

Article

Using Flint Maize for Developing New Hybrids: A Case Study in Romania

Roxana Elena Călugăr ¹, Andrei Varga ¹, Carmen Daniela Vana ^{1,*}, Loredana Ancuța Ceclan ¹,
Felicia Chețan ², Andras Fodor ¹ and Nicolae Tritean ¹

¹ Laboratory of Maize Breeding, Agricultural Research and Development Station Turda, 401100 Turda, Romania; roxana.calugar@scdaturda.ro (R.E.C.); andrei.varga@scdaturda.ro (A.V.); anca.ceclan@scdaturda.ro (L.A.C.); andras.fodor@scdaturda.ro (A.F.); nicu.tritean@scdaturda.ro (N.T.)
² Laboratory of Technology and Mechanization, Agricultural Research and Development Station Turda, 401100 Turda, Romania; felicia.chetan@scdaturda.ro
* Correspondence: carmen.vana@scdaturda.ro

Abstract

Maize, one of the most cultivated crops worldwide, has multiple uses, one of which is human food. Maize flour intended for human consumption is preferably produced from var. *indurata*. This maize variety, although it has some desirable traits, generally has a lower yield capacity. In order to obtain high-yielding hybrids that would have some traits necessary to obtain flour for human consumption, fourteen lines with dent or semi-dent grains were crossed with four inbred lines with flint grain in a cyclic system. The 56 resulting hybrids were tested in two experimental years for yield, the percentage of unlodged plants, grain dry matter at harvest, as well as other traits, such as ASI (anthesis-to-silking interval), the interval from sowing to the appearance of stigmas and to physiological maturity, and plant senescence. The maternal lines A478 and A480 were noted for transmitting higher yields. Three hybrids were identified with higher yields, good silking–flowering coincidence, stay-green, and a high unlodged plants percentage: A478 × D328, A480 × B330, and A480 × D328.

Keywords: maize; flint; *indurata*; yield; semi-dent



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1. Introduction

Maize (*Zea mays* L.), a plant with high yield potential, is one of the species cultivated on the largest areas worldwide, currently surpassed only by wheat, which it surpasses in terms of total production [1]. This plant has very high phenotypic and genetic diversity, so its uses are multiple in human food, animal feed, and industry (production of biofuels, starch for fabrics, high-quality oil, and alcoholic coolants, as well as the production of raw materials in the pharmaceutical and cosmetic industries [2]). This crop is subject to a series of stress factors, so new genotypes must cope as well as possible. In the maize breeding process, breeders must consider, in addition to obtaining the highest possible yields, tolerance to biotic and abiotic stress factors. The ever-changing climate is one of the main factors to consider in maize breeding, with adverse conditions often occurring during critical periods of development [3,4]. One of the most critical periods for this crop is anthesis, and if drought occurs during maize flowering, pollination can be significantly affected, leading to very high production losses. The maximum temperature for achieving anthesis should not exceed 37 °C [3], but even at temperatures above 35 °C, physiological processes are affected, with pollen viability decreasing considerably [5]. Another aspect

that must be taken into account is the apical dominance found in maize, with the silk being more sensitive to a lack of water, so that under unfavorable conditions it develops after flowering. Delayed silk emergence (high ASI) can cause losses of up to 20–50% [6,7]. ASI therefore represents an index that must be carefully monitored, both for parental genotypes and for the transmission of a reduced interval in hybrids.

Genetic narrowing of the germplasm is possible, especially due to the use of a limited number of elites or sources for the improvement of new genotypes [2], but in the case of maize, it is desirable to use the natural variability given by local populations and backcrosses, as is the use of diverse sources as initial material for the creation of new inbred lines. With the introduction of American maize hybrids to Europe after 1950 [8,9], the proportion of dentiformis genotypes increased significantly, with many local populations being contaminated over time with this type of germplasm. The use of European-origin flint maize can bring improvements to the genetic base of this plant, and due to genetic differences, crosses can result in hybrids with high heterosis.

Zea mays var. *indurata* (flint maize) is of particular interest to maize growers due to its characteristics. The *indurata* genotypes have a smaller plant size, somewhat lower yields [10], and rounded upper grains that are smooth, shiny, and hard to break (soft starch in the middle surrounded by a hard shell) [11], hence the name “hard corn”. This type of maize is specific to local populations in Europe, with most Romanian populations also being flint [12,13]. It has more beneficial properties compared to var. *indentata* (dent grains), with superior quality and a specific pleasant taste, being rich in fiber, protein, carbohydrates, and lipids. It is ideal for polenta, containing proteins, lipids, minerals, and vitamins A, B1, B2, B6, E, and PP [14]. Another advantage of flint maize is the lower incidence of pest attack on the cobs, due to the fact that the grain is harder [15]. European flint maize is characterized by early vigor and good cold tolerance [16], while dent maize can bring increased yield potential to hybrids [17]. In many areas of Europe, low temperatures can occur in the early part of the maize growing season, a situation often encountered in the experimental area. According to data obtained from the weather station [18] located near the fields, almost every year, temperatures below 0 are recorded in April. Also, both in April and even in May, the minimum temperature is often below 10 °C. The higher tolerance of flint maize to low temperatures (compared to var. *indentata*) [19], as well as the ability to recover from cold stress [20–22], makes it a good source for improving new genotypes. To obtain hybrids that retain the desirable characteristics of var. *indurata*, but also have a higher yield, crosses with genotypes with dent or semi-dent grains can be made [23].

Even though the seed offer in Romania includes flint or semi-dent hybrids [24–27], their proportion in the portfolio of companies is 5–20% of the total hybrids. In Romania, polenta and other corn-based products are quite popular, while the demand for gluten free products is increasing. According to the National Institute of Statistics [28], in Romania, the average consumption of maize and maize products is 35.1–35.8 kg/capita (grain equivalent), so there is a niche for such hybrids.

Although var. *indentata* is used more often in hybrid combinations, due to its productive superiority, at the Agricultural Research and Development Station (ARDS) Turda, *indurata* inbred lines and their crossing with dent or semi-dent lines are used. ARDS Turda is one of the main agricultural research stations in Romania, with a history of almost 70 years in maize breeding. The germplasm collection contains over 1460 inbred lines and their number varies annually, depending on the number of newly developed lines in the selection department. The collection also includes a significant number of local populations (391) from many regions of the country: 6 varieties and 72 synthetic populations. To date, 47 commercial hybrids have been registered and cultivated, of which 28 have dent grains, 14 semi-dent, 2 flint and 4 sweet corn. The latest hybrids developed are all dented, so it is

also necessary to focus on flint maize as well. Given that, usually, the indurata maize from the in-house collection has a shorter vegetation period, a series of hybrid combinations were made to obtain earlier, qualitative hybrids, which also have good yield.

Thus, in order to obtain productive hybrids with flint grains, 14 high-yielding inbred lines (dent or semi-dent) were crossed in a cyclic crossing system with 4 flint lines. The 56 hybrids thus obtained were cultivated in 2 experimental years, and the yield, dry matter of grains at harvest (DM), and percentage of unlodged plants (UP) were analyzed. The period required to reach the BBCH 65 phenophase (flowering) (sowing–flowering interval), as well as the flowering-to-silking interval (ASI), was also monitored.

2. Materials and Methods

2.1. Biological Material

Although in the ARDS Turda germplasm collection there are about 100 flint inbred lines, 4 were chosen for this study, considering their yield capacity, a number of previously known traits (tolerance to abiotic factors, high percentage of unlodged plants at maturity, ear size) and also their genetic divergence. The lines used in crosses were classified into the germplasm group based on previous studies [29] and verified based on crosses with different heterotic groups. These lines were used in cyclic crosses (line × tester) with 14 dent or semi-dent lines, thus resulting in 56 hybrids. Several traits of the parental inbred lines are presented in Table 1.

Table 1. Inbred lines used in crosses and their cob traits.

Inbred Line	Germplasm Group	Kernel Type	Kernel Color	Cob Color	Ear Average Length	No Kernel Rows	No Kernels/Row
Maternal inbred lines							
E292	Iodent + Mo17	Dent	Dark yellow	Red	16	12–14	24
C344	Iodent	Semi-dent	Dark yellow	Red	18	16–18	24
D394	Iodent	Semi-dent	Dark yellow	Red	13	18–20	27
E347	Iodent	Semi-dent	Dark yellow	White	16	16–18	29
E348	Iodent	Semi-dent	Dark yellow	White	13	16–18	21
E359	Iodent	Semi-dent	Dark yellow	Red	13	14–16	22
E372	Mo17 + C103	Dent	Dark yellow	Red	15	18–20	31
E376	Iodent	Dent	Dark yellow	Red	20	18–20	40
E378	Iodent	Dent	Dark yellow	Red	18	14–16	35
E385	B73	Dent	Yellow	Pink	13	18–20	22
A447	Iodent + Oh43	Dent	Dark yellow	Red	13	16–18	25
A478	Iodent	Semi-dent	Dark yellow	Pink	12	16–18	27
A480	Iodent	Semi-dent	Dark yellow	White	12	18–20	30
A483	Iodent + B73	Dent	Dark yellow	Pink	20	16–18	35
Paternal inbred lines							
D298	Mo17 + C103	Flint	Dark yellow	Dark red	18	14–16	28
B330	W153 + D105	Flint	Orange	White	16	12–14	17
C336	W153 + Lo3	Flint	Yellow	White	16	12–14	26
D328	Mo17 + C103	Flint	Dark yellow	Red	13	14–16	24

2.2. Experimental Design and Cultivation Technology

The maize hybrids resulting from the cyclical crossing of the previously mentioned lines were tested over two years, 2021 and 2022, in the experimental field of the Maize breeding laboratory (ARDS Turda), located on the upper terrace of the Arieș River, north-western part of Turda, Cluj County, Romania (longitude 23°48′ E, latitude 46°35′ N).

According to the analyses carried out by the Pedological and Agrochemical Studies Office in Cluj-Napoca [30], the dominant soils in the experimental fields are vertical clay-

iluvial chernozems (approximately 56% clay and 0.7% coarse sand), pH 6.2–6.8. The values of total nitrogen, phosphorus availability, potassium availability, carbonates and humus (at a maximum depth of 30 cm) are 0.2%, 65 ppm, 400 ppm, 0.7% and 3.5%, respectively. The reported values are averages, as they have not changed significantly over the tested years (analysis is performed every 4 years).

The hybrids were sown in both years in the first week of May, using the Monoseed DT seeder (Wintersteiger, Ried im Innkreis, Austria) [31], at a density of 70,000 plants ha⁻¹ (19 cm between plants, 0.7 m between rows). Each hybrid was sown in four 5 m rows (only the middle rows were harvested and analyzed), in three replications, and the plots were arranged according to the randomized block design.

In the experimental field, a three-year crop rotation is used: soybean–winter wheat–maize. In autumn, ploughing was carried out, and in the spring, a pass was made with the combiner in order to level the land, simultaneously with the application of 400 kg⁻¹ of NPK 27:13.5:0 fertilizer [32] (Azomureș, Târgu Mureș, Romania). Weed control was carried out using two herbicides: pre-emergence 1.5 L ha⁻¹ (active substance S-metolachlor, 960 g L⁻¹) (Tender 960 EC, by Syngenta Crop Protection AG, Basel, Switzerland) and post-emergence 1.5 L ha⁻¹ (active substances tembotrione (44 g L⁻¹) and isoxadiphen-ethyl (22 g L⁻¹)) (Laudis66OD, by BayerAG, Crop Science Division, Monheim am Rhein, Germany).

Observations to determine the ASI index were made in two-day intervals, noting the date when 75% of the plants in the rows were in flower or silken. Observations of physiological maturity (BBCH 67) [33] were also made every two to three days, noting the date when 75% of the plants had dry husks, validated by visual inspection of the black layer. The stay-green genotypes have green leaves until physiological maturity, while for the non-stay-green ones, the leaves begin to dry about a month after anthesis [34]. Genotypes that had delayed leaf senescence, with green leaves at physiological maturity, were noted as stay-green (SG), those that had dry leaves up to the level of ear insertion were noted as medium stay-green (MSG), and those that had dry leaves above the ear were considered non-stay-green (NSG)

Before harvesting, fertile and sterile plants (without cobs) were counted, as well as lodged ones (plants with broken stems). The harvesting was performed mechanically with the Wintersteiger Classic combine for experimental plots (Wintersteiger, Ried im Innkreis, Austria) [35]. The yield was determined using the weight of the grains in each experimental plot, related to the number of plants counted; grain moisture was determined using the analyzer included in the combine for experimental plots (also verified with a Granomat plus analyzer, Pfeuffer GMBH, Kitzingen, Germany [36]). For the calculation of yield, the moisture was brought to the standard of 14%. The dry matter was determined by calculating the difference from the grain moisture, measured at harvest.

2.3. Meteorological Conditions

The climate of the area where the experiment was carried out is continental, with the highest temperatures being recorded in July and August, while precipitation is more abundant in June.

One of the best years for maize cultivation in the experimental area was 2021, especially due to the combination of abundant precipitation with high temperatures. This year was favorable starting from the first part of the growing season (April and May), with temperatures and precipitation specific to the area. In June and July, high temperatures were recorded, 1.9 and 3 °C, respectively, above the multiannual average (65 years). The maximum temperatures in July, during anthesis, ranged between 21.7 and 33.7 °C. Although in June, rainfall was below the average for the area (−39.8 mm), in July, it exceeded this

value by 46 mm. August and September had values close to the multiannual averages, both for precipitation and for temperature (Figure 1a).

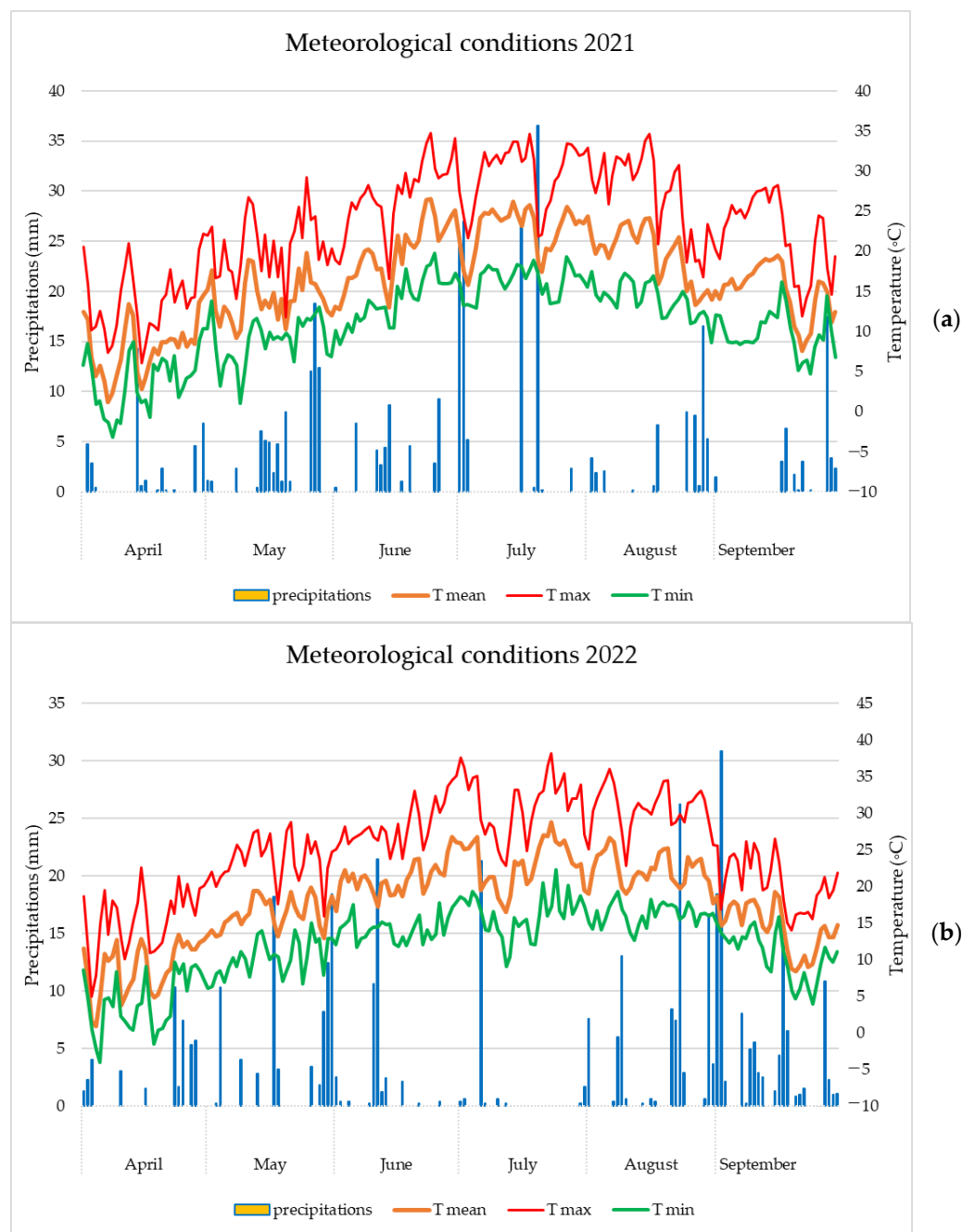


Figure 1. Meteorological conditions, Turda, (a) 2021 and (b) 2022.

One of the most unfavorable years for maize cultivation was 2022, especially due to the climatic conditions, with water scarcity and excessive temperatures being recorded throughout the year (Figure 1b). Although during the sowing period, conditions were favorable for this crop, the extreme water deficit in June and July, associated with high temperatures, affected the crop. In June and July, the multiannual average temperature was exceeded by 3.1 and 3.3 °C, respectively, while rainfall was 42.8 and 52.8 mm lower than normal for the area. July was one of the hottest months in the area, with maximum temperatures ranging from 22.8 to 38.2 °C. August continued with extreme temperatures (+2.8 °C compared to the multiannual average) and lack of precipitation. Heavy rainfall

(+38.5 mm) was recorded, especially in the last days of the month and in September (+77.5 mm), but it was already too late for maize [18].

2.4. Data Processing and Statistical Methods

ANOVA was performed on data, using Past 4 (<https://past.en.lo4d.com/windows>, accessed on 12 September 2025). The Fisher test for least significant differences (LSDs) was used to assess differences between genotypes at p -values of 0.05, 0.01 and 0.001. The general combining ability (GCA) was inferred from the variances of maternal and paternal effects, and the specific combining ability (SCA) was determined based on the interaction between the parental lines. The Duncan test was applied to identify differences between GCA effects of maternal and paternal lines. The hybrids were ranked based on year-wise yield and the average of the years: the average UP and DM, respectively.

3. Results

3.1. Plant Phenology

Both the inbred lines and the hybrids created at ARDS Turda are generally classified in the semi-early maturity group, this being the most optimal for the cultivation area. The hybrids studied needed 66–73 days between sowing and flowering (Figure 2). Some maternal lines have been noted that have transmitted a reduced number of days required for flowering to hybrids: E385, E359, E376 and E378, and also the paternal line C336. The shortest number of days required for flowering was observed for hybrids E378 $\times$ C336 and E385 $\times$ C336. The hybrids resulting from the crossing of testers with the maternal lines A447, A478, A480 and A483 generally needed more days to flower.

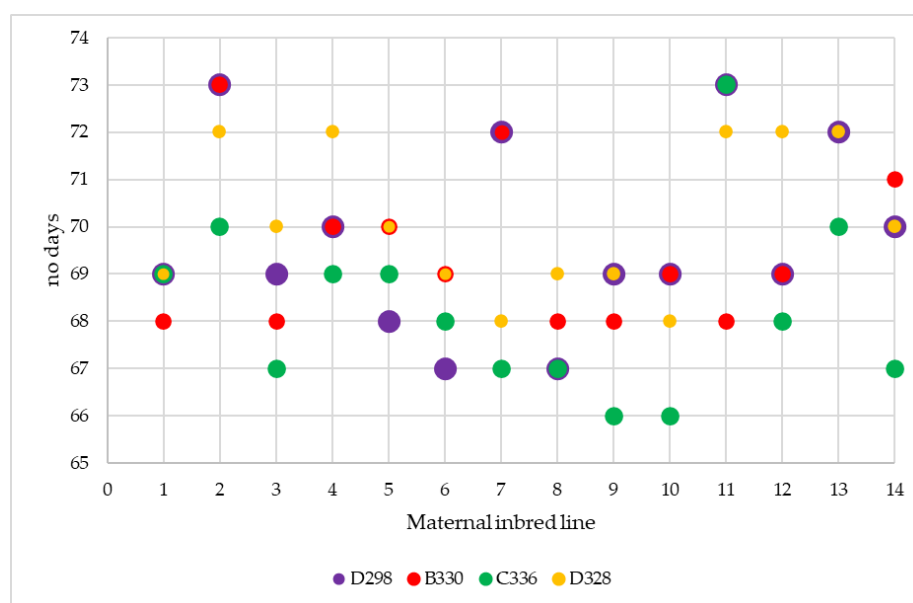


Figure 2. Sowing to flowering interval 1. E292; 2. C344; 3. D394; 4. E347; 5. E348; 6. E359; 7. E372; 8. E376; 9. E378; 10. E385; 11. A447; 12. A478; 13. A480; 14. A483.

The anthesis-to-silking interval (ASI) is a crucial metric for determining the coincidence of reproductive development and is a good indicator for the crop's tolerance or sensitivity to drought and heat [37,38]. In general, the hybrids had a good coincidence between the appearance of silk and flowering, showing good heat tolerance. The inbred line E347 was noted for the good anthesis–silking coincidence that it transmitted to hybrids. Even though a delay of 3 days was observed in the case of hybrids E359 $\times$ C336 and E376 $\times$ D298, the difference is not large enough to affect pollination (Figure 3).

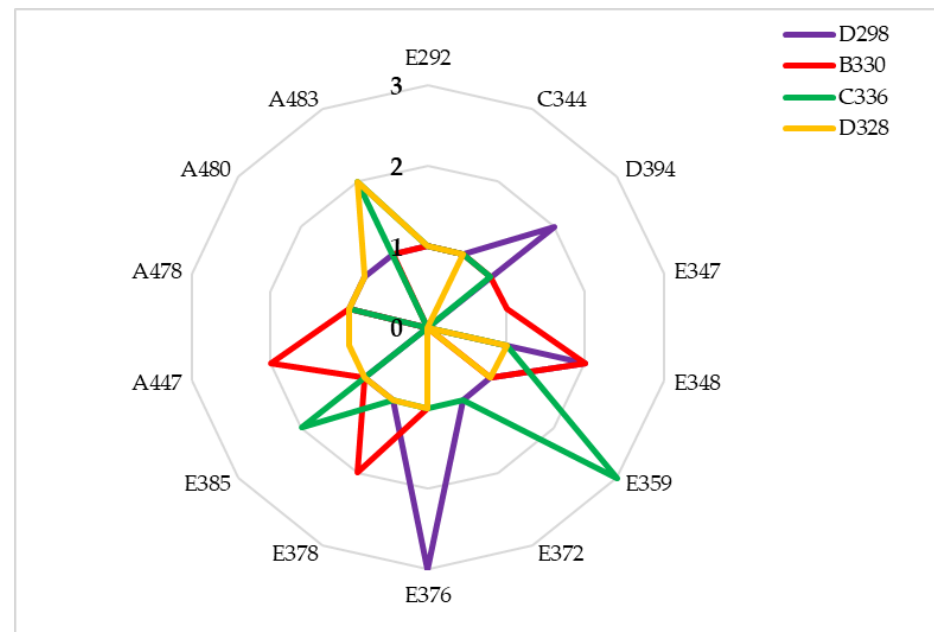


Figure 3. Anthesis to silking interval. 0 = flowering and silking coincidence, 1–3 = number of days between flowering and silking.

Regarding the physiological maturity period (Figure 4), it took between 104 (E376 × C336) and 127 (E378 × D328) days to reach this phenophase. The genotypes noted as SG maintained all their leaves green at physiological maturity, or had 1–2 dry leaves; MG hybrids had half green–half dry leaves, while NSG genotypes had 50–100% dry leaves. A483 exhibited a longer period required to reach physiological maturity, but the plants of these hybrids had a higher number of green leaves per plant and thus stayed green for a prolonged period, a trait that indicates a longer period for the photosynthetic activity [39]. The stay-green ability is one of the traits monitored when breeding new maize genotypes. Inbred lines D328 and E347 can be used to create new hybrids or to improve new inbred lines with a shorter vegetation period, and are also used for their prolonged photosynthetic capacity. A total of 22 of the hybrids studied were SG at physiological maturity, while 31 had half of their leaves dry, being noted as MSG. Three hybrids were noted as NSG (A447 × D298, E372 × B330 and A478 × C336) at the moment of physiological maturity, with early senescence having already been observed.

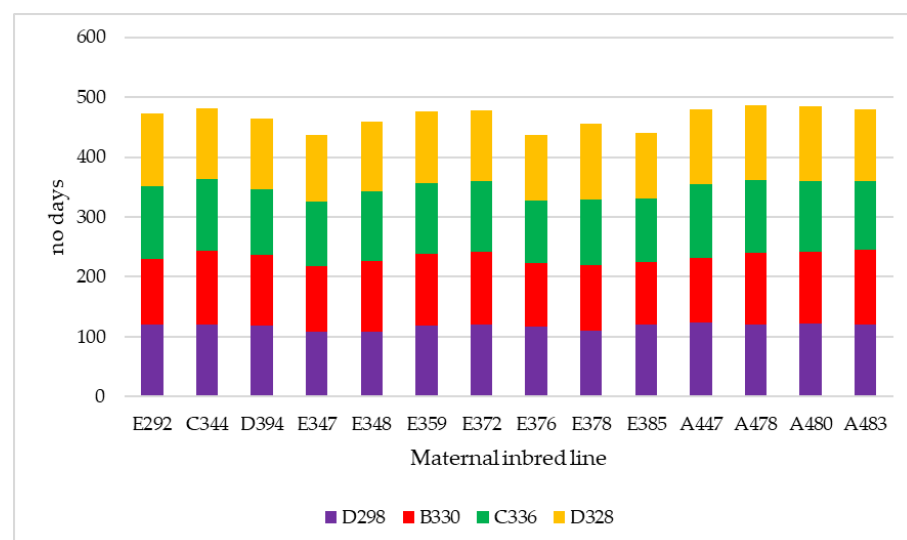


Figure 4. Sowing-to-physiological maturity interval.

3.2. Hybrid Yield, Dry Matter at Harvest and Unlodged Plants

The ANOVA analysis (Table 2) indicates a clear influence of the year factor for yield, DM and UP. All the traits studied were also significantly influenced by both hybrid and inbred lines (parental genotypes). This aspect is consistent with the premises of the study. The two years were very different in terms of favorability for the maize crop, and regarding the genotypes studied, ANOVA confirms the diversity of the biological material used.

Table 2. ANOVA for yield, DM and UP—F values.

Source of Variability	DF	Yield	DM	UP
Year (Y)	1	3795 **	1421 **	364.6 **
Hybrid (H)	55	17.9 **	18.3 **	1377 **
Maternal genotype (M)	13	31.1 **	94.3 **	1396 **
Paternal genotype (P)	3	135.4 **	37.7 **	1319 **
Y × H	55	0.3	3.2 **	0.1
Y × M	13	0.4	1.1	0.2
Y × P	3	0.4	2.3 **	0.2

** Fisher test, significant for $f = 0.01$.

The additive action for A478 and A480 (Table 3) indicates the higher yield that the two maternal lines transmit. These lines were also noted for their high SCA in crosses with D328, B330 and D298. A447 and A483 were also noted for their GCA. All four lines are elite inbred, used in recent years for a large number of crosses.

Table 3. GCA and SCA effects for yield.

No.	Maternal Inbred Line	SCA Effects Paternal Inbred Line				Maternal Inbred Line GCA
		D298	B330	C336	D328	
1	E292	679	416	651	243	497 gh
2	C344	45	−142	−1019	−296	−353 bcd
3	D394	−457	−131	−818	1122	−71 bc
4	E347	288	380	−764	−256	−88 de
5	E348	62	−3	−2323	86	−545 bc
6	E359	502	538	−1571	−425	−239 cde
7	E372	−292	−658	−1263	−199	−603 b
8	E376	210	−284	−123	158	−10 ef
9	E378	−944	−527	−1129	−2492	−1273 a
10	E385	465	−252	−1040	1166	85 ef
11	A447	533	854	−96	416	427 gh
12	A478	1372	1251	−123	2107	1152 i
13	A480	955	1646	−1243	1525	721 h
14	A483	428	1164	−870	456	295 fg
Paternal inbred line GCA		275 b	304 b	−838 a	258 b	

The letters following the numbers represent the significance between the variants, according to Duncan's test.

The inbred line C336 was obtained by crossing a local population of Romanian flint and an inbred line from the Lo3 group; although this line stood out for other traits, in the case of yield, its use had an unfavorable effect. However, the inbred line B330, which also originates from the same local population, was noted for its good SCA in crosses with A480, A478 and A483. The flint inbred lines D298 and D328 (improved by recurrent selection from hybrids) had good GCA and stood out in crosses with A478 and A480 for the highest yields.

The hybrids studied were ranked according to the yield per year, and also the average of the two experimental years (Table 4). The A478 × D328 hybrid achieved the highest yield in the favorable year 2021, and was second in the unfavorable year. This hybrid combination is considered the best in the experimental system, with the highest average yield, 10,168 kg ha^{−1}. Second ranked in the first year, and in terms of yearly average, is A480 × B330, with an average yield of 9707 kg ha^{−1}. In 2021, the top two ranked hybrids

exceeded the yield of the two controls, Turda 332 and Turda 335, but were less productive in unfavorable conditions. In the unfavorable year 2022, the highest yield was achieved by the A480 × D328 hybrid, which ranked third in 2021 and for the yearly average. The hybrids resulting from crossing of the maternal lines A478 and A480, respectively, with three of the paternal lines (D298, B330 and D328) were ranked among the top 10 hybrids, demonstrating the superiority of the two lines.

Table 4. Hybrid yield, by year, average and rank.

Hybrid	Yield 2021	Yield 2022	Average Yield	Rank 2021	Rank 2022	Average Rank
E292 × D298	11,066 ± 20	6415 ± 306	8740	12	11	11
C344 × D298	10,565 ± 326	5648 ± 346	8106	25	29	28
D394 × D298	9963 ± 463	5244 ± 275	7604	43	42	42
E347 × D298	10,708 ± 364	5991 ± 167	8350	22	23	22
E348 × D298	10,482 ± 81	5765 ± 126	8123	28	27	27
E359 × D298	10,855 ± 354	6271 ± 141	8563	18	13	15
E372 × D298	10,128 ± 241	5411 ± 163	7770	40	38	39
E376 × D298	10,630 ± 275	5913 ± 280	8272	23	24	24
E378 × D298	9476 ± 140	4759 ± 245	7118 *	49	47	48
E385 × D298	11,019 ± 487	6034 ± 196	8526	13	22	16
A447 × D298	10,921 ± 217	6268 ± 192	8594	15	14	14
A478 × D298	11,725 ± 318	7142 ± 273	9434 **	5	3	4
A480 × D298	11,375 ± 122	6658 ± 93	9016 *	9	9	9
A483 × D298	10,885 ± 540	6094 ± 475	8490	16	19	18
E292 × B330	10,836 ± 218	6118 ± 318	8477	20	18	20
C344 × B330	10,278 ± 337	5560 ± 303	7919	35	33	34
D394 × B330	10,389 ± 143	5472 ± 30	7931	30	35	33
E347 × B330	10,800 ± 151	6081 ± 431	8441	21	20	21
E348 × B330	10,417 ± 422	5700 ± 258	8058	29	28	29
E359 × B330	10,958 ± 289	6240 ± 433	8599	14	15	13
E372 × B330	10,193 ± 310	4614 ± 304	7404	37	49	44
E376 × B330	10,136 ± 430	5418 ± 101	7777	39	37	38
E378 × B330	9893 ± 183	5175 ± 498	7534	44	43	43
E385 × B330	10,334 ± 305	5284 ± 127	7809	31	40	36
A447 × B330	11,274 ± 43	6557 ± 488	8916	10	10	10
A478 × B330	11,738 ± 380	6886 ± 63	9312 **	4	5	5
A480 × B330	12,300 ± 354	7115 ± 77	9707 ***	2	4	2
A483 × B330	11,584 ± 397	6866 ± 240	9225 *	7	6	7
E292 × C336	11,071 ± 267	6353 ± 304	8712	11	12	12
C344 × C336	9400 ± 262	4684 ± 518	7042 *	50	48	49
D394 × C336	9602 ± 422	4885 ± 450	7243	46	45	46
E347 × C336	9656 ± 293	4937 ± 342	7297	45	44	45
E348 × C336	8197 ± 124	3279 ± 386	5738 ***	55	55	55
E359 × C336	8683 ± 248	4298 ± 113	6490 ***	54	53	54
E372 × C336	9157 ± 390	4439 ± 481	6798 **	53	52	53
E376 × C336	10,297 ± 514	5580 ± 305	7938	34	31	31
E378 × C336	9291 ± 170	4573 ± 233	6932 **	52	50	51
E385 × C336	9514 ± 346	4528 ± 134	7021 *	48	51	50
A447 × C336	10,324 ± 188	5606 ± 281	7965	32	30	30
A478 × C336	10,297 ± 291	5579 ± 361	7938	33	32	32
A480 × C336	9344 ± 234	4293 ± 131	6819 **	51	54	52
A483 × C336	9550 ± 99	4832 ± 140	7191	47	46	47
E292 × D328	10,563 ± 187	6045 ± 97	8304	26	21	23
C344 × D328	10,124 ± 81	5406 ± 181	7765	41	39	40
D394 × D328	11,542 ± 471	6825 ± 416	9184 *	8	8	8
E347 × D328	10,164 ± 211	5446 ± 419	7805	38	36	37
E348 × D328	10,506 ± 240	5789 ± 159	8148	27	26	26
E359 × D328	9995 ± 185	5277 ± 29	7636	42	41	41
E372 × D328	10,221 ± 269	5504 ± 272	7862	36	34	35
E376 × D328	10,578 ± 268	5861 ± 102	8220	24	25	25
E378 × D328	7928 ± 56	3211 ± 380	5569 ***	56	56	56
E385 × D328	11,616 ± 93	6838 ± 210	9227 *	6	7	6
A447 × D328	10,836 ± 228	6118 ± 411	8477	19	17	19
A478 × D328	13,161 ± 213	7176 ± 107	10,168 ***	1	2	1
A480 × D328	11,945 ± 327	7228 ± 397	9586 **	3	1	3
A483 × D328	10,876 ± 185	6158 ± 321	8517	17	16	17
CT1 Turda 332	10,716 ± 457	8315 ± 376	9516			
CT2 Turda 335	11,060 ± 346	8172 ± 241	9616			

$p = 0.05$ (*), $p = 0.01$ (**) and $p = 0.001$ (***).

The percentage of dry matter at harvest indicates the precocity of a genotype; thus, several sources have been identified that can be used for the precocity of the genotypes: E376, E385 and D394. In the case of the paternal lines, although the differences were statistically significant, they had low values. The hybrids that ranked higher for DM were E376 × C336, E376 × B330, E385 × C336, D394 × D328, E376 × D328 and E385 × B330. The lower DM values for hybrids with maternal lines A478 and A480, respectively, indicate the late-maturing group of the higher yielding genotypes.

As for the percentage of unlodged plants at harvest, although the values were close to the experimental average, it is nevertheless worth mentioning the maternal lines E359 and A447 transmit a lower percentage of lodged plants, as does the paternal line D298. The first two ranked hybrids had A447 as their maternal line (A447 × D298 and A447 × C336), and the hybrid resulting from the cross with D328 (the highest yielding one) was 10th. Three of the hybrids whose maternal genotype is the E359 line were ranked 7th and 9th, while several of the hybrids in the top positions in the ranking had D298 as their paternal line. One of the problems raised by the use of local populations in maize breeding is precisely the low resistance to stem breakage [40], an aspect also observed in the case of line B330, which was obtained following selection from a local population; crosses with this line had lower values of UP (Table 5).

Table 5. Hybrid dry matter and unlodged plants, by year, average and rank.

Hybrid	DM 2021	DM 2022	Average DM	DM Rank	UP 2021	UP 2022	Average UP	UP Rank		
E292 × D298	74.0 ± 0.3	76.1 ± 0.1	75.1	***	55	97.8 ± 1.3	96.8 ± 0.8	97.3	19	
C344 × D298	77.0 ± 0.2	80.0 ± 0.4	78.5		29	99.1 ± 0.0	98.2 ± 1.8	98.7	6	
D394 × D298	77.8 ± 1.1	80.4 ± 0.3	79.1		24	98.4 ± 0.7	97.5 ± 1.6	97.9	16	
E347 × D298	78.7 ± 0.6	81.8 ± 0.3	80.2		18	97.3 ± 0.0	96.4 ± 0.8	96.9	24	
E348 × D298	79.2 ± 0.5	82.0 ± 0.3	80.6	*	15	99.2 ± 0.0	98.3 ± 1.7	98.8	4	
E359 × D298	79.9 ± 0.7	82.6 ± 0.1	81.3	**	7	99.1 ± 0.0	98.2 ± 1.3	98.7	7	
E372 × D298	79.4 ± 0.2	82.1 ± 0.3	80.7	*	10	98.5 ± 0.6	97.7 ± 1.5	98.1	12	
E376 × D298	79.2 ± 0.3	81.9 ± 0.5	80.6	*	15	97.1 ± 1.3	96.3 ± 1.3	96.7	25	
E378 × D298	77.5 ± 0.6	80.6 ± 0.4	79.0		25	92.0 ± 0.7	91.1 ± 2.8	91.6	50	
E385 × D298	79.0 ± 0.6	82.5 ± 0.3	80.7	*	12	96.3 ± 0.7	95.3 ± 0.6	95.8	33	
A447 × D298	75.6 ± 0.6	78.5 ± 0.3	77.1	*	45	99.2 ± 0.0	98.5 ± 1.5	98.9	1	
A478 × D298	76.6 ± 0.5	79.4 ± 0.3	78.0		33	98.4 ± 0.7	97.5 ± 1.3	97.9	15	
A480 × D298	74.5 ± 0.6	80.2 ± 0.2	77.4		40	100.0 ± 0.0	97.5 ± 1.6	98.7	5	
A483 × D298	79.3 ± 0.4	82.3 ± 0.3	80.8	*	9	96.4 ± 0.7	95.6 ± 1.0	96.0	31	
E292 × B330	76.4 ± 0.3	79.4 ± 0.3	77.9		36	87.7 ± 3.8	86.7 ± 2.3	87.2	**	54
C344 × B330	75.8 ± 0.3	78.8 ± 0.4	77.3		42	96.9 ± 1.4	96.0 ± 1.9	96.4		28
D394 × B330	78.0 ± 0.6	81.0 ± 0.4	79.5		21	85.5 ± 2.7	84.6 ± 1.8	85.0	***	55
E347 × B330	78.2 ± 0.8	81.1 ± 0.6	79.6		20	96.8 ± 1.3	94.4 ± 3.2	95.6		35
E348 × B330	76.7 ± 0.7	79.7 ± 0.3	78.2		32	95.0 ± 3.2	94.2 ± 0.8	94.6		39
E359 × B330	77.8 ± 0.2	80.8 ± 0.6	79.3		23	99.1 ± 0.0	98.2 ± 1.4	98.7		7
E372 × B330	76.2 ± 0.1	79.4 ± 0.3	77.8		38	89.5 ± 3.1	88.6 ± 2.2	89.0	*	53
E376 × B330	81.4 ± 0.6	84.1 ± 0.2	82.8	***	2	83.8 ± 2.3	80.7 ± 1.0	82.3	***	56
E378 × B330	75.1 ± 0.4	78.1 ± 0.4	76.6	**	48	92.0 ± 0.6	91.3 ± 1.7	91.6		48
E385 × B330	80.1 ± 0.0	82.7 ± 0.1	81.4	**	6	95.1 ± 2.6	92.4 ± 1.4	93.8		41
A447 × B330	75.8 ± 0.5	78.4 ± 0.2	77.1	*	44	93.1 ± 0.4	90.4 ± 1.5	91.8		47
A478 × B330	74.9 ± 0.2	80.2 ± 0.6	77.6		39	92.3 ± 0.6	91.3 ± 0.7	91.8		46
A480 × B330	73.9 ± 0.4	75.0 ± 0.2	74.4	***	56	96.6 ± 1.3	94.1 ± 2.0	95.3		36
A483 × B330	75.1 ± 0.5	77.7 ± 0.6	76.4	**	51	97.1 ± 1.8	94.4 ± 3.6	95.8		33
E292 × C336	77.6 ± 0.6	83.0 ± 0.3	80.3	*	17	96.4 ± 0.6	95.6 ± 1.7	96.0		29
C344 × C336	75.3 ± 0.7	78.3 ± 0.1	76.8	**	46	97.9 ± 1.3	97.0 ± 0.7	97.4		17
D394 × C336	79.2 ± 0.9	82.3 ± 0.4	80.7	*	10	95.5 ± 1.2	94.6 ± 1.5	95.0		38
E347 × C336	79.1 ± 0.4	82.1 ± 0.4	80.6	*	13	98.5 ± 0.6	97.6 ± 1.6	98.0		14
E348 × C336	75.9 ± 0.2	78.8 ± 0.4	77.3	*	41	96.3 ± 1.4	95.4 ± 1.3	95.8		32
E359 × C336	76.8 ± 0.2	80.0 ± 0.3	78.4		30	100.0 ± 0.0	97.2 ± 1.4	98.6		9
E372 × C336	76.5 ± 0.3	80.0 ± 0.3	78.3		31	94.7 ± 1.7	93.9 ± 3.1	94.3		40
E376 × C336	82.2 ± 0.2	84.9 ± 0.3	83.6	***	1	92.2 ± 0.7	91.4 ± 1.3	91.8		45
E378 × C336	78.0 ± 1.4	80.6 ± 0.4	79.3		22	98.5 ± 0.7	97.7 ± 1.4	98.1		11
E385 × C336	80.6 ± 0.7	83.4 ± 0.2	82.0	***	3	94.1 ± 3.4	93.3 ± 0.5	93.7		42
A447 × C336	76.5 ± 0.6	79.1 ± 0.2	77.8		37	99.2 ± 0.0	98.4 ± 0.9	98.8		2
A478 × C336	75.9 ± 1.0	76.7 ± 0.7	76.3	***	52	97.7 ± 1.4	96.8 ± 0.9	97.2		22
A480 × C336	73.9 ± 1.0	77.3 ± 0.4	75.6	***	53	96.4 ± 0.7	95.6 ± 1.8	96.0		30
A483 × C336	77.2 ± 0.2	79.9 ± 0.4	78.5		28	98.5 ± 0.6	97.7 ± 0.2	98.1		12

Table 5. Cont.

Hybrid	DM 2021	DM 2022	Average DM	DM Rank	UP 2021	UP 2022	Average UP	UP Rank
E292 × D328	74.9 ± 0.6	78.4 ± 0.3	76.7 *	47	90.8 ± 1.3	90.0 ± 1.8	90.4 *	52
C344 × D328	79.1 ± 0.4	82.1 ± 0.1	80.6	14	92.6 ± 3.6	91.7 ± 1.4	92.1	44
D394 × D328	80.1 ± 0.5	83.1 ± 0.5	81.6 **	4	92.0 ± 0.5	91.2 ± 1.5	91.6	49
E347 × D328	77.2 ± 0.3	80.6 ± 1.1	78.9	26	97.7 ± 0.7	96.8 ± 1.3	97.2	21
E348 × D328	76.7 ± 0.4	79.3 ± 0.2	78.0	33	97.2 ± 0.7	95.8 ± 2.2	96.5	26
E359 × D328	78.5 ± 0.4	81.2 ± 0.2	79.9	19	97.8 ± 0.6	96.9 ± 2.4	97.4	18
E372 × D328	79.8 ± 0.5	82.5 ± 0.3	81.1 **	8	94.0 ± 2.2	91.6 ± 1.0	92.8	43
E376 × D328	80.0 ± 0.0	83.1 ± 0.2	81.5 **	5	92.2 ± 0.7	90.7 ± 2.0	91.5	51
E378 × D328	74.2 ± 0.8	76.9 ± 0.5	75.6 ***	54	98.3 ± 1.7	95.8 ± 1.1	97.0	23
E385 × D328	76.9 ± 0.1	80.9 ± 0.4	78.9	27	96.8 ± 0.0	93.6 ± 1.3	95.2	37
A447 × D328	76.6 ± 0.5	79.4 ± 0.3	78.0	35	99.5 ± 0.7	96.8 ± 2.2	98.1	10
A478 × D328	76.1 ± 0.2	78.5 ± 0.2	77.3	42	96.9 ± 2.3	96.0 ± 2.5	96.4	27
A480 × D328	73.6 ± 0.3	79.6 ± 0.3	76.6 *	49	97.7 ± 0.7	96.8 ± 1.7	97.3	20
A483 × D328	74.9 ± 0.1	78.3 ± 0.1	76.6 *	50	99.2 ± 0.0	98.4 ± 0.9	98.8	3
CT1 Turda 332	78.0 ± 0.8	81.8 ± 0.5	79.9		97.1 ± 1.8	97.0 ± 1.5		
CT2 Turda 335	78.9 ± 0.7	81.4 ± 0.7	80.2		100 ± 0.0	97.7 ± 1.2		

$p = 0.05$ (*), $p = 0.01$ (**) and $p = 0.001$ (***).

4. Discussion

In many areas of Romania, including that where the experiment was conducted, there is a high probability that low temperatures will occur in the first period of maize vegetation. This happened in the two experimental years, when temperatures below 10 degrees were recorded even in May. In the context of an ever-changing climate, the likelihood of low temperatures will continue in the future, both in Romania and in many other areas of Europe [20,41]. European flint germplasm is often described as being more tolerant to this abiotic factor [20,21]. Several breeders have reported positive results in terms of the tolerance to low temperatures of hybrids obtained by crossing flint lines with dent lines [19].

One of the main problems that compromises the maize crop is the occurrence of high temperatures, often associated with water scarcity, in one of the most sensitive periods: anthesis [42,43]. In recent years, in Romania, this phenomenon has been encountered; in many areas of the country, the yield was significantly affected or even totally compromised [44]. In order to develop maize hybrids that continue to achieve good yields, despite unfavorable conditions, it is necessary to understand and explore maize germplasms to identify tolerant genotypes. Improving drought tolerance in maize uses both conventional techniques (backcrossing, double-haploidy) and modern genomics-assisted methods (marker-assisted selection, genome-wide association studies for gene identification, genomic selection, genome editing technologies) [45–48]. Flint maize can perform well, even at high temperatures [49], conditions encountered in the two experimental years. The tested hybrids performed quite well at high temperatures during the anthesis period, as evidenced by the ASI index values [50]. From this point of view, E347 stood out, as this line transmitted the best flowering–silking coincidence, while no differences were observed for the paternal lines. Even though, in some hybrids, the silk appeared late compared to flowering, the difference was not large enough to negatively influence pollination. With the increase in the incidence of higher temperatures during the summer, the objectives of the Maize breeding laboratory at ARDS Turda include the creation of genotypes with a lower ASI index. For both inbred lines and hybrids, the date of silk emergence and flowering is noted every year.

High temperatures and prolonged drought can cause physiological changes in maize plants, with some showing early senescence. In breeding programs, it is desired to avoid early senescence, as the quality of the grains decreases in this case. Stay-green indicates good plant health and post-anthesis drought tolerance [34] and is often linked to higher yields [43,51–53]. Tolerance to high temperatures and prolonged drought has been observed

in some hybrids that remained green at physiological maturity. The three highest yielding hybrids, A478 × D328, A480 × B330 and A480 × D328, were noted as SG, senescence being observed only after physiological maturity.

Flint inbred lines can be used in crosses to transmit favorable traits, especially for the grains (texture, color and composition), but these genotypes also come with a series of less desirable traits, such as lower yield, small grains and lower plant growth [10]. Compensation for lower yields can be achieved by using productive lines as maternal genotypes, either with a dentate grain or a combination of it with flint. The resulting hybrids can meet both the requirements of the food industry (texture and color of flour for polenta or other products made from maize flour) and a higher yield, which will please farmers. Following the crossing of elite lines with flint testers, some hybrids were higher yielding, compared to both the other hybrids tested and some hybrids registered and cultivated in the area. The three highest yielding hybrids, A478 × D328, A480 × B330 and A480 × D328, had an average yield of 10,168 kg ha⁻¹, 9707 kg ha⁻¹ and 9586 kg ha⁻¹, respectively. Productive superiority over the controls was observed only in the favorable year 2021, while in 2022, both controls had yield of about 1000 kg ha⁻¹ higher.

In maize breeding it is very difficult to combine several characters of interest, since they are most often negatively correlated. Higher yielding hybrids with a reduced number of lodged plants have a lower DM (Figure 5). Even if the DM is lower, the values are consistent with the hybrids generally cultivated in the area, as they can reach technical maturity before frost. Sometimes hybrids with larger cobs are more prone to plant lodging, but the values obtained for UP still indicate the superiority of the studied genotypes from this point of view as well. The number of lodged plants is still quite low.

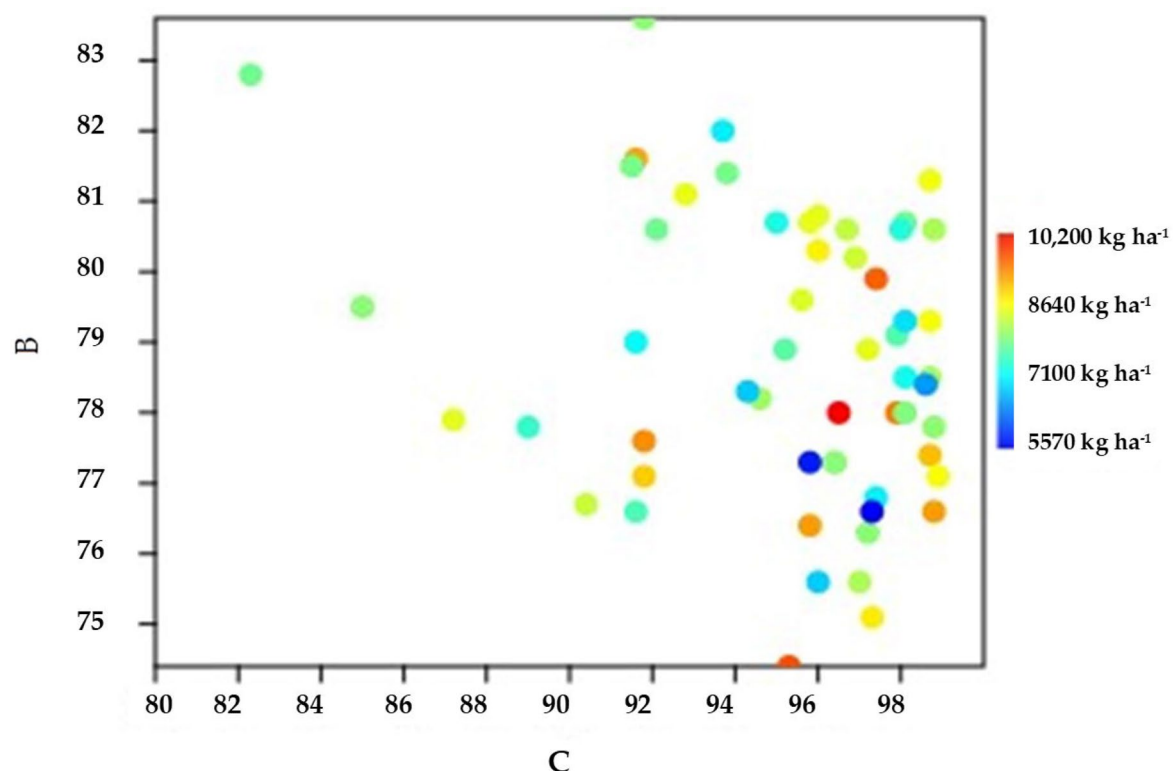


Figure 5. Yield, DM and UP of the studied hybrids.

Among the 56 hybrids studied, three stood out (A478 × D328, A480 × B330 and A480 × D328) for yield, stay-green status, good coincidence at anthesis and a high percentage of unlodged plants (96.4%, 95.3% and 97.3%). Even though the DM accumulated in the grains until harvest had slightly lower values compared to the rest of the hybrids, the

association of the other favorable traits led us to continue testing them and even to propose one them (A478 $\times$ D328, Figure 6) for registration testing.



Figure 6. An ear of A478 $\times$ D328 (semi-dent), the highest yielding hybrid.

5. Conclusions

The maternal lines A478 and A480 were noted for a higher GCA and yield, and can still be used as elite lines for other crosses. The highest yielding hybrids, A478 $\times$ D328, A480 $\times$ B330 and A480 $\times$ D328, exceeded the experimental average in favorable conditions, even with the registered hybrids used as controls. These hybrids were tested in the following years, and the best one, A478 $\times$ D328, is to be tested for registration. Considering that most hybrids in our country have dent grains and that the supply of flint maize hybrids is limited in Romania, the new hybrid could have the potential to be registered and cultivated.

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Abbreviations

The following abbreviations are used in this manuscript:

ARDS	Agricultural Research and Development Station
GCA	General combining ability

SCA	Specific combining ability
UP	Unlodged plant
DM	Dry matter
ASI	Anthesis-to-silking interval

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