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The grain yield ability of early maize hybrids under combined high densities and water stress environments

Capacitatea de producție a unor hibrizi timpurii în condiții de stres combinat: desime ridicată și stres hidric

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ABSTRACT

Breeding early maize hybrids with high genetic potential for grain yield and stability, under different environmental conditions is an important objective for this crop management. Consequently, this study was carried out to evaluate in Transylvania-Romania: 1) the behaviour of ten early maize hybrids developed at the Agricultural Research and Development Station (A.R.D.S.) Turda, in different plant densities and water stress environments; 2) the relationship between grain yield and other agronomic traits and 3) identify the hybrids with the highest yield and the most stable under stress conditions. A split-plot design was used in a completely randomized block arrangement with three replications. The main plots were assigned to plant densities and secondary plots to ten maize hybrids. This study was conducted in three years 2021, 2022, and 2023. The hybrids were evaluated in four plant densities: low (LD = 60,000), medium (MD = 70,000), high (HD1 = 80,000 and HD2 = 90,000) plants/ha, in combination with three climatic regimes, non-irrigated. Among the recently registered maize hybrids, the following stood out for their superior grain yield, tolerance to high-density and water stress: Turda 380, Turda 2020, Turda 335, and the new hybrid SUR 18 /399. The high grain yield as well as the tolerance to high density and water stress of Turda 380, Turda 2020, and Turda 344, demonstrated the genetic value of the common parental line, to simultaneously inherit these traits.

Keywords: yield, hybrids, maize, tolerance

REZUMAT

Ameliorarea hibrizilor timpurii de porumb cu potențial genetic ridicat pentru producția de boabe și stabilitatea acesteia, în diferite condiții de mediu, este un obiectiv important pentru gestionarea acestei culturi. Acest studiu a fost realizat pentru a evalua în Transilvania-România: 1) comportarea a zece hibrizi timpurii de porumb, creați la Stațiunea de Cercetare Dezvoltare Agricolă (S.C.D.A.) Turda, la diferite desimi ale plantelor și medii de stres hidric; 2) determinarea relației dintre producția de boabe și alte trăsături agronomice și 3) identificarea hibrizilor cu cea mai ridicată producție și cea mai bună stabilitate în condiții de stres. Experiența a fost așezată conform metodei blocurilor randomizate, în trei repetiții. Principalele parcele au fost atribuite desimilor, iar subparcelele la zece hibrizi de porumb. Acest studiu a fost realizat în trei ani experimentali: 2021, 2022 și 2023. Hibrizii au fost evaluați la patru desimi: scăzută (LD = 60.000), medie (MD = 70.000), ridicată (HD1 = 80.000 și HD2 = 90.000) plante/ha, în combinație cu trei regimuri climatice, în sistem neirigat. Dintre hibrizii studiați s-au remarcat prin producții superioare, toleranță la densitatea ridicată și stresul hidric: Turda 380, Turda 2020, Turda 335 și noul hibrid SUR 18 /399. Producția ridicată de boabe, precum și reacția de toleranță la densitatea ridicată și stresul hidric al hibrizilor studiați Turda 380, Turda 2020 și Turda 344, au demonstrat valoarea genetică a liniei parentale comune, pentru a moșteni simultan aceste caracteristici.

Cuvinte cheie: producția, hibrizi, porumb, tolerant

INTRODUCTION

The climate changes over the last two decades, characterized by an increase in the frequency of hot and dry summers (Bruce et al., 2002; Cairns et al., 2013, Has et al., 2022) have led to different responses in maize hybrids. In recent years, active temperatures (≥10 °C) have increased, while total precipitation and sunshine hours have decreased. This climate change led to a reduction in potential yield, especially for very early and early maize hybrids (FAO groups 100-280), in Transylvania-Romania. This fact aroused the interest of breeders in finding new methods to select productive maize hybrids, even under heat and water stress conditions (Has et al., 2008; Tokatlidis, 2013; Nasser et al., 2020). Cooper et al. (2014) considered that important changes in maize breeding strategy are necessary to improve grain yield in drought-prone regions and proposed wide-area testing in multi-environment trials. In field trials, water stress drastically affected yield and assimilation rate, more than high density. Badu-Apraku et al. (2022) proposed an indirect selection method to improve maize yield under stress conditions, by exploiting genotypes with specific adaptations and selecting maize hybrids with better stability.

Creating new climate-adapted maize hybrids is a potential solution, but this approach requires understanding the complex adaptive agronomic characteristics of maize under climate change conditions (Bonea and Urechean, 2020). Several researchers indicated that hybrids suffer more from poor precipitation distribution, especially in the critical phenophases for maize development, with rainfall being insufficient for normal densities, although the total amount of precipitation required during the entire maize vegetation period was adequate (Sangoi et al., 2002; Carena et al., 2009; Fasoula and Tollenaar, 2005). Therefore, when drought prevails, lower densities are indicated to ensure survival and achieve production capacity (Duvick, 2005; Berzsenyi and Tokatlidis, 2012).

Selecting genotypes with high yield under drought stress is a stated objective of many maize breeding programs. However, direct selection is difficult in many years and locations because of the irregular occurrence of drought, which can be severe one season and nonexistent in the next. Alternatively, breeders have attempted to use physiological or morphological measures other than yield under water stress in selecting for drought tolerance such as the date of silk emergence tends to be delayed more than the date of anthesis, when plants are stressed, also the selection of hybrids for improved drought resistance can be done by screening pre-commercial hybrids at high plant densities (Bonea and Urechian, 2020). Badu-Apraku et al. (2022, 2023) recommended selection for several characteristics that contribute to improving grain yield and its stability under drought stress: high kernels number per ear, rolling and erect leaves, higher leaf and stem length, low tassel size and number of branches.

The yield increase observed in recent years is attributed to genetic improvement, improved crop management practices (Al-Naggar et al., 2016a) and greater tolerance of modern hybrids to low soil-moisture stress (Dwyer et al., 1992) and weed interference (Tollenaar and Lee, 2002). According to Duvick (2005), although breeders have always had the primary objective of improving maize grain yield, the need to simultaneously select hybrids with increasing stress tolerance was added. Newer hybrids yield more under both favorable and unfavorable growing conditions. The improved grain yield per unit area of modern maize hybrids is due to increased optimal plant density rather than the improved grain yield per/plant (Tokatlidis et al., 2011).

Selection for tolerance of the newer hybrids to various stresses has played an important role in the improvement of maize yields (Tollenaar and Lee, 2002; Duvick, 2005; Kamara et al., 2006). Individual hybrids respond differently to plant densities (Sangoi et al., 2002; Has et al., 2008) and periodic reassessment of optimal hybrid plant density is necessary (Widdicombe and Thelen, 2002).

Plant density for maximum grain yield varies depending on water availability, soil fertility, maturity (FAO group), sowing date and row spacing. When the number of individuals per area increases above the optimal plant density, some consequences are detrimental to ear ontogeny and result in barren plants, increased anthesis-silking interval (ASI) (Sangoi et al., 2002; Tokatlidis et al., 2010; Sher et al., 2017), reduced kernel number per unit area, respectively the main yield component of maize. High plant density affects plant architecture and alters growth and development patterns (Câmpean, 2009; Sarca, 2004; Tokatlidis and Koutroubas, 2004, Has et al., 2021). The effective use of increased densities requires, first, the modification of leaf orientation. Plants with leaves above the ear oriented at 20-30° from the vertical along their entire length will be the most advantageous structures of the fields (Sangoi, 2001; Haș et al., 2021).

Newer hybrids have reduced lodging at higher plant densities and a better ability to withstand abiotic stress, resulting in fewer barren plants. By using high plant densities ≥70000 plants/ha, some productivity elements (ear and kernels weight/ear, ear length and the number of rows/ear) decreased, but the final yield was not influenced, as the yield/plant losses were compensated by the higher number of plants per area. It is well established that newer maize hybrids achieve maximum crop yield at a narrow density range, becoming density-dependent and vulnerable to environmental variation (Fasoula and Tollenaar, 2005; Tokatlidis et al., 2015).

Fasoula and Fasoula (2002) explained that tolerance to higher plant densities is mainly the result of the incorporation of genes that confer tolerance to various biotic and abiotic stresses, due to the screening applied under a wide range of environmental conditions. According to Tokatlidis and Koutroubas (2004), the yield capacity of maize hybrids has not changed, what would differentiate the recently developed hybrids from older ones would be their tolerance to high crop density, which in the last 70 years has increased (Kamara et al., 2006; Tokatlidis et al., 2015).

One of the major improvement objectives pursued at the Maize Breeding Laboratory at A.R.D.S. Turda – Romania is to create maize hybrids that are drought tolerant, but also early enough to complete their life cycle in a certain season (Has, 2001; Has et al., 2020). However, when the amount of precipitation is higher than average, precocity implies a yield "penalty".

Therefore, the objectives of the present study were: (I) to evaluate, under conditions of high densities and water stress, the grain yield of early-maturing maize hybrids developed in recent years, (II) to determine the relationship between grain yield and other agronomic traits of early-maturing maize hybrids and (III) identify high-yielding and stable hybrids under high densities and water stress environments for trade in the region of Transylvania-Romania.

MATERIAL AND METHODS

Study site

Field research was conducted at the Agricultural Research and Development Station (A.R.D.S.) Turda, in the Transylvanian Plain, Romania. The climatic conditions of the area correspond to the FAO groups 250-380, in spring usually there are low temperatures, and the growing season is shorter compared to the rest of the country (Has et al., 2022) so early and semi-early hybrids are recommended. Therefore, the shorter growing season in the central and northern areas of the country forces farmers to cultivate early maize hybrids, at higher densities, optimal for those regions (60,000 - 70,000 plants/ha) (Câmpean, 2009; Ghețe et al., 2021; Vana et al., 2022).

Cultivation technology

The soil analysis in the experimental plots indicated that the dominant soils in the area are vertical-illuvial chernozem, the most important biochemical indices have the following average values: humus content over 3.5%, mobile phosphorus content is 4.5 mg P_2O_5/100 g soil and mobile potassium content is over 30 mg K_2O/100 g sol and the soil reaction is neutral, between 6.2 and 6.8 pH units (Tinca, 2017).

The field was ploughed in the fall and a shallow pass was made with the combinator in the spring. The previous crop at the test site was winter wheat. Before sowing 400 kg/ha of complex mineral fertilizer NPK 27:13.5:0 was applied. Weeds were controlled using two herbicides, as follows: 1.2 L/ha a.s. dimethenamid-p (720 g/L) at preemergence (Spectrum, produced by BASF, Germany) and 2 L/ha a.s. tembotrione (44 g/L) and isoxadiphen-ethyl (22 g/L) at post-emergence (Laudis 66 OD, produced by Bayer AG, Germany).

The field trials were sown in the last decade of April 2021-2023. There were four rows in each sub-plot; the rows were 7.0 m long and were spaced 0.70 m apart.

Experimental design

The experimental field had the following three factors:

1) experimental year, with three graduations: 2021, 2022, and 2023;
2) plant density, with four graduations: low density (LD) = 60,000 plants/ha, medium density (MD) = 70,000 plants/ha, and two high plant density (HD1) = 80,000 and (HD2) = 90,000 plants/ha;
3) maize hybrids, with ten graduations. The ten hybrids studied (Table 1) were developed and registered by A.R.D.S. Turda – Romania.

Table 1. Hybrids used in the study

Hybrid	Registration year	Hybrid* type	FAO group
Turda 248	2012	SC	300
Turda Star	2005	TC	370
Turda 332	2014	SC	380
Turda 344	2017	TC	380
Turda 335	2021	SC	380
Turda 2020 Nrf	2021	SC	380
Turda 2020 cms	2021	SC	380
Turda 380	2022	SC	380
SUR 18/399	New	SC	380
HST 148	New	SC	380

*SC = single cross; TC = three-way cross; Nrf = seed production detasseling; cms-C = seed production of the hybrid by using cytoplasmic male sterility maternal line (Columbia-type)

Data analysis

Grain yield was recorded from the two central rows of each split-plot that were harvested separately, and the grain yield per hectare (ha) computed at the 14.0% grain moisture, was calculated from the shelled grain. The total number of plants and ears were counted in each plot before harvest. The number of ears/plant was calculated as the total number of ears at harvest divided by the total number of plants. The ears harvested from each plot were shelled and the grain moisture was determined using a Granomat moisture tester (PFEUFFER – GMBH –Germany).

The following yield-related traits were measured using 20 random ears/plots at harvest: Grain yield per plant (GYP) (g); Number of rows per ear (NRE) and ear length (EL) (cm). 1000 – kernels weight (TKW) (g) adjusted at 14.0% grain moisture was determined using shelled grains of each plot. Grain yield per hectare (GYA) (tons/ha) was determined by adjusting grain yield/plot to grain yield kg/hectare. Barren stalks (BS) (%) represent the percentage of plants bearing no ears relative to the total number of plants in the plot. SE, the number of small ears at harvest was also counted.

The stress tolerance index (STI) modified from the equation suggested by Fageria (1992) (according to Naggar et al., 2016) was used to classify genotypes for tolerance to high-density stress. The formula used is as follows:

$$STI = \frac{Y_1}{AY_1} \times \frac{Y_2}{AY_2}$$

where, Y_1 = grain yield mean of a genotype at non-stress; AY_1 = average yield of all genotypes at non-stress; Y_2 = grain yield mean of a genotype at stress; AY_2 = average yield of all genotypes at stress. When *STI* is ≥ 1.0, it indicates that the genotype is tolerant (T), If *STI* is < 1, it indicates that the genotype is sensitive (S).

Comparing the effect of combining the two stresses (plant density and water stress) in 11 environments (from E2 to E12), with the control non-stressed environment (E1) (2021 LD), expressed in change percentages gives a picture of the effects of the high stress on the 8 studied traits (GYA, GYP, EL, NRE, GM, TKW, BS, SE) was calculated by the formula used by Al Naggar et al. (2016)

$$Change = \frac{100 \times (E_1 - RE)}{E_1}$$

where, *RE* = Respective environment, *E1* = normal environment.

Comparison of means was performed by least significant difference (LSD) after analysis of variance for a three-factor split-plot design: years × density × hybrid.

Analysis of the variance (ANOVA) was performed using Past4. The significance of the factors was achieved by the difference from the factor mean, based on the least significant difference (LSD), after calculating the factors (variances) analysis for a multifactorial experiment with split plots.

Climatic conditions

The meteorological data (Figure 1) used were obtained from Turda Meteorological Station, longitude 23°47`, latitude 46°35`, altitude 427 m.

The year 2021, favorable for maize cultivation, can be assessed as warm (+131.5 °C compared to a normal multiannual of 62 years) in terms of the evolution of temperatures between April and August, and regarding the rainfall regime, a slightly rainy year (+3.8 mm), dry conditions in June, and excessively rainy in July.

The year 2022 can be characterized as warm (+293.3 °C compared to the normal multiannual temperature of 62 years, and with a rainy regime (+30.8 mm), during the maize vegetation period, but the months of June and July, considered two important months for achieving the harvest, were excessively dry, while the last week of August and September were excessively rainy, which led to the extension of the maize vegetation period, by slowing down the rate of loss of water from the grains.

The year 2023 can be characterized as warm, with a poor water supply in May and the first decade of June, which influenced the uneven emergence of plants and a low rate of vegetative development. August and September were rainy, which influenced the extension of the maize vegetation period.

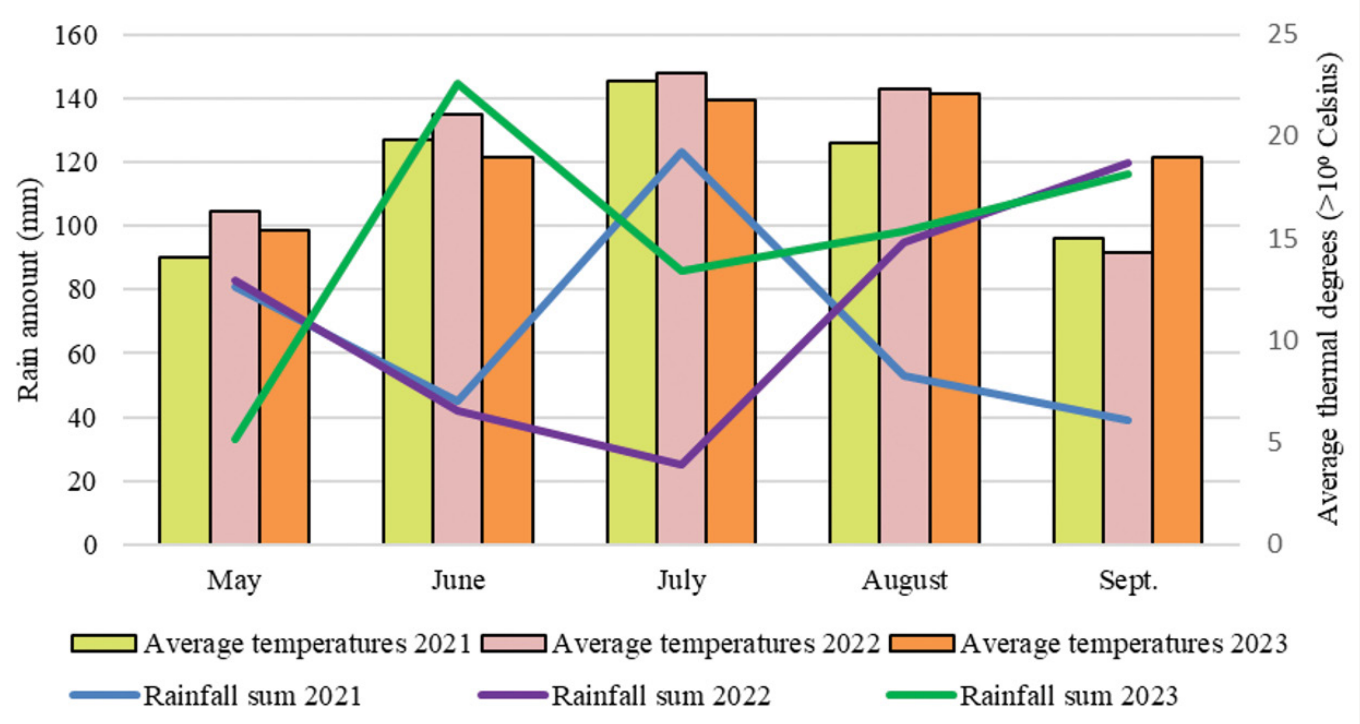

Figure 1. Average utility thermal degrees and rainfall during the maize growing season, in Turda (2021-2023)

RESULTS AND DISCUSSION

Analyses of variance and genotype by environment interaction of grain yield and other agronomic traits

Analysis of variance over three years (Y) of the split-plot design for the studied 10 maize hybrids (H), under four plant densities (D) is presented in Table 2.

The results of the combined analysis of variance (ANOVA) in the three-years and four densities environments showed significant ($P < 0.05$) mean squares for: year, density and hybrid, for grain yield and other measured traits. Additionally, significant mean squares were observed for Y × H, and D × H interactions, for grain yield and most measured traits. Similarly, significant ($P < 0.05$) mean squares for Y × D × H interaction were observed for most traits, except for the number of rows/ears and small ears at harvest which were not significant.

The mean squares due to the Y × D × H were significant or highly significant for all traits studied, indicating that the reaction of maize hybrids differs from one density to another and from one year to another. Several researchers have supported the idea that selection for the simultaneous improvement of several characters is complicated, under only the influence of high plant densities (Duvick et al., 2003; Has et al., 2008; Al-Naggar et al., 2015).

The effect of climate change

In the areas relying on growing season rainfall, such as Turda-Romania, there is considerable interannual variation in the quantity of total rainfall and its distribution (Has et al., 2022; Simon, 2022), an aspect noted also between 2021-2023. In some years, grain yield can be significantly reduced by transient water limitations of varying critical timing, duration, and severity (Table 3).

The hybrids achieved lower grain yields in 2022 and 2023 compared to 2021, due to the water stress, especially at the higher densities. In July 2022, total precipitation was deficient, at only 25.2 mm, most of which occurred in the first ten days of the month, while in July 2021, higher precipitation (123.1 mm) and above the normal amounts of the month were recorded. The drought in June (-42.8 mm) and July (-52.8 mm) 2022 were more damaging to maize because they coincided with a critical period for

Table 2. Combined analysis of variance across 2021-2023 (% sum of squares) of split plot design for studied 10 maize hybrids under four plant densities

Source of variance	df	GYA	GYP	EL	NRE	GM	TKW	BS	SE
		s^2 (Mean squares)							
Year (Y)	2	743.06^{**}	$96,721^{**}$	919.2^{**}	0.1	$1,088^{**}$	$53,449^{**}$	$4,249^{**}$	$1,889^{**}$
Error (Y)	4	0.38	103	0.1	1.3	0.1	117	182	49
Density (D)	3	3.07^{**}	$5,734^{**}$	24.9^{**}	4.2^{**}	2.0^{**}	$1,575^{**}$	698^{**}	155^{**}
Y × D	6	4.73^{**}	390^{ns}	4.5^{**}	1.8^{*}	8.8^{**}	$1,550^{**}$	67^{ns}	23^{ns}
Error (D)	18	0.32	133	0.6	0.3	0.3	144	50	30
Hybrid (H)	9	24.77^{**}	$1,292^{**}$	31.5^{**}	67.6^{**}	17.1^{**}	$19,046^{**}$	454^{**}	285^{**}
Y × H	18	4.64^{**}	$1,015^{**}$	4.3^{**}	4.9^{**}	5.4^{**}	$3,440^{**}$	242^{**}	22^{ns}
D × H	27	1.11^{**}	244^{**}	0.7^{**}	0.8^{*}	1.0^{**}	177^{ns}	40^{**}	44^{**}
Y × D × H	54	0.99^{**}	279^{**}	0.9^{**}	0.6^{ns}	0.7^{**}	215^{*}	39^{**}	17^{ns}
Error (H)	216	0.17	110	0.3	0.5	0.4	134	21	20

GYA, grain yield/area (kg/ha); GYP, grain yield/plant (g/plant); EL, ear length (cm); NRE, number of rows per ear (no.); GM, grain moisture at harvest (%); TKW, 1000-kernel weight (g); BS, barren stalks (%); SE, small ear; *,** significant at $P < 0.05$ and $P < 0.01$ probability levels; ^{ns} = non-significant plant development, when reproductive organs appear, pollination, grain formation and filling occur (Sarca, 2004; Has et al., 2022).

The experimental mean yield was higher in the first year, at 10.53 tons/ha compared with the second and third years, when the mean yields were 5.92 tons/ha and 6.62 tons/ha, respectively. The highest yield was obtained in 2021, 56% higher than in 2022, respectively 63% higher than in 2023. The mean grain yield differences between the three years were significant, with LSD being 0.22 tons/ha.

The climatic conditions during the three years influenced the grain yield, mainly due to the uneven distribution of precipitation. The response of the hybrid plants to the drought and heat of 2022 and 2023 was different. The hybrids Turda 380, Turda 2020 and the new hybrid SUR 18/399 significantly exceeded the average of the ten hybrids, in all three years of experimentation. Nevertheless, considering the LSD of 0.34 tons/ha (for Y × H interactions), only hybrids Turda 380, Turda 335, and SUR 18/399 had significantly higher yields both in 2022 and 2023.

Effect of high plant density

The effects of higher plant density on the means of studied characters are presented in Table 4. The environments LD (60,000 plants/ha) and MD (70,000 plants/ha) represent the non-stressed ones, while HD1 (80,000 plants/ha) and HD2 (90,000 plants/ha) represent higher plant densities (stressed) environments. The increased plant density and water stress at the flowering stage in maize caused a decrease in grain yield/plant. The hybrids Turda 2020 and Turda 380 had the highest yield at the four densities, whereas Turda Star had the lowest yield at the LD and both high plant densities. Mean grain yield/plant was significantly reduced due to the increase in plant density from LD to MD, HD1 and HD2, by 8%, 10%, and 17%, respectively (Tables 4 and 5).

Table 3. Mean grain yield (tons/ha) of each hybrid under four plant densities and across three years

Hybrid	Experimental year					
	2021		2022		2023	
	tons/ha	± average	tons/ha	± average	tons/ha	± average
Turda 248	8.91	-1.62^{000}	5.22	-0.70^{000}	6.42	-0.17^{ns}
Turda Star	8.09	-2.44^{000}	4.92	$-1,00^{000}$	5.67	-0.93^{000}
Turda 332	11.08	0.55^{**}	5.17	-0.76^{000}	4.71	-1.88^{000}
Turda 344	10.31	-0.22^{ns}	6.01	0.09^{ns}	6.50	-0.09^{ns}
Turda 335	11.29	0.77^{***}	6.36	0.44^{*}	6.97	0.38^{*}
Turda 2020 Nrf	11.25	0.72^{***}	6.29	0.37^{*}	7.59	1.00^{***}
Turda 2020 cmsC	10.57	0.04^{ns}	5.65	-0.28^{ns}	7.23	0.63^{***}
Turda 380	12.12	1.59^{***}	7.53	1.60^{***}	7.37	0.78^{***}
SUR 18/399	10.93	0.40^{*}	6.49	0.56^{**}	7.33	0.73^{***}
HST 148	10.74	0.21^{ns}	5.60	-0.33^{0}	6.15	-0.44^{00}
Average - check	10.53	-	5.92	-	6.60	-
LSD $_{0.05}$	Y = 0.22; H = 0.19; Y × H = 0.34					

$^{*, **, ***/ 0, 00, 000}$ significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, positive, respectively negative values; + = increase, - = decrease; ^{ns} = non-significant

This reduction was associated with reductions in all yield components, namely NRE, EL and TKW, indicating the importance of these traits as measures of tolerance to high density. At higher plant densities, the reduced grain yield also results from the increased anthesis-silking interval and the higher percentage of the barren stalks. This was also reported by Carena and Cross (2003), Tokatlidis and Koutroubas (2004), Has et al. (2008), Tokatlidis et al. (2010) and Al-Naggar et al. (2016a). The higher grain yield/ha (GYA) was obtained at MD, and the best hybrids were Turda 380 and Turda 2020, significantly superior to the average (Table 5).

Table 4. Differences (±) in means of studied traits from low density to medium (MD) and the two high densities (HD1 and HD2) across all studied hybrids 2021-2023

Plant density	GYA	GYP	EL	NRE	GM	TKW	BS	SE
	tons/ha	g	cm	no.	%	g	%	%
LD (check)	7.80	137.72	17.38	17.98	19.16	261.88	11.48	14.63
MD	$+0.08^{ns}$	-10.82^{000}	-0.70^{000}	-0.22^{ns}	$+0.04^{ns}$	0.04^{ns}	$+2.54^{*}$	$+1.75^{*}$
HD1	-0.27^{00}	-14.32^{000}	-0.92^{000}	-0.36^{00}	0.06^{ns}	-4.84^{0}	$+4.25^{***}$	$+2.05^{*}$
HD2	-0.28^{00}	-23.67^{000}	-1.56^{000}	-0.63^{000}	$+0.28^{**}$	-8.60^{000}	$+6.61^{***}$	$+3.16^{**}$
$LSD_{0.05}$	0.18	4.58	0.31	0.24	0.18	3.75	2.21	1.72

GYA, grain yield/area (tons/ha); GYP, grain yield/plant (g/plant); EL, ear length (cm); NRE, number of rows per ear (no.); GM, grain moisture at harvest (%); TKW, 1000-kernel weight (gram); BS, barren stalks (%); SE, small ear (gram); $^{*, **,***/0,00,000}$ significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, positive, respectively negative values; ^{ns} = non-significant; + = increase, - = decrease

Table 5. Mean grain yield (tons/ha) of each hybrid under four plant densities and across three years

Hybrid	Plant density							
	LD-60,000 pl/ha		MD-70,000 pl/ha		HD1-80,000 pl/ha		HD2-90,000 pl/ha	
	tons/ha	Signif.	tons/ha	Signif.	tons/ha	Signif.	tons/ha	Signif.
Turda 248	6.16	-1.64^{000}	7.54	-0.34^{ns}	6.81	-0.72^{000}	6.90	-0.62^{00}
Turda Star	6.70	-1.10^{000}	6.20	-1.68^{000}	6.21	-1.32^{000}	5.80	-1.72^{000}
Turda 332	7.58	-0.22^{ns}	7.48	-0.40^{0}	6.46	-1.07^{000}	6.42	-1.10^{000}
Turda 344	8.14	0.34^{ns}	7.91	0.03^{ns}	7.22	-0.31^{ns}	7.15	-0.37^{ns}
Turda 335	8.30	0.50^{**}	8.20	0.32^{ns}	7.96	0.43^{*}	8.38	0.86^{***}
Turda 2020 Nrf	8.35	0.55^{**}	8.54	0.66^{***}	8.26	0.73^{***}	8.37	0.85^{***}
Turda 2020 cmsC	8.03	0.24^{ns}	7.70	-0.18^{ns}	7.73	0.20^{ns}	7.80	0.28^{ns}
Turda 380	8.62	0.82^{***}	9.21	1.33^{***}	9.25	1.72^{***}	8.95	1.43^{***}
SUR 18/399	8.24	0.44^{*}	8.14	0.26^{ns}	8.26	0.74^{***}	8.35	0.83^{***}
HST 148	7.86	0.06^{ns}	7.88	0.00^{ns}	7.14	-0.39^{0}	7.10	-0.42^{0}
Average - check	7.80	-	7.88	-	7.53	-	7.52	-
$LSD_{0.05}$	D = 0.18; H = 0.19; D × H = 0.39							

$^{**, **, ***/0, 00, 000}$ significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, positive, respectively negative values; ^{ns} = non-significant

The higher plant densities (HD1 and HD2) caused a significant increase in GYA for some hybrids compared to LD, moreover, higher plant density caused a significant increase in barren stalks, small ears and higher grain moisture at harvest. Similar results were previously reported by other studies (Fasoula and Fasoula, 2002; Tokatlidis and Koutroubas, 2004; Câmpean, 2009; Al-Naggar et al., 2016a).

The grain yield capacity of the Turda 380 and Turda 2020 hybrids was the highest at MD, 9.21 tons/ha and 8.54 tons/ha, respectively. The hybrid Turda 380 was noted for achieving the highest grain yield, at all four densities (Table 5).

Turda Star and Turda 332 achieved significantly lower grain yield, at all four densities than the more recently registered hybrids. The yield capacity of the Turda 248 hybrid increased from 6.16 to 7.54 tons/ha when the density increased from LD to MD.

The analysis of the results on some components of the grain yield showed that the effect of the increased combined stress (high density and water stress) caused a decrease in GYA, EL, NRE, GYP and TKW (Table 6). The percentage of BS and SE increased from 8% to 18% and from 14.6% to 17.8%, respectively. The low number of ears per plant, a measure of sterility, strongly influenced grain yield.

The hybrids Turda 335 (3.6%), Turda 2020 (3.1%), and Turda 380 (3.1%) recorded a significant decrease in barren stalks, while other hybrids recorded significant increases at high densities, ranging from 9.3% to 12%. This suggests that reduced barrenness at high plant densities is linked to tolerance of maize hybrids to high plant densities (Sarca, 2004).

Optimum density

The dependence of maize hybrid yield on plant density is acknowledged because seed companies always recommend the optimum plant density for newer hybrids, each hybrid shows a different and very narrow optimum density, indicating that this should be considered in the development, selection, and evaluation of hybrids (Sangoi et al., 2002; Has et al., 2008; Tokatlidis et al., 2011). Tokatlidis (2000) suggested that selection under higher plant densities could be a means of improving maize grain yield.

Table 6. The effect of combined plant density and water stress environments (YxDxH) on some yield components of “Turda” maize hybrids

Hybrid	GYA	GYP	EL	NRE	GM	TKW	BS	SE
Turda 248	6.85^{000}	128	18.0^{***}	18.6^{***}	18.9^{00}	219^{000}	8.5^{ns}	19.67^{**}
Turda Star	6.23^{000}	116^{00}	18.3^{***}	16.4^{000}	18.5^{000}	240^{000}	7.8^{ns}	1.23^{ns}
Turda 332	6.99^{000}	113^{000}	15.4^{000}	18.4^{***}	19.1^{ns}	263^{ns}	12.0^{***}	3.64^{***}
Turda 344	7.61^{ns}	125^{ns}	16.9^{ns}	18.2^{*}	18.6^{000}	241^{000}	7.2^{ns}	-0.37^{ns}
Turda 335	8.21^{***}	132^{*}	15.8^{000}	15.3^{000}	19.5^{*}	295^{***}	3.6^{00}	-2.00^{ns}
Turda 2020 Nrf	8.38^{***}	134^{**}	16.8^{ns}	19.8^{***}	19.1^{ns}	250^{00}	3.1^{00}	-2.45^{0}
Turda 2020 cmsC	7.81^{ns}	126^{ns}	16.5^{ns}	19.3^{***}	18.9^{00}	249^{000}	6.3^{ns}	-1.29^{ns}
Turda 380	9.01^{***}	133^{*}	17.2^{***}	15.7^{000}	19.1^{ns}	278^{***}	3.1^{00}	-3.29^{00}
SUR 18/399	8.25^{***}	128^{ns}	16.4^{ns}	15.9^{000}	20.3^{***}	274^{***}	5.4^{ns}	-2.73^{0}
HST 148	7.50^{ns}	120^{ns}	14.5^{000}	19.3^{***}	20.5^{***}	277^{***}	9.3^{*}	3.95^{***}
Average (check)	7.68	126	16.6	17.7	19.2	259	6.6	16.37
$LSD_{0.05}$	0.19	6.01	0.33	0.42	0.28	5.38	2.11	2.08

$^{*, **, ***/0, 00, 000}$ significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, positive, respectively negative values; ^{ns} = non-significant

Among the ten hybrids tested, Turda 380 and Turda 2020 could be considered “independent to density” terms used in literature by Sangoi (2001) and Tokatlidis et al. (2011). The latest registered hybrids (Turda 335, Turda 2020, Turda 380), produce more ears/plant than the older hybrids. The highest yield was noted at MD, but the impact of increasing the plant densities was insignificant. At the same time, the grain moisture, barren stalk and small ears at harvest showed an increasing trend from non-stressed to high-density stressed environments. The hybrids Turda Star, Turda 332, and Turda 248, selected at medium population density, are intolerant to high plant populations of 90,000 plants/ha (Vana et al., 2024). Each of the ten hybrids studied showed a different and very narrow optimum density, indicating that the optimum plant density should be considered in the development and evaluation of hybrids (Sangoi et al., 2002; Has et al., 2008; Tokatlidis et al., 2011).

Significant increases in GYP, EL, NRE, and TKW and decreasing in BS, contributed to obtaining the highest grain yield for four hybrids (Turda 380, Turda 2020, Turda 335 and SUR 18/399), at the increased plant density combined with water stress (WS-HD). For each hybrid, it was possible to identify the optimal environment for obtaining the highest yield/ha. The results indicate that maize plants exposed to high plant density stress have reduced ears/plant, kernels/plant and kernel weight.

Hybrids tolerant to high plant densities were also noted for some plant characteristics, such as erect leaves (leaves above the ear oriented at 20-30° from the vertical along their entire length), high plant height and main ear insertion and the reduced number of ramifications in the tassel, and last but not least, for their prolificacy (Turda 380) (Tinca, 2017; Has et al., 2021; Haș et al., 2022).

Superiority of tolerant hybrids

To evaluate the tolerance of maize hybrids to environmental conditions, the stress tolerance index (STI) was calculated, using the equation suggested by Fageria (1992), under the stressed environments MD, HD1, and HD2 presented in Table 7.

The highest STI at medium and high density were calculated for the hybrids Turda 380 (1.31) and Turda 2020 (1.17), followed by hybrids Turda 335 (1.12) and SUR 18/399 (1.13) (table 8). These three hybrids had STI values greater than unity under the two stress environments and therefore could be considered tolerant to medium and high plant density stress. On the contrary, the hybrids Turda Star, Turda 248 and Turda 332 exhibited STI values less than unity under both stressed environments and therefore could be considered sensitive to medium and high plant density stress. Turda 344 hybrid was considered tolerant to MD, under both stress environments, and showed increases for the GYA, EL, and NRE, On the other hand, the most sensitive hybrid under both stress environments was Turda Star.

For GYA, GYP and TKW, the hybrids Turda 248, Turda Star, and Turda 332 showed a decrease due to the combined stress, with an optimum environment at E1 (low density, 2021); all three hybrids showed an increased index (STI) for BS and SE. Turda 344 had a high STI for NRE, EL, and GYA, in all environments. Regarding GYA, GYP, and TWK, Turda 335, Turda 380 and SUR 18/399 showed an increased STI in all combined stress environments.

The superiority of tolerant hybrids in GYA, under combined stress, was due to their superiority in GYP (Turda 2020 Nrf = 1.12%; Turda 380 = 1.12%; SUR 18/399 = 1.11%; Turda 335 = 1.08%) and was associated with superiority in TKW (Turda 335 = 1.28%; Turda 380 = 1.15% and SUR 18/399 = 1.10%), NRE (Turda 2020 Nrf = 1.26%), and EL (SUR 18/399 = 1.07%; Turda 380 = 1.06%; Turda 2020N = 1.02%). The superiority of the tolerant hybrids may be attributed to their high water use efficiency. These results are in agreement with those reported by Al-Naggar et al. (2016a), Al-Naggar et al. (2016b) and Tinca (2017).

Effects of stressed environments across all genotypes and years, for the studied traits on the 12 environments arranged (based on GYA) from the normal environment (E1) to highly stressed conditions (E8 and E12) (Table 9). E1 represents the unstressed environment (WW-LD

- well-watered and low plant density) and was used as a controlled environment, E2 represents WW-MD, E3 and E4 represent WW-HD1 and WW-HD2, in 2021. E5 represents only water stress from 2022 (WS1-LD), E6 represents WS1-MD, E7 is WS1-HD1, while E8 represents WS1-HD2. E9 represents only water stress from 2023 (WS2-LD), E10 represents WS2-MD, and E7 and E8 represent water stress/2023 combined with the two high-densities.

Table 7. Stress tolerance index (STI) for grain yield of maize hybrids under two factors of stress: density (MD, HD1, and HD2) and water stress (two years: 2022 and 2023), compared to the non-stress year (2021)

	Check (tons/ha)				STI							
	2021 (non-stress year)				2022				2023			
Hybrid / Plant density	LD	MD	HD1	HD2	LD	MD	HD1	HD2	LD	MD	HD1	HD2
Turda 248	6.51	10.39	9.82	8.92	0.78	0.80	0.83	0.79	0.90	1.00	0.93	0.90
Turda Star	9.22	8.23	7.78	7.15	0.66	0.62	0.66	0.66	0.68	0.66	0.63	0.62
Turda 332	12.18	12.20	10.24	9.69	0.96	0.89	0.81	0.92	0.89	0.77	0.71	0.57
Turda 344	11.70	10.95	9.40	9.18	1.14	1.08	0.93	0.95	1.03	1.01	0.93	0.90
Turda 335	11.63	11.62	10.55	11.39	1.04	1.14	1.11	1.24	1.05	1.04	1.14	1.21
Turda 2020 Nrf	11.57	11.55	11.32	10.56	1.04	1.17	1.14	1.13	1.17	1.24	1.21	1.27
Turda 2020cms C	11.30	10.41	10.17	10.40	0.94	0.86	0.97	0.97	1.07	1.05	1.05	1.13
Turda 380	11.76	13.32	11.88	11.53	1.26	1.46	1.45	1.41	1.11	1.18	1.33	1.29
SUR 18/399	11.51	10.72	10.99	10.51	1.15	1.07	1.13	1.11	1.19	1.06	1.17	1.19
HST 148	11.72	11.27	10.59	9.38	1.10	1.01	1.05	0.88	0.95	1.01	0.98	1.03

Table 8. Stress tolerance index (STI) for yield and yield components of maize hybrids under medium and high-density stressed environments

Hybrid	GYA	GYP	EL	NRE	GM	TKW	BS	SE
Turda 248	0.87	1.02	1.17	1.10	0.94	0.71	1.68	1.56
Turda Star	0.65	0.83	1.19	0.86	0.92	0.87	1.18	1.24
Turda 332	0.82	0.84	0.87	1.07	0.99	1.03	2.05	1.51
Turda 344	1.00	0.99	1.03	2.08	0.94	0.88	0.99	0.97
Turda 335	1.12	1.08	0.90	0.75	1.03	1.28	0.41	0.66
Turda 2020 Nrf	1.17	1.12	1.02	1.26	0.99	0.94	0.45	0.64
Turda 2020 cmsC	1.01	1.01	1.00	1.21	0.97	0.95	1.12	0.92
Turda 380	1.31	1.12	1.06	0.78	0.99	1.15	0.23	0.69
SUR 18/399	1.13	1.11	1.07	0.82	1.11	1.11	1.09	0.58
HST 148	1.00	0.94	0.84	1.18	1.12	1.15	1.35	1.51

GYA, grain yield/area GYP, grain yield/plant; EL, ear length; NRE, number of rows per ear; GM, grain moisture at harvest; TKW, 1000-kernel weight; BS, barren stalks; SE, small ear

Table 9. Change (%) in stress tolerance of the hybrids due to high plant density combined with deficit water across hybrids and seasons

Hybrid	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
	WW			WS1				WS2			
	MD	HD1	HD2	LD	MD	HD1	HD2	LD	MD	HD1	HD2
Turda 248	-1.69	-0.49	-1.08	14.78	11.67	8.60	13.19	1.10	-9.89	-2.20	1.10
Turda Star	2.25	4.97	5.84	16.06	21.08	16.43	16.46	13.92	16.46	20.25	21.52
Turda 332	3.71	8.60	10.77	11.52	17.93	25.19	14.81	17.59	28.70	34.26	47.22
Turda 344	-0.81	7.10	7.69	-12.97	-6.59	7.89	5.94	-1.98	0.00	7.92	10.89
Turda 335	-4.81	-4.92	-13.22	-3.59	-14.40	-11.43	-24.00	-5.00	-4.00	-14.00	-21.00
Turda 2020 Nrf	-4.04	-2.53	-5.19	-1.31	-14.07	-10.23	-9.71	-13.59	-20.39	-17.48	-23.30
Turda 2020Cms	5.90	1.63	-0.40	9.20	16.98	5.55	5.83	-3.88	-1.94	-1.94	-9.71
Turda 380	-9.48	-7.06	-7.18	-17.99	-36.28	-35.82	-31.78	-3.74	-10.28	-24.30	-20.56
SUR 18/399	7.91	-0.95	3.63	-9.39	-2.03	-7.78	-5.71	-13.33	-0.95	-11.43	-13.33
HST 148	2.23	-4.36	0.78	-5.32	3.05	-0.97	15.38	8.65	2.88	5.77	0.96

(%) Change = 100 × (E1–RE)/E1, RE = Respective environment; WW = Well watered (2021), WS = water stress (1 = 2022 and 2 = 2023)

The best behavior under stress conditions was noted for Turda 380 and Turda 335, both hybrids standing out for their better tolerance both to higher densities and unfavorable climatic conditions, as well as to the combination of the two stress factors. Turda 2020 Nrf and Sur18/399 were also noted for their smaller change due to stress factors.

The differential response of the best hybrids for different grain yields/ha, suggests that the highest yield per area is determined by the genetic background of the hybrid, plant density, water regime and yield components (Tokatlidis et al., 2015; Al-Naggar et al., 2016a; Al-Naggar et al., 2016b). The plant density of 90,000 plants/ha is not the optimal plant population for the hybrids evaluated, as the hybrids were selected at a medium density of 70,000 plants/ha. To improve maize grain yield at high plant densities, we recommend that new hybrids be selected at high plant densities.

The hybrids Turda 380, Turda 2020, and Turda 335 had the highest GYA under medium-MD and high-HD, i.e. they could be considered the most density-efficient and the most responsive genotypes in this study. The high grain yield as well as the tolerance to high density and water stress of the studied hybrids Turda 380, Turda 2020, and Turda 344, demonstrated the genetic value (general combining ability) of the parental line, common to the three hybrids, to simultaneously inheritance of the ability high production as well as stress tolerance for different environmental conditions. The value of a genotype is given not only by its per se potential but especially by the combining ability, preferably for as many characters as possible (Muntean et al., 2014).

CONCLUSIONS

Maize breeding for grain yield ability has consistently improved plant stress to higher density and drought tolerance. Selecting for grain yield alone is inefficient due to the low heritability under stress conditions.

High-yielding maize hybrids, tolerant to high plant densities, under normal environmental conditions in 2021 Turda - Romania, tended to perform relatively well under the same densities and the water stress conditions in 2022 and 2023.

The superiority of modern maize hybrids and tolerance to high plant density may also be attributed to decreased plant barrenness, selection of genotypes with erect leaves, increased prolificacy, and synchronization of anthesis and silk.

The hybrids Turda 380, Turda 2020 and SUR 18/399 significantly exceeded the average, in all three years of field trials; these hybrids were noted not only for the stability of the grain yield but also for the vegetative characteristics of the plants, very different from those of the old hybrids. These hybrids reacted significantly positively to the interaction with the four plant densities and could be considered "density-independent".

The high grain yield and the tolerance to high density and water stress of hybrids Turda 380, Turda 2020 and Turda 344 demonstrated the genetic value of the common paternal parental line of the three hybrids, to simultaneously inheritance the high production capacity as well as stress tolerance for different environmental conditions.

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