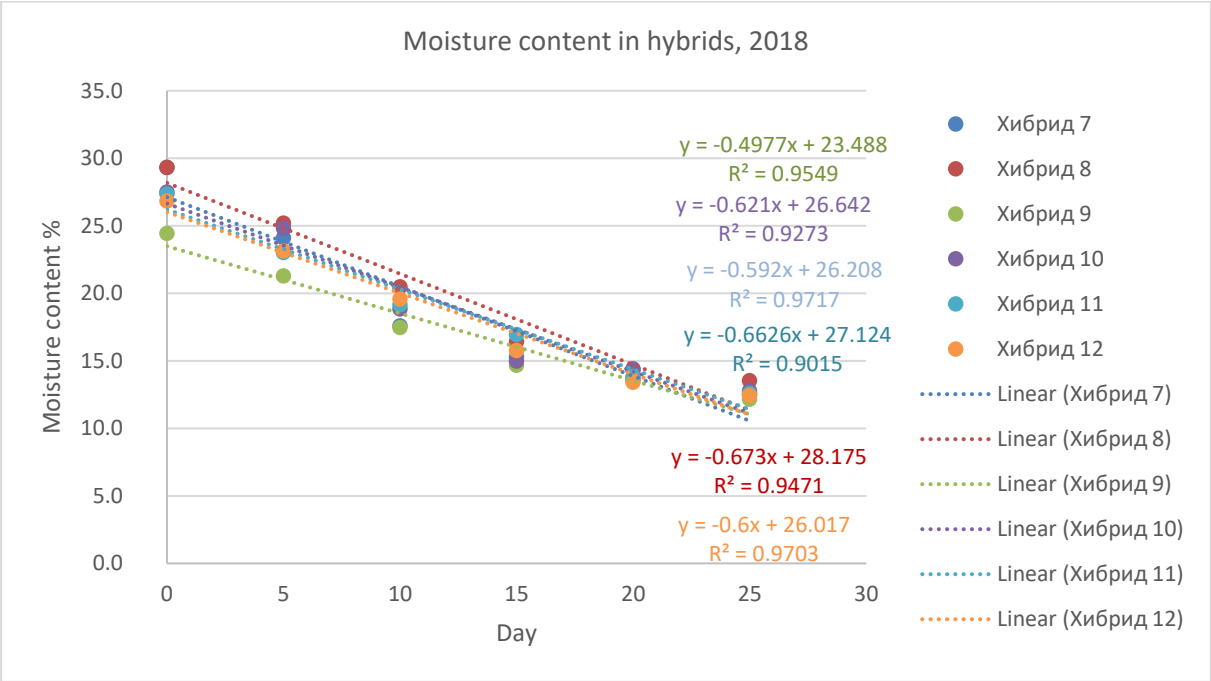


Figure 8. Moisture release in variants 7 to 12 in the 2018 trial

Figure 9 shows the trend lines for maize Hybrids 13, 14, 15, 16, 17 and 18 for 2016. Hybrid 13 is characterized by the lowest rate of moisture release for the studied period. On the other hand, Hybrid 18 and Hybrid 15 prove to have the fastest moisture release. Hybrids of this group differ significantly in terms of moisture content at the first sampling. Between 83% and 96% of the moisture values of the studied hybrids are determined by the duration of the period after the first sampling.

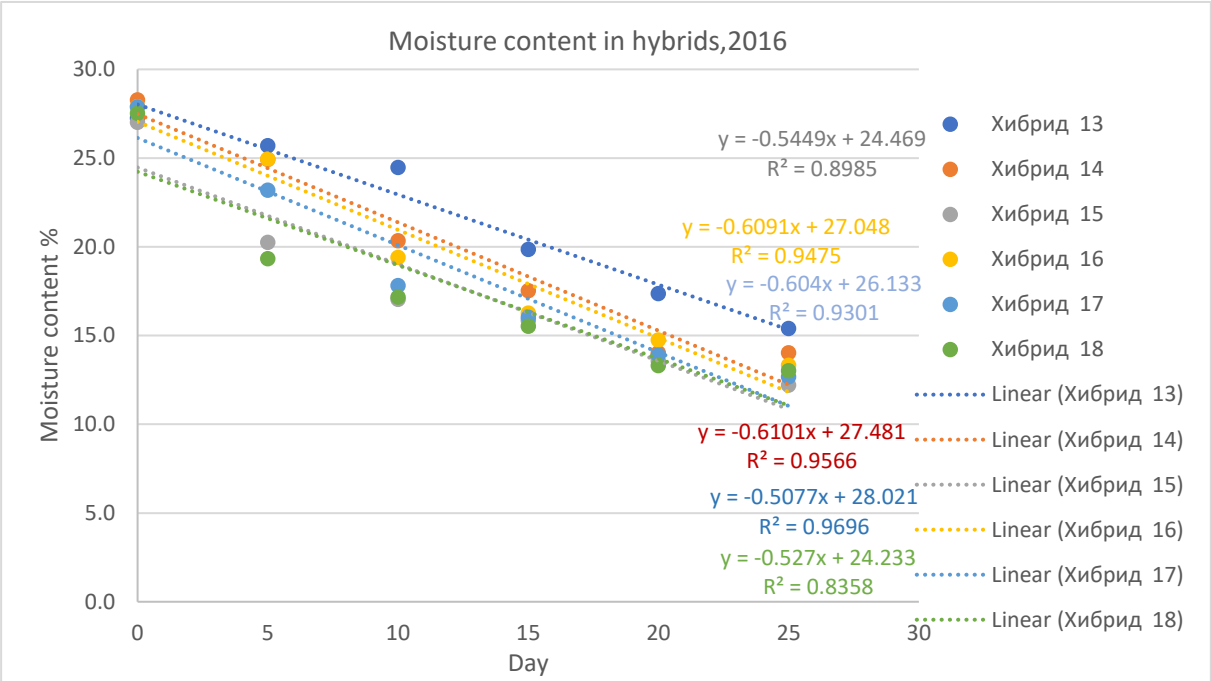


Figure 9. Moisture release in variants 13 to 18 in the 2016 trial

Figure 10 presents the regression equations for changes in moisture recorded in the studied Hybrids 13, 14, 15, 16, 17 and 18 for 2017. In Hybrid 16, 99% of the decrease in moisture content is due to the duration of the period after the first sampling. Hybrid 17 is characterized by the fastest rate of moisture release, which is maximum at the beginning of the period and minimum at the end of the period, compared to the other studied hybrids.

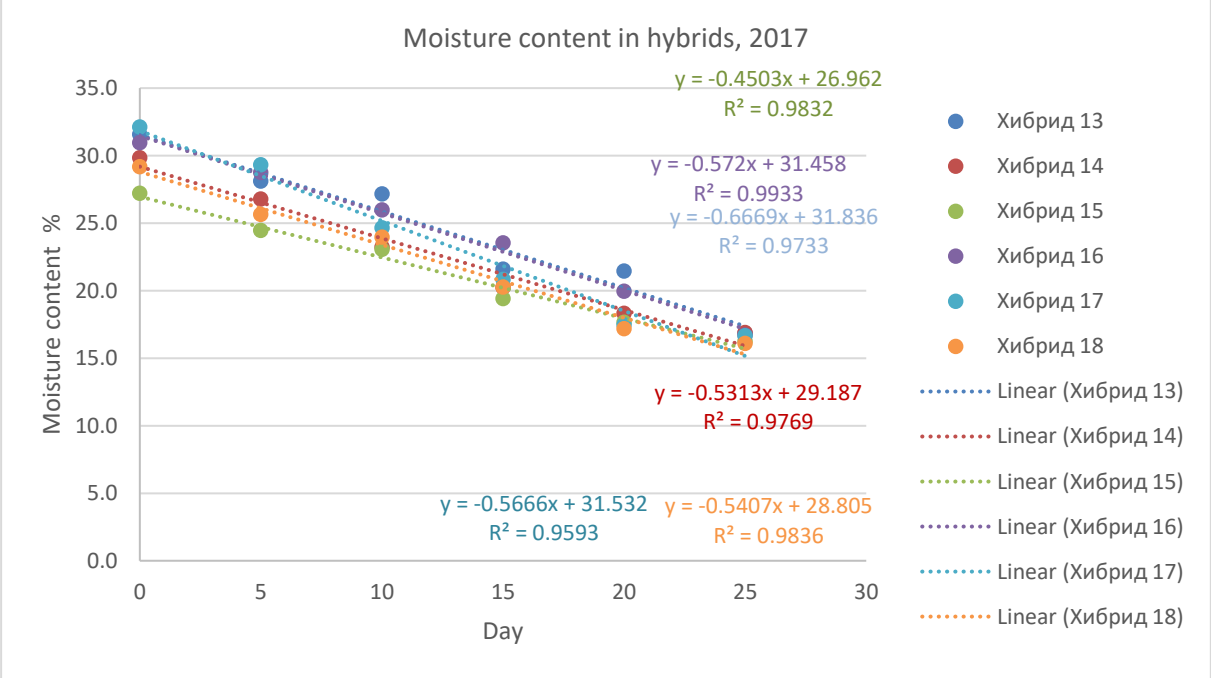


Figure 10. Moisture release in variants 13 to 18 in the 2017 trial

Figure 11 shows the regression equations for changes in moisture recorded in the studied Hybrids 13, 14, 15, 16, 17 and 18 in 2018. In Hybrid 15, 97% of the change in grain moisture content is due to

the duration of the period after the first sampling. Hybrid 13 exhibits the fastest rate of moisture release and Hybrid 15 the slowest.

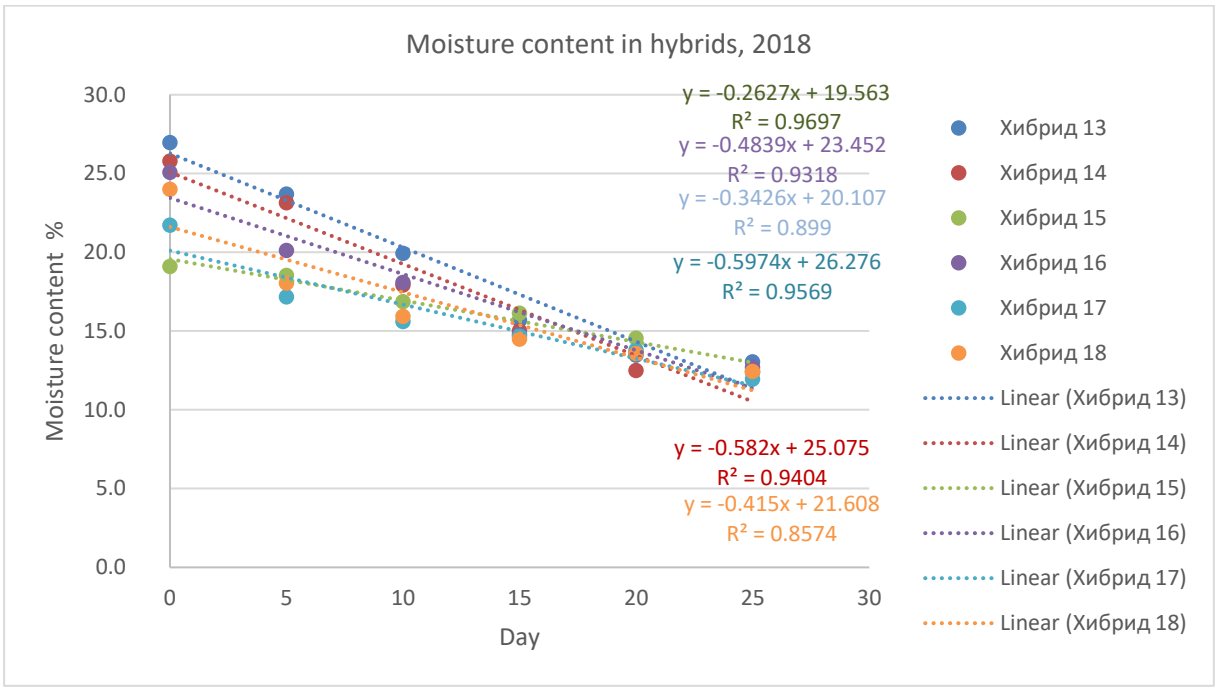


Figure 11. Moisture release in variants 13 to 18 in the 2018 trial

Figure 12 shows the regression equations for the change in moisture recorded in the studied Hybrids 19, 20, 21, 22, 24 and Check 23 for 2016. Hybrid 21 is proven to have the fastest rate of moisture release, which is maximum at the beginning of the period and minimum at the end of the period, compared to the other studied maize forms studied. Given the minimum slope of the trend line characterizing the rate of moisture release of Hybrid 20, this should be considered the hybrid with the lowest moisture release rate. Again, significant differences in moisture content are observed at the time of the first sampling.

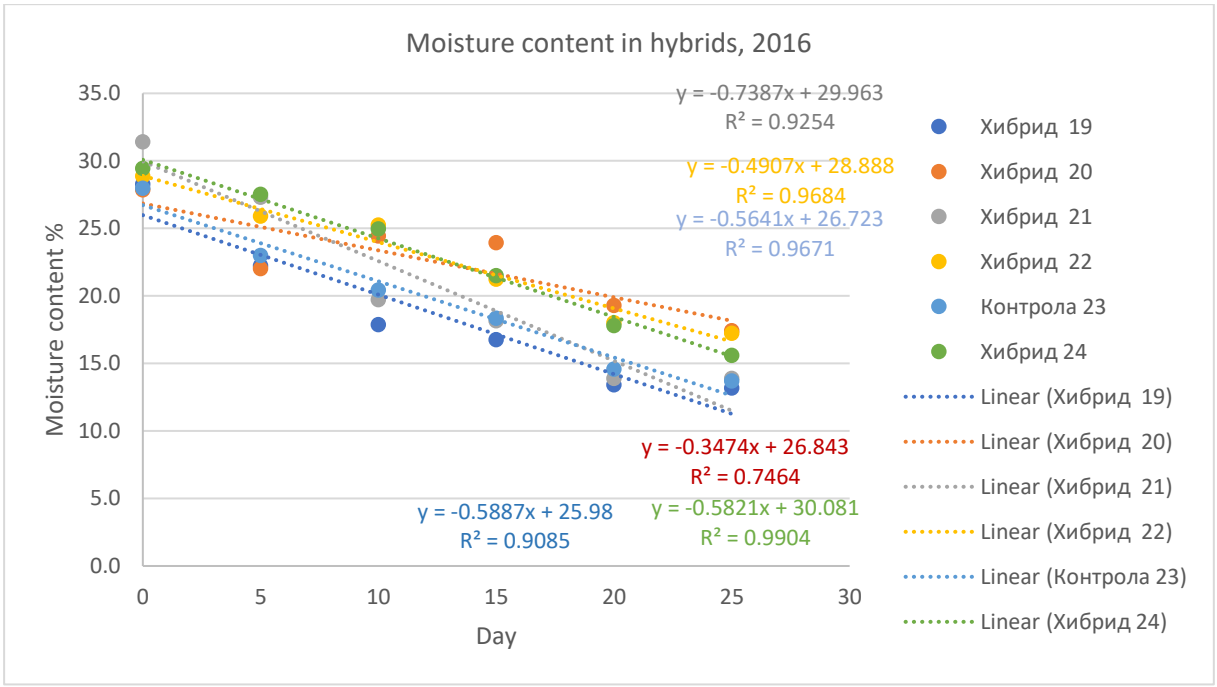


Figure 12. Moisture release in variants 19 to 24 in the 2016 trial

Figure 13 shows the regression equations for the change in moisture content recorded in the studied Hybrids 19, 20, 21, 22, 24 and Check 23 for 2017. This graph, as well as the previous ones, including in 2016, show that the rate of moisture release from the grain does not depend on its moisture content at the time it reaches physiological maturity, but that these are two completely separate indicators. Hybrid 22 stands out as having the highest moisture content at the beginning of the sampling period and also has the highest moisture content at the end of the 25-day period.

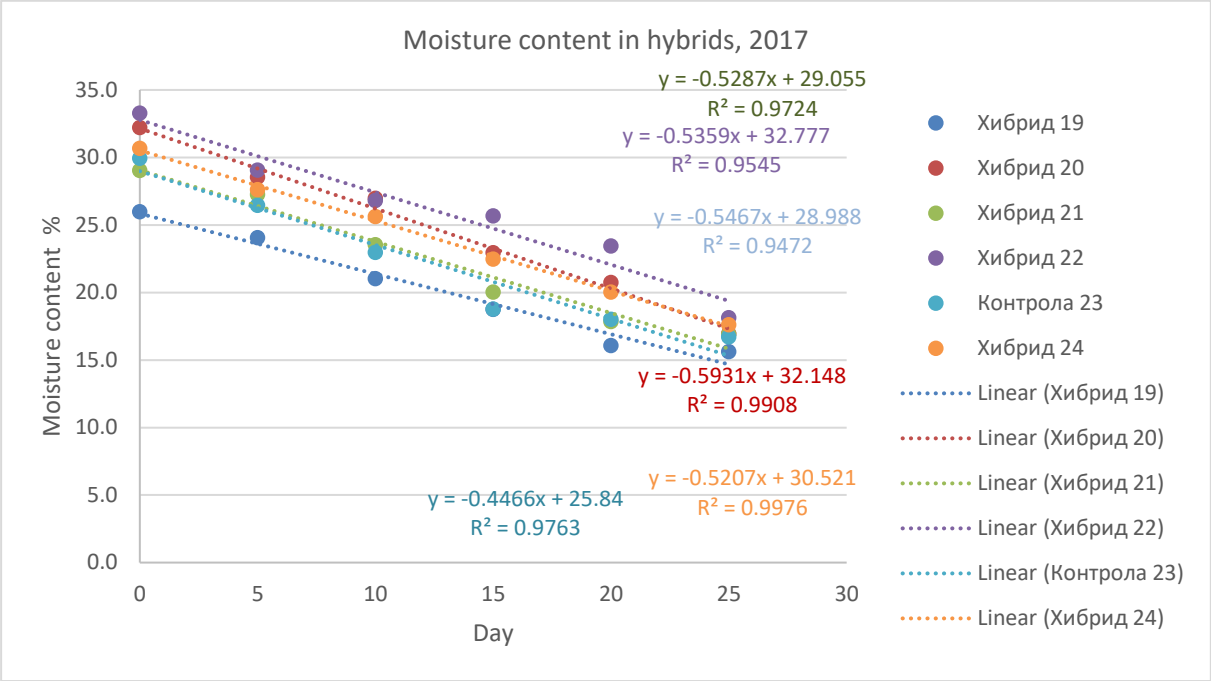


Figure 13. Moisture release in variants 19 to 24 in the 2017 trial

It can be seen from Figure 14 that the trend of moisture release of the studied Hybrids 19, 20, 21, 22, 24 and Check 23 is almost the same. At the beginning of the period, Hybrid 20 is characterized by the highest moisture content, and at the end of the 25-day period, the moisture content of Hybrid 22 is higher, with Hybrid 20 exhibiting a relatively higher rate of moisture release. The starting values of grain moisture content at the first sampling have a very large amplitude - over 8%. Trait values are most unstable in Hybrid 19. This hybrid severely slows the rate of moisture release already at trait values of about 20%.

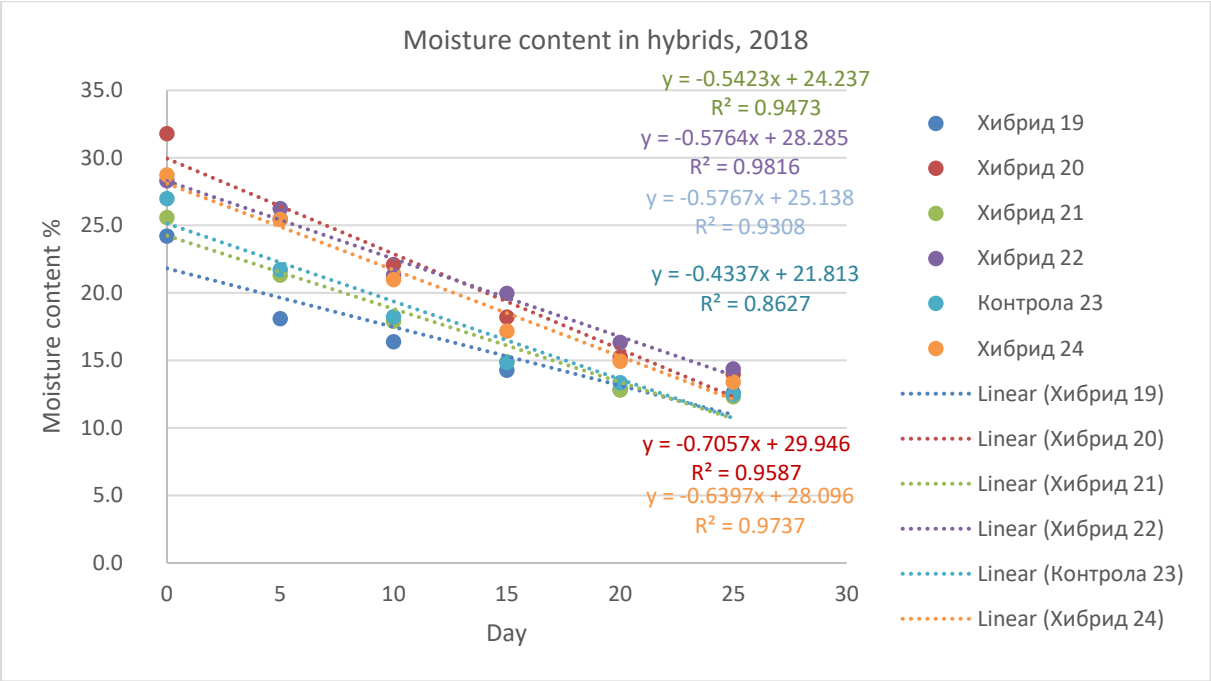


Figure 14. Moisture release in variants 19 to 24 in the 2018 trial

It can be seen from Figure 15 that the trend of moisture release of the hybrids studied in 2016 (Hybrids 25, 26, 27, 28, 29 and 30) is similar. The differences between the studied hybrids in terms of moisture content at first sampling are again significant, which confirms the results of the work showing that different forms reach physiological maturity at different grain moisture contents (Cross, 1985).

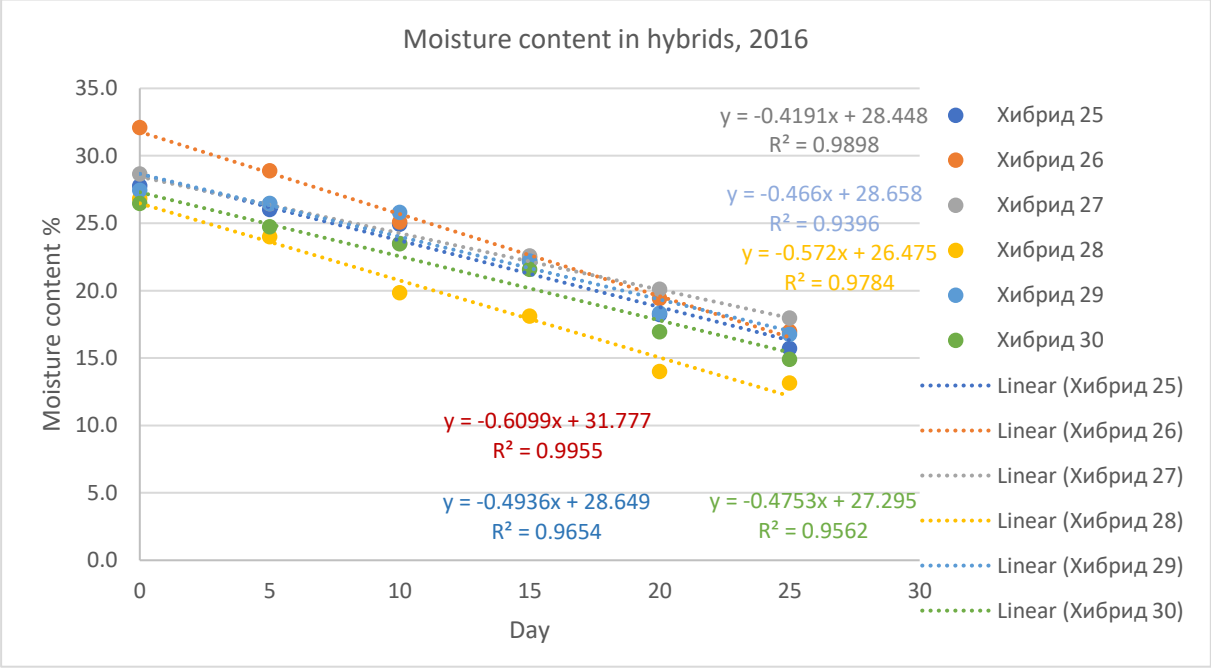


Figure 15. Moisture release in variants 25 to 30 in the 2016 trial

Figure 16 traces the trend of moisture release rate in Hybrids 25, 26, 27, 28, 29 and 30 for 2017. At the beginning of the moisture reporting period Hybrid 27 is demonstrated to have the highest moisture content and Hybrid 25 - the lowest. The values of the coefficients of determination /R^2/ prove that

between 94% and 99%, the rate of moisture release is determined by the duration of the period after the first sampling.

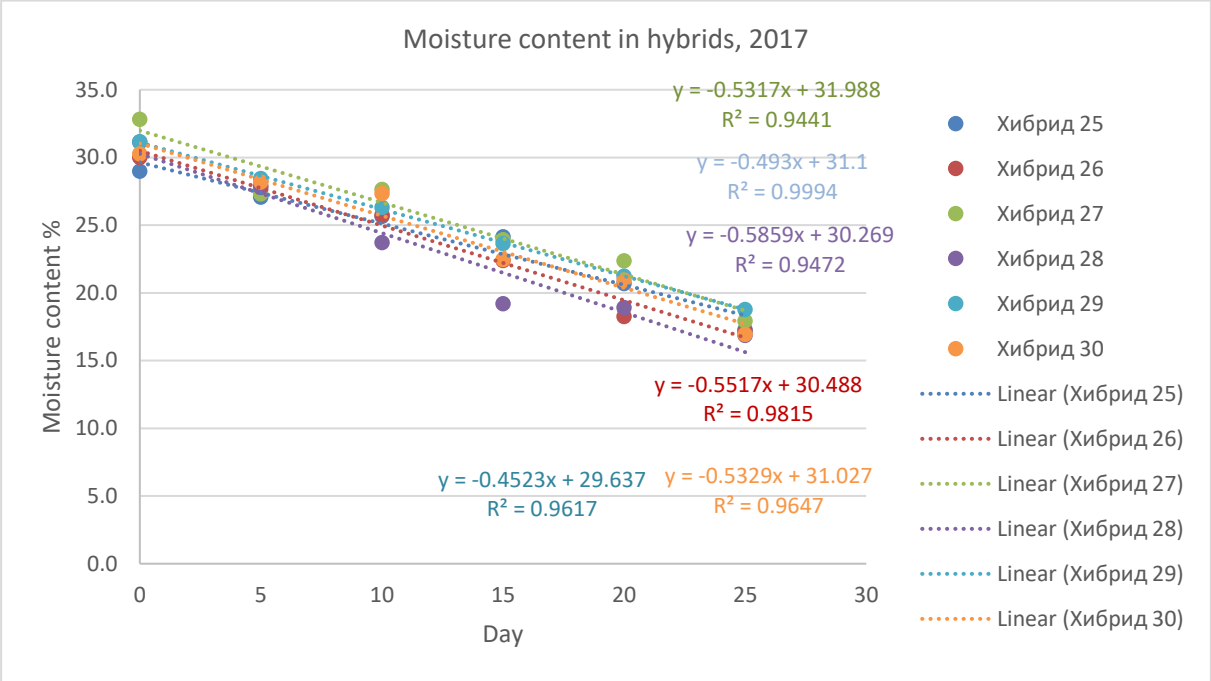


Figure 16. Moisture release in variants 25 to 30 in the 2017 trial

In 2018, in the group of studied Hybrids 25, 26, 27, 28, 29 and 30, the slowest moisture release is observed in Hybrid 28, as its trend line has a minimum slope (Figure 17). The remaining hybrids have approximately the same linear rate of moisture release for the studied period, despite the relatively wide amplitude of starting moisture content during the first sampling.

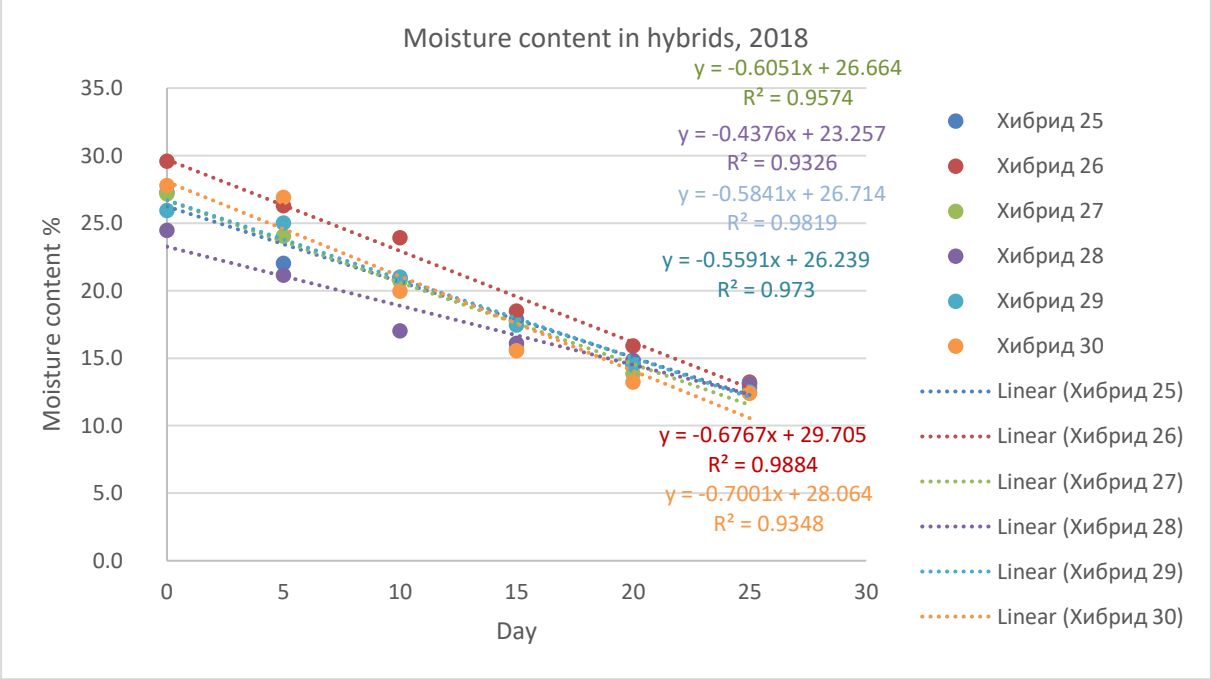


Figure 17. Moisture release in variants 25 to 30 in the 2018 trial

Figure 18 shows the regression equations for changes in moisture recorded in the studied maize Hybrids 31, 32, 34, 35, 36 and Check 33 for 2016. At the beginning of the period, Hybrid 36 stands out with the highest moisture content, and at the end of the 25-day period, Hybrid 32 has a higher moisture content.

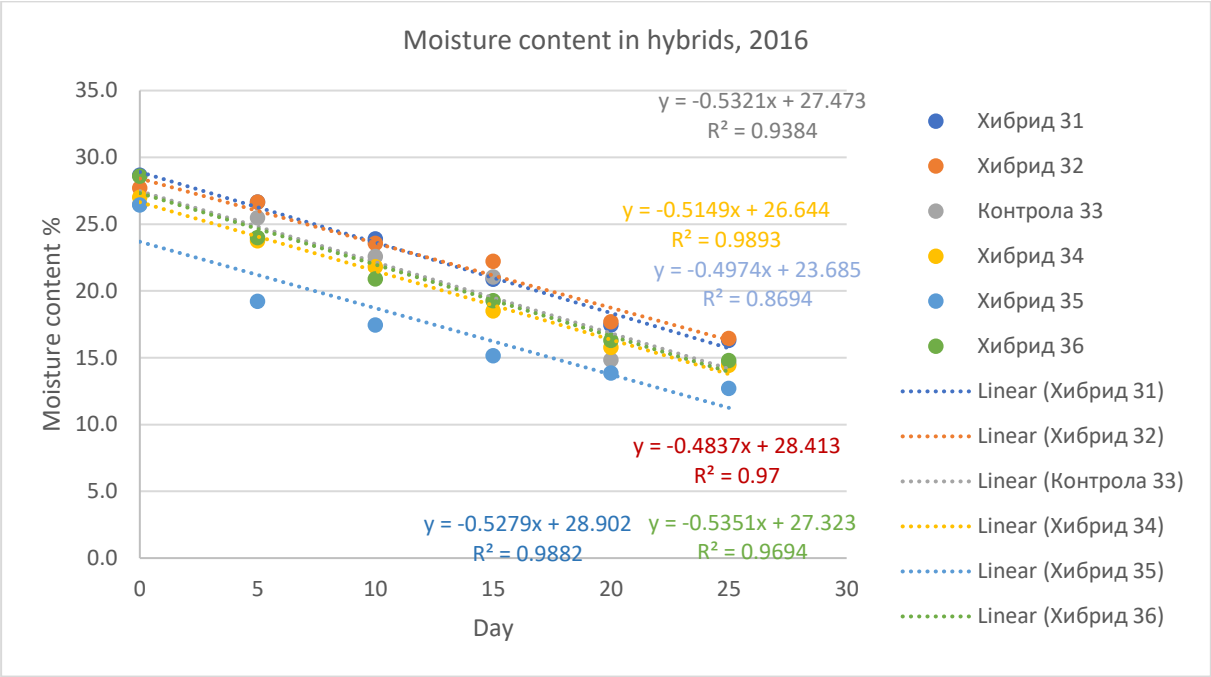


Figure 18. Moisture release in variants 31 to 36 in the 2016 trial

Figure 19 shows the regression equations for changes in moisture recorded in the studied Hybrids 31, 32, 34, 35, 36 and Check 33 in 2017. In Hybrid 34, 99% of the decrease in moisture content is found to be due to the duration of the period after the first sampling. Hybrid 35 is proven to have the fastest rate of moisture release, which is maximum at the beginning of the period and minimum at the end of the period, compared to the other studied hybrids.

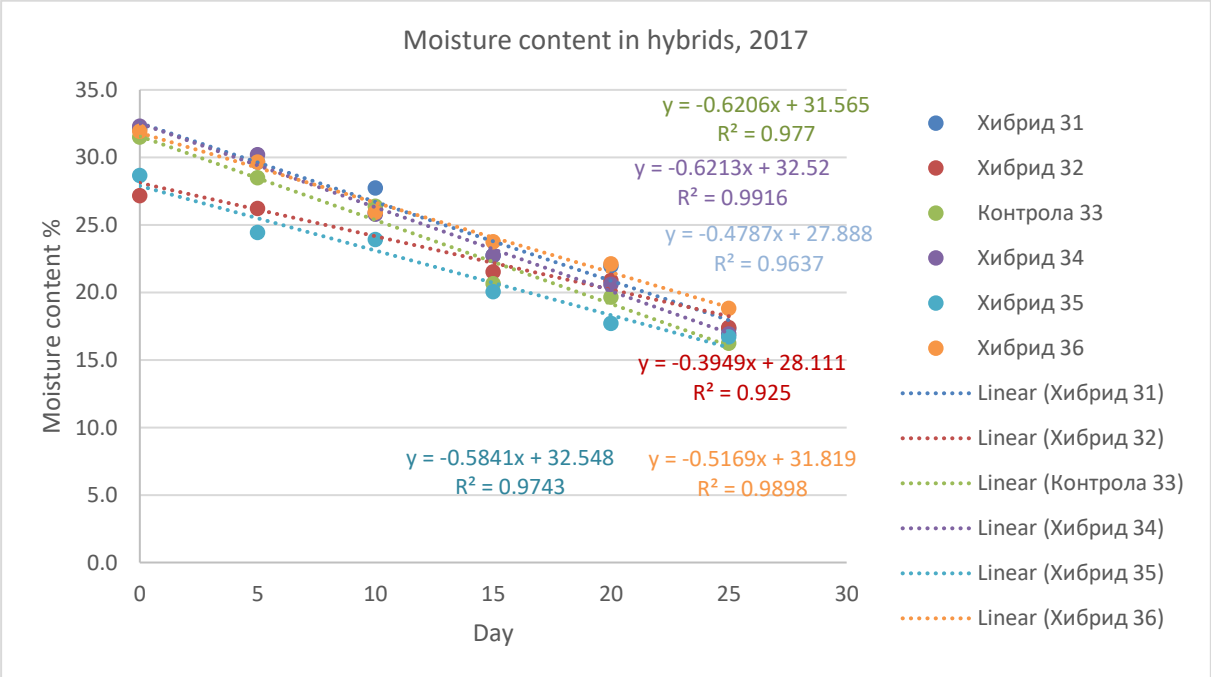


Figure 19. Moisture release in variants 31 to 36 in the 2017 trial

Figure 20 shows the regression equations and lines for changes in moisture recorded in the studied variants from 31 to 36 in 2018. In addition, hybrid 31 begins to slow the rate of moisture release from the grain much earlier than the other hybrids in the group, which also contributes to its higher grain moisture content at harvest.

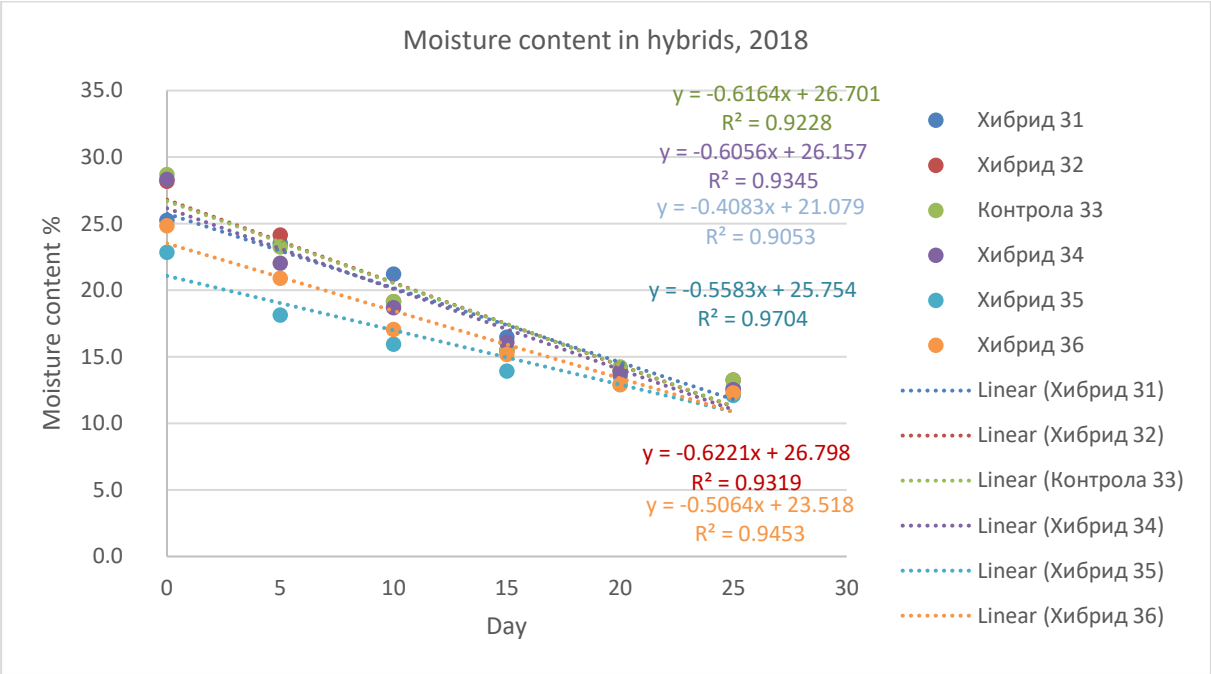


Figure 20. Moisture release in variants 31 to 36 in the 2018 trial

It can be seen from Figure 21 that Hybrid 38 exhibits a fast rate of moisture release at the beginning, which has a slight retention at the end of the reporting period (for 2016), occurring well before that of the other hybrids in the group. In the remaining Hybrids 37, 40 and Check 39, the trend of the moisture release does not differ much. The starting moisture measured varies within a narrower range.

The average daily rate of moisture release in grain is lower than that found in most studies for the USA and Bulgaria. Our research shows an average grain drying rate per day close to that found by Nielsen R. L. (Nielsen, 2012) of about 0.5% per day.

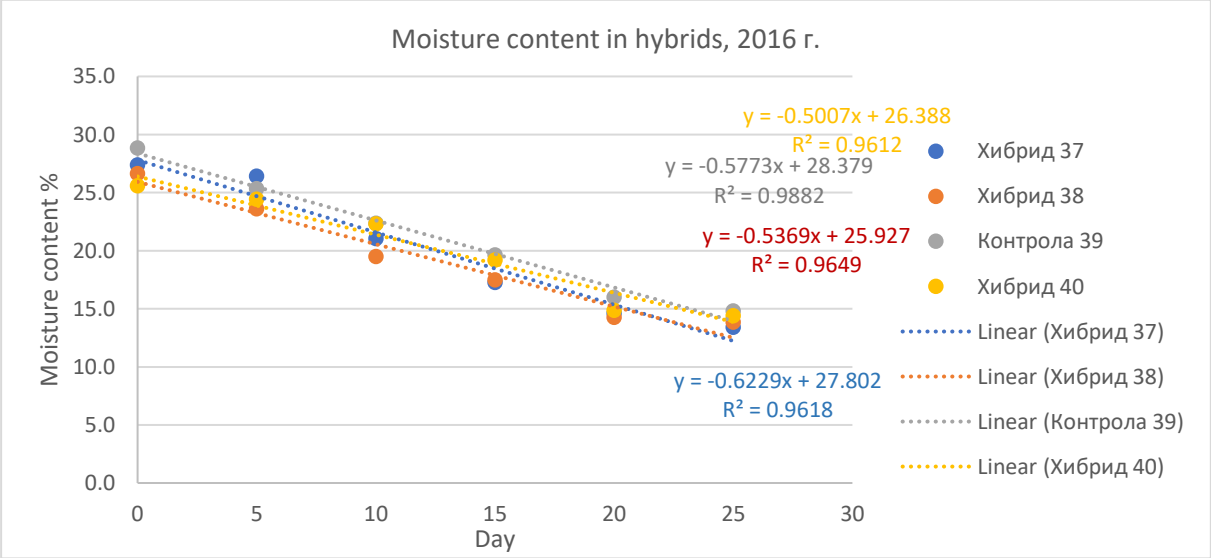


Figure 21 Moisture release in variants 37 to 40 in the 2016 trial

It can be seen from Figure 22 that Hybrid 40 is proven to have a fast rate of moisture release rate for 2017. This variant exhibits high grain moisture content at the beginning of the study period and minimal moisture content at the end. Hybrid 38 is characterized by slight moisture retention after the 15th day even when reaching trait levels of around 20%. For the other hybrids and Check 39, the trend of moisture release is not very different, although the maize hybrids stand out within 6% in terms of moisture content at the first sampling and that corresponds to almost 300 FAO units, which in turn does not correspond to their vegetation period, which is quite close - within 120-150 FAO units.

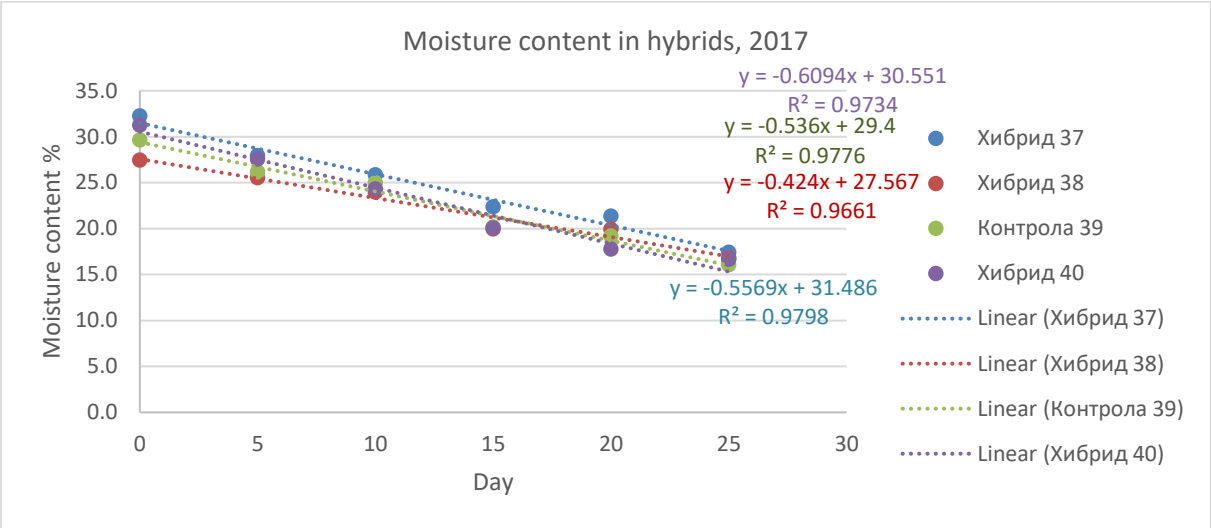


Figure 22. Moisture release in variants 37 to 40 in the 2017 trial

It can be seen from Figure 23 that Hybrid 37 exhibits a fast rate of moisture release, which has little retention at the end of the reporting period (in 2018). In the remaining Hybrid 38, 39, 40 and Check 39, the trend of moisture release does not differ much. Throughout the entire 25-day reporting period, the maximum amount of moisture is found in Hybrid 38, which also has the highest starting moisture content at the first sampling.

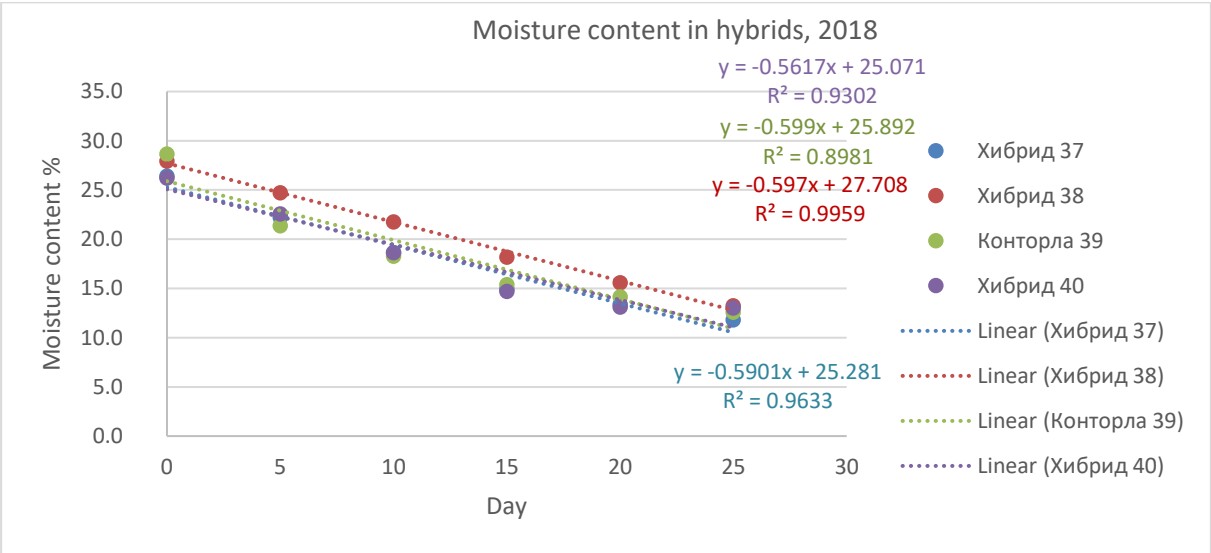


Figure 23. Moisture release in variants 37 to 40 in the 2018 trial

5.6 Tracking trends in the rate of moisture release in the parental lines of the studied maize hybrids

The trend of decreasing moisture in all lines is expected given the environmental conditions. The high degree of accuracy, respectively the high value of the coefficient of determination proves that the compiled mathematical models could serve for the fairly accurate prediction of the grain moisture content of a given line under conditions close to the objective ones, i.e. to the conditions under which the experimental values were obtained. Between the lines, there are very different rates of water release from the grain, something that has been established by other researchers (Petrov, 1995).

As expected, in all regression equations the regression coefficient in front of the independent variable /x/ has been found to have a negative sign. This is due to the negative effect of the duration of the period after the date of the first sampling on the moisture content /%/ in the grain.

In 2016, the maternal lines (Figure 24) demonstrate slower moisture release in line GWC06021 throughout the 25-day study period. Line GW57091 is characterized by faster moisture release at the beginning of the reporting period and moisture retention at the end of the period. In the other inbred lines, the trend of moisture release does not differ much. Within the range of 91% to 98%, the rate of moisture release is determined by the duration of the period after the first sampling, with the greatest dependence in line GWC06021.

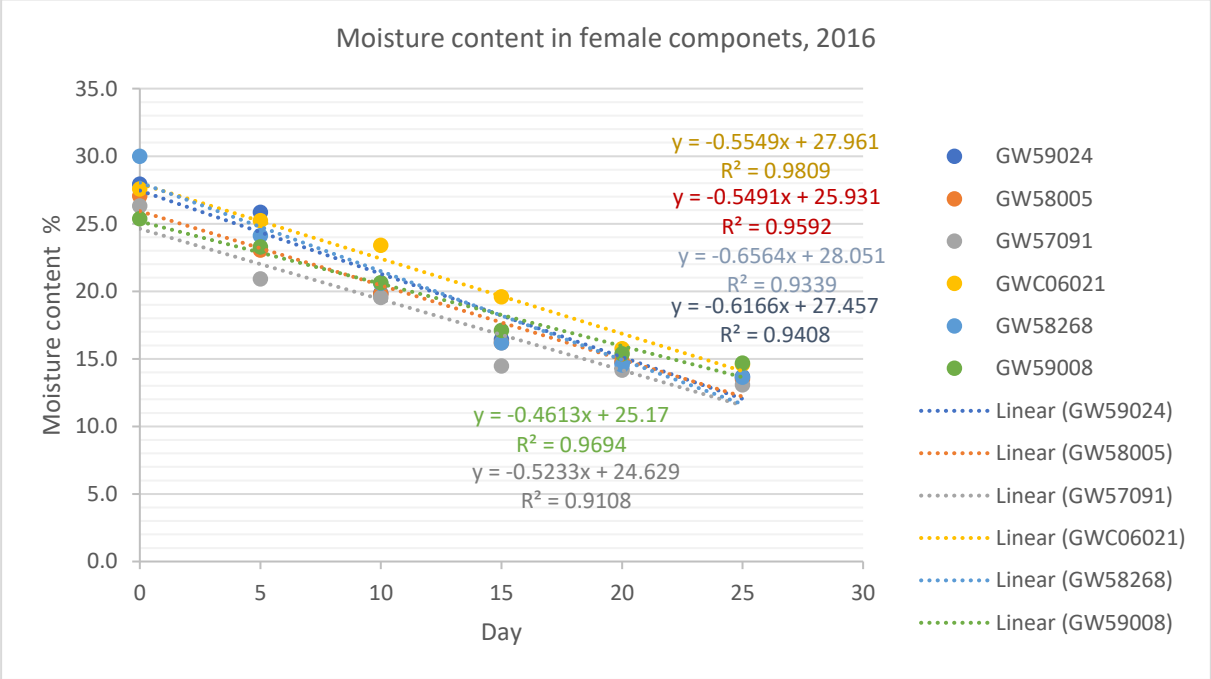


Figure 24. Moisture release in maternal lines in 2016

In Figure 25 it can be seen that the rate of moisture release of the studied parent components is practically the same, although the differences in the starting values of the trait at the first sampling are quite different. The trend lines visualising the change in moisture content over time are located almost parallel, as in 2016. At the beginning of the period line GW58268 stands out with the highest moisture content, and at the end of the 25-day period GW57091 has a higher moisture content. The values of the coefficients of determination prove that between 96% and 98% the specific moisture content of the grain is determined by the duration of the period after the first sampling.

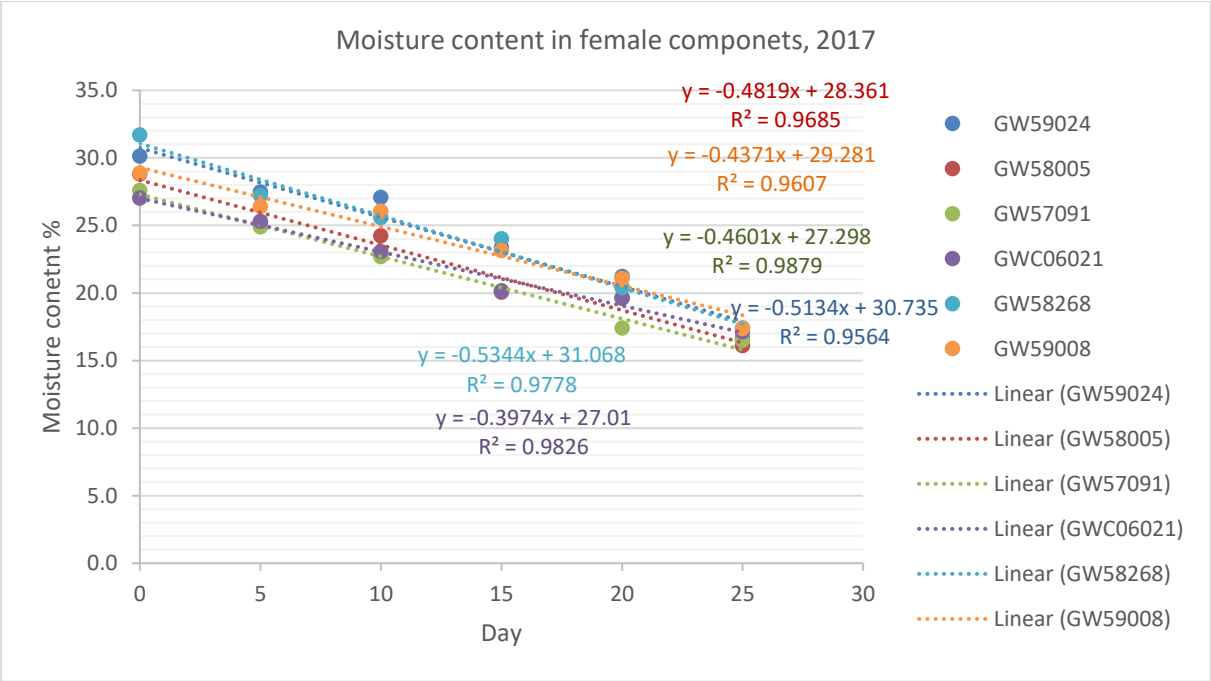


Figure 25. Moisture release in maternal forms in 2017

Figure 26 shows the change in moisture content in 2018 for the maternal components over 25 days. The trend of decreasing moisture between the lines has much more variable regressions compared to their hybrids.

Line GW57091 is proven to have the fastest rate of moisture release, which is maximum at the beginning of the period and minimum at the end of the period, compared to the other studied lines. The lowest moisture release rate is observed in line GW59008, which shows the lowest moisture content in the grain at the beginning of the sampling period and above average at the end. This is the line that starts to slow down the rates of moisture release much earlier than the other lines. The amplitude of the grain moisture content at the first sampling in the different maternal lines is very large - over 10%, which is not observed in their hybrids.

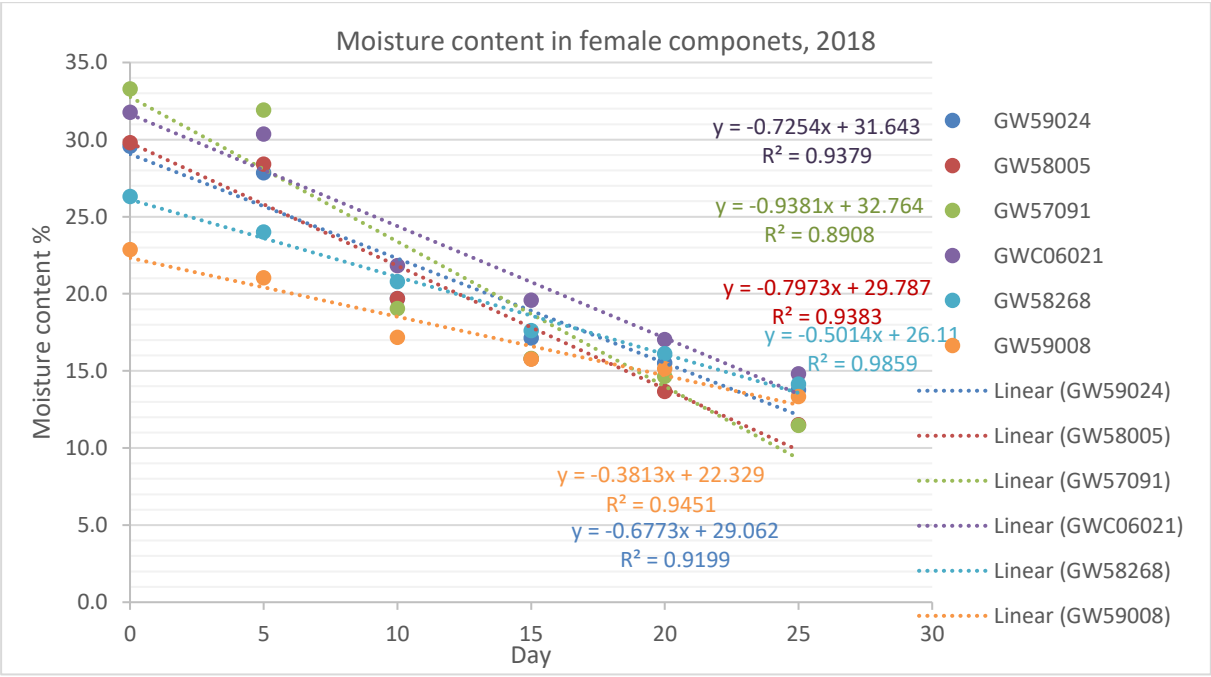


Figure 26. Moisture release in maternal forms in 2018

It can be seen from Figure 27 that line GW52090 has the fastest rate of moisture release compared to the other paternal lines studied for the period of 2016. Line GW58012 shows a fast initial rate of moisture release, but at the end of the pre-harvest study it shows a persistent retention of the studied trait i.e. its moisture release is non-linear in nature. The GW57010 line shows very low moisture at grain harvest, which is due not so much to the high rates of moisture release from the grain as to the extremely low starting moisture content at the first sampling. This maize line is one practical proof of the importance of grain moisture content at or around the period of reaching physiological maturity for the success of low moisture breeding. Similar values of the rate of moisture release were found in the experiments of Petrov (1995), where the studied trait varied from 0.39% to 0.62%.

Between 86% and 99%, the rate of moisture release of the tested parental components depends on the duration of the period after the first sampling. In the GW58012 line, the moisture content of the grain is the most unstable.

In both maternal and paternal components, it is even more evident than in their hybrids that the different lines reach a period of slowing down (non-linearity) in the rates of moisture release at very different values of this trait.

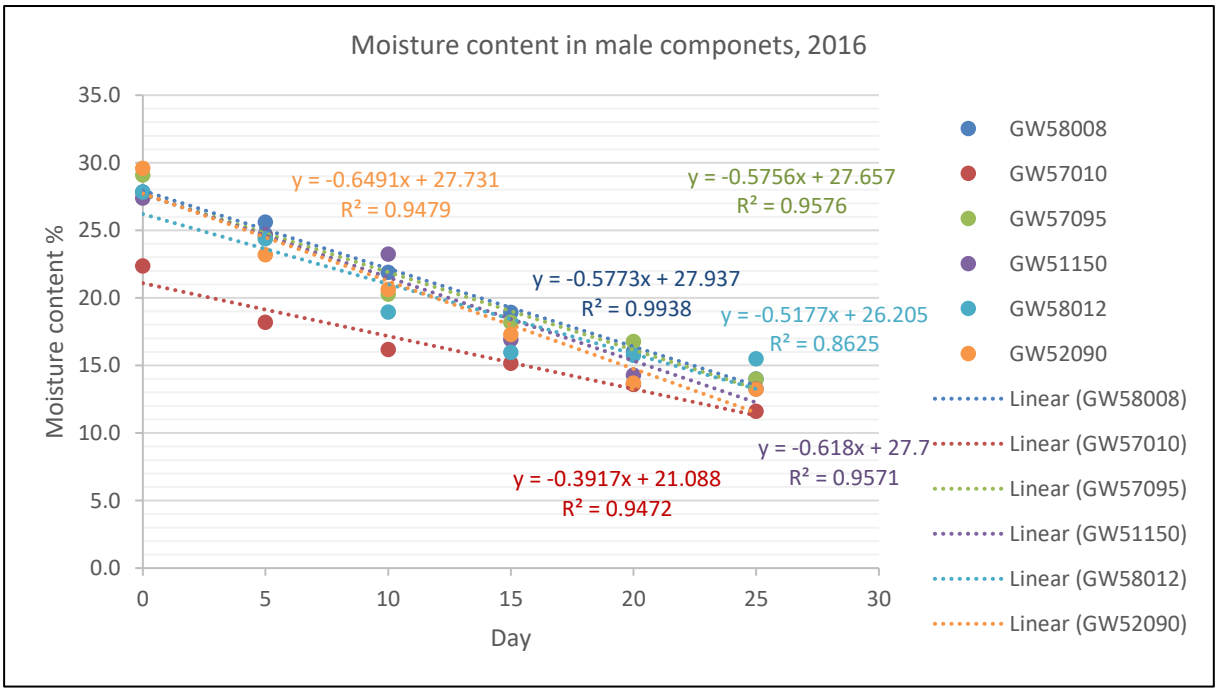


Figure 27. Moisture release in paternal lines for 2016.

In the paternal components of the crosses in 2017 (Figure 28), the slowest moisture release is observed in the maize line GW58008, with this trend persisting until the 20th day after sampling and accelerating only at the end of the sampling period. The highest moisture release rate is in line GW52090, the same as in 2016. As in the previous year, line GW57010 has the lowest starting moisture content in the grain at the time of the first sampling and it exhibits medium to low rates of moisture release, but retains the linear nature of its moisture release (no slowing of rates) even at indicator levels below 15%.

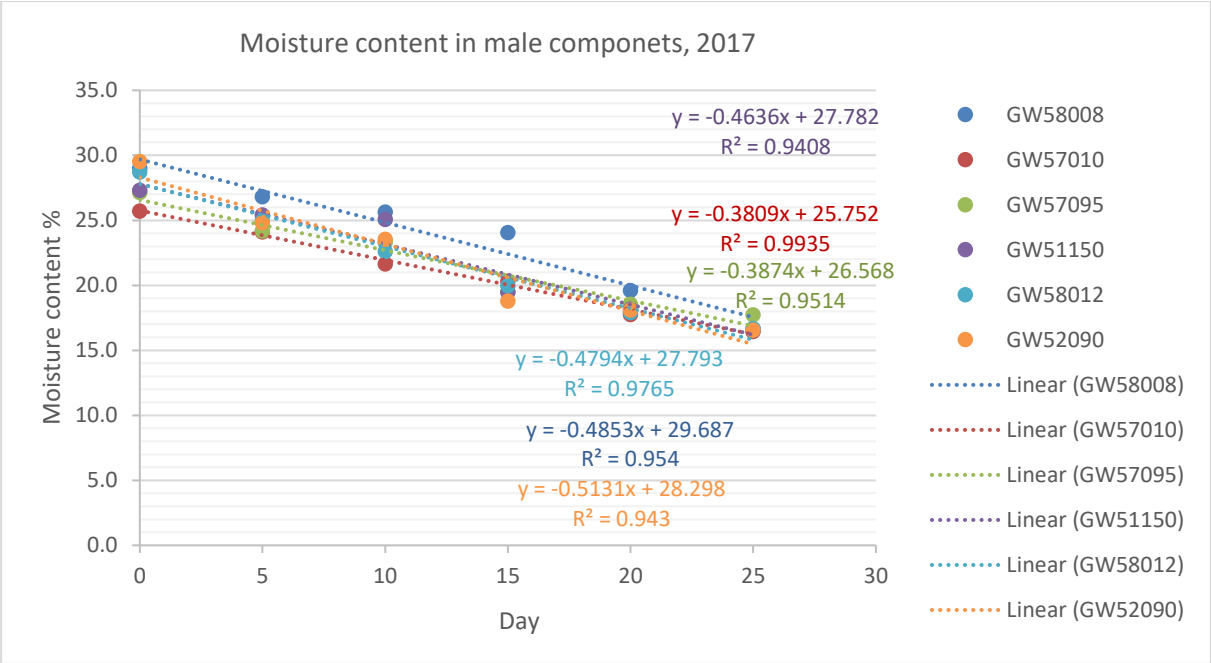


Figure 28. Moisture release in paternal forms in 2017

Similarly, the variations of their regressions with respect to the rates of moisture release are greater in the paternal lines (Figure 29) than in the hybrids. For the period of 2018, line GW58008 stands out with the weakest rate of moisture release, and line GW52090 has the strongest. The remaining lines have an approximately similar linear rate of moisture release for the studied period. GW57010 has the lowest moisture content over the entire 25-day study period, but the trend line has a minimal slope, demonstrating the slowest rate of moisture release at least at drying rates below 17%. The differences between the lines in terms of grain moisture content at the first sampling exceed 14%.

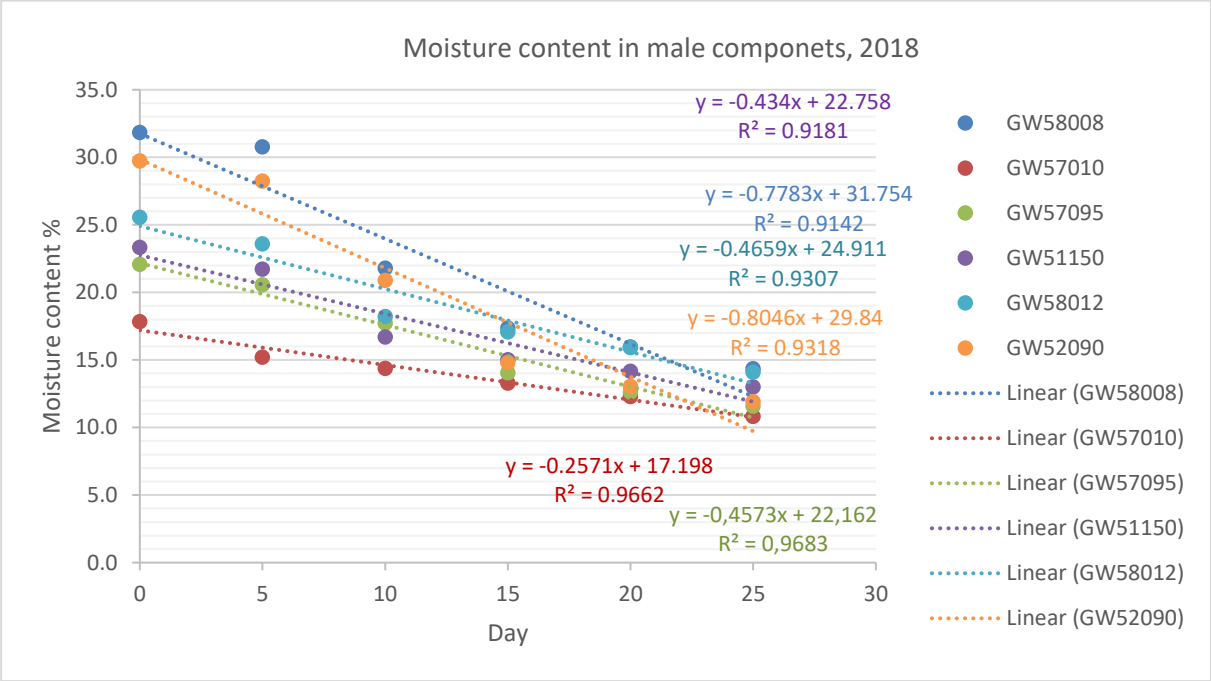


Figure 29. Moisture release in paternal forms in 2018

Conclusions

1. The rates of moisture release in maize grain are predominantly linear in the drying range from 30-32% to about 20 and even below 15%, corroborating the results of most other studies that moisture loss immediately after the onset of physiological maturity is in the form of a linear regression. After that, drying slows down and becomes non-linear, and at different times in different genotypes (or at different trait magnitudes).

2. A much higher, positive correlation is found between the moisture content of the parental components on the one hand and their maize hybrids on the other than in most previous studies, which may be due both to the specificity of the lines or climatic conditions used, as well as to the set crossing scheme rather than the diallel crossing scheme at work. Our research shows that breeding of lines per se for low grain moisture content at harvest can also be an effective breeding approach for the creation of low-moisture hybrids.

3. The economically valuable maize hybrids are crosses with relatively high yields, medium to low grain moisture content, and high stability of expression of these traits across replications, although the variation per year is not different from the average values. For this reason, the assessment of hybrids under a variety of environmental conditions from the earliest stages of improvement work is very important.

4. The clustering of maize hybrids with respect to the trait "grain moisture content" and yield is not affected to a very large extent by grain yield in materials with a close vegetation period, which is proven by the fact that both high and low yielding hybrids can fall into the same cluster. However, hybrids with low grain moisture content tend to have lower yields at harvest.

5. In the group of 40 maize hybrids studied, there are no significant differences in the values of the standard deviation in terms of moisture content, which proves that over the three-year period of the experiment, there are no strong dips or peaks observed in moisture values i.e., moisture when harvesting maize can also be very strongly influenced by external conditions, but the influence of these conditions in the different hybrids is approximately equal and unidirectional.

6. The analysis shows that, in practical breeding work, the use, mainly as maternal components of maize hybrid crosses, of lines per se characterised by low grain moisture content can help in the creation of products that are also characterized by low moisture.

7. When grain moisture content varies, the direct positive maternal effect has the strongest influence in two of the three years of the study, while the direct positive paternal effect has a weaker influence on their hybrids. This is evidence that in the inheritance of grain moisture content in maize there are also many non-nuclear heritable factors involved.

8. The clustering of parental lines by grain moisture content at harvest shows that lines from different heterotic groups fall into all clusters. That is, lines with high and low grain moisture content at harvest and acceptable yields can be created in any heterotic group, including the Lancaster heterotic group. Low grain moisture content is not a special attribute of any of the heterotic groups studied.

9. The moisture content of grain at harvest in maize depends on four factors at least - the length of the vegetation period, the moisture content when physiological maturity has been reached, the rates of grain drying thereafter, and the trait values at which these rates maintain their linear nature before slowing down. Breeding of lines with low moisture content at physiological maturity and continuous linear moisture release would be very useful for creating hybrids with low moisture content in the grain at harvest i.e. the breeding should take into consideration not only the lines with a fast rate of moisture release after the onset of physiological maturity, which is very often stated as the N1

objective of low moisture breeding, but also the initial moisture content and the length of time over which the linear nature of the rate of moisture release is maintained.

10. The variation among maize hybrids in both grain moisture content at first sampling and in rates of moisture release is much smaller than the variation in the relevant indicators of their parental lines. This, together with the high correlation of grain moisture content between lines and their hybrids, leads to an apparent trend of averaging moisture content in maize crosses compared to their parental lines.

11. The rate of moisture release from the grain does not depend on the its moisture content at the time it reaches physiological maturity - these are completely separate traits. A completely separate trait is the percentage of moisture in the grain (or the duration of the period) at which a line starts to slow down its rate of moisture release during the drying process.

12. Our work shows that the breeding of maize should be guided by considering the productive potential first and drought resistance only second. Therefore, assessing maize hybrids under non-irrigated conditions alone is flawed as an approach because it requires the trials to be exposed to a variety of climatic conditions over the years, and this would consume a lot of time.

13. Maize lines GW57010 and GW57091 were identified as lines harvested with low moisture content in the grain and consistently transmitting this indicator to their hybrid combinations. As a result of the recommendation to use them as recurrent parents in the creation of new breeding, the modern mid-early lines from the Lancaster group of Golden West Seed Bulgaria contain GW57010 plasma in over 60% of the cases, and those from Iodent contain GW57091 plasma in over 18%.

14. Maize Hybrid 24 which exhibits medium to high grain moisture content and very high yield has been successfully registered by IASAS and commercialized as GW2122.

Contributions

With theoretical nature

1. A stronger direct positive maternal effect is found in two of the three years of the study, which is evidence that a number of non-nuclear heritable factors are influential in the inheritance of grain moisture content in maize.

2. Through hierarchical cluster analysis, it has been found that there is no direct correlation between the heterotic group, the genetic affiliation of the lines and the grain moisture content. As a direct result of the work on the dissertation, lines have been created that transmit to hybrids low or high moisture grain content from both of the major heterotic groups for the breeding of mid-early maize.

3. Different maize hybrids have been found to retain their linear (strong) grain moisture release to different trait values. Hybrids decreasing their moisture linearly to lower trait values are valuable for breeding because they can be harvested with lower grain moisture content.

4. It has been found that the moisture content of maize at harvest depends mainly on four factors:

- duration of the vegetation period
- moisture content at physiological maturity
- rates of grain drying after the onset of physiological maturity
- a trait value at which these rates retain their linear nature before slowing down

With applied nature

1. A new maize hybrid GW2122 (exp. Hybrid 24) has been created and successfully registered by IASAS, which exhibits medium to high grain moisture content and produces very high yields.
2. Maize line GW57010 has been identified as having low grain moisture content at harvest and transmitting this trait consistently in its hybrid combinations. It is used as a recurrent parent in the creation of new breeding projects. The modern mid-early lines from the Lancaster group of Golden West Seed, Bulgaria in more than 60% of cases contain plasma from GW57010.
3. Maize line GW57091, which belongs to the Iodent heterotic group, also stands out with its low grain moisture content and transmits this trait in its hybrid combinations. It is included in the composition of 18% of the lines from the Iodent heterotic group of Golden West Seed, Bulgaria.
4. The study has found that breeding of lines per se for low grain moisture content at harvest can be an effective breeding approach in creating maize hybrids with low moisture content.
5. After the analysis of the information from the conducted study, it is recommended that lines per se characterized by low grain moisture content at harvest should be included as maternal components of hybrid crosses, as this leads to the creation of hybrids with the same indicators.

Publications related to the dissertation

1. Tsoneva K. 2023. Inheritance of moisture release rates in mid-early maize hybrids from the Iodent x Lancaster heterotic pattern, Crop Science 60(3), 31-39.

2. Tsoneva., K. (2023). Research of combining ability of mid-early maize lines. Grain Crops. 2022. 6 (2). 57–60.https://doi.org/10.31867/2523-4544/0232.

Study of moisture release rates of inbred maize lines for use in breeding programmes

Summary of PhD Thesis
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The object of the research is the rate of moisture release from the grain after the onset of physiological maturity in 36 maize hybrids and the moisture content of the grain at harvest of the investigated hybrids and their parental components. During the period 2016-2018, the moisture release rates (MRR) of 36 hybrid combinations of the heterosis model Iodent x Lancaster were studied. The grain moisture content of the parental forms and their hybrids, the correlation between the grain moisture content of the parental components and their hybrids, the rate of moisture release from the grain of the studied 36 hybrids and their parental components were studied. Classification of hybrids and lines by grain moisture content at harvest was done. The comparative evaluation of the hybrids by moisture content and average yield was carried out. Lines with low grain moisture content which stably inherited this parameter in hybrid combinations were selected for the purpose of recurrent selection.

The results showed a much higher, positive correlation between the moisture content of the parental components on one hand and their maize hybrids on the other.

Grain moisture content at harvest depends on at least four factors—the length of the growing season, the moisture content at physiological maturity, the rates of grain drying thereafter, and the trait values at which these rates maintain their linear nature before slowing down.