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### THE INFLUENCE OF PLANT CUTTING ON GRAIN YIELD TRAITS IN MAIZE

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## **Abstract**

In this research an influence of the strong source restriction meaning cutting off the whole plants at the first internodes 5 (5DAPt), 10 (10DAPt) and 15 (15DAPt) days after pollination on grain yield and its corresponding traits in maize were tested. Control represented plants harvested at full maturity. Four inbred lines were used, two historical ones (Mo17 and B73) and two commercial ZP inbreds (ZPL and ZPB). The experiment was conducted at Zemun Polje, Serbia, in 2014 and 2015. The trait of particular importance was the number of kernels per ear and its average values were 37.73 at 5DAPt, 115.14 at 10DAPt and 175.20 at 15DAPt, being sufficient for planting next generation of breeding. According to the results obtained, ZPL represented an improved Lancaster line over Mo17 regarding drought tolerance, that could not be stated for ZPB over B73. Hybrid among these two lines is drought tolerant due to heterosis (epistatic effects) or dominant origin of ZPLs good response to drought stress. Values for seed set and eventually for grain yield per plant were 0.00 for line B73 at 5DAPt in 2015. An improved breeding scheme for increased drought tolerance could be proposed, namely self-pollination of border plants on high density sown selfing progenies or dihaploid (DH) lines, cutting off selfed plants at 15 DAP and evaluating their kernel properties. Openpollinated progeny would serve to estimate other important traits for selection. From the chosen progenies kernels of selfed and cut-off plants should be used for the next generation of breeding.

**Keywords**: *drought*, *grain filling*, *Zea mays L*.

### Introduction

Drought is one of the most important limiting factors in maize production, and new methods for increasing drought tolerance in maize are searched for. Maize plants are most susceptible to drought at the flowering time, resulting in low kernel number and grain yield per plant (Grant *et al.*, 1989). Following in sensitivity is grain filling phase, while the vegetative phase is the least sensitive (Fereres and Villalobos, 2016). Some researchers have suggested that the early grain filling phase is very suitable for breeding for drought tolerance (Grant *et al.*, 1989; Nesmith and Ritchie, 1992). However, kernel number is highly correlated with grain yield under drought stress (O'Neill *et al.*, 2004).

The source-sink interaction in plants represents the connectivity between the source of assimilated material and the pathway of this material to the sink, a region where carbon-based products are metabolized and energy (in the form of ATP) is synthesized. Source-to-sink ratio is very important in all cereals in determining grain yield. The supply of assimilates from mother plant (source) or the capability of the reproductive ear (sink) to accumulate assimilates limits growth of maize kernels (Gambín *et al.*, 2006). Assimilate supply can be increased by the environment (nitrogen N supply) and genotype. Under normal growing conditions maize yield is mostly sink limited (Borrás *et al.*, 2004). Drought and/or deficiencies in N supply decrease grain yield by reducing kernel number per plant (poor synchronization between silking and pollination) and greater kernel abortion (poor grain filling). The same authors denoted maize as source-limited crop under stress conditions. Maize plants set sink potential early during grain filling, indicating importance of studying first stages of this phenophase.

Yield responses of major crops to different source-sink manipulations were often studied. Usually, this was done in five ways: 1) stand density manipulations (Hernández *et al.*, 2014; Solomon *et al.*, 2017); 2) induced drought stress at flowering time (Oveysi *et al.*, 2010; Siahkouhian *et al.*, 2013); 3) pollination treatments during seed set (Borrás *et al.*, 2003a, 2003b), 4) seed removal (Jones and Brenner, 1987) or truncation of the ear (Seebauer *et al.*, 2010), and 5) manipulation of N supply in the soil (D'Andrea *et al.*, 2008).

The objective of our research was to estimate the effects of cutting off the whole maize plants at the first internodes at different days after pollination (DAP) on grain yield traits. This procedure would provide a high source restriction, and make an impact on grain filling and other kernel traits. Finally, it could serve as a tool for estimating drought tolerance in maize.

### **Materials and Methods**

The four maize inbred lines were used: two historical ones (Mo17 of Lancaster and B73 of BSSS origin) and two commercial ZP inbred lines (ZPL of Lancaster and ZPB of BSSS origin) which are components of the commercial late maturity drought tolerant ZP hybrid. The trial was conducted at Zemun Polje, Serbia, in 2014 and 2015 according to the Split-Plot Randomized Complete Block Design (RCBD) in two replications. Three treatments (sets) were applied: cutting off plants at first internodes at 5 (5DAPt), 10 (10DAPt) and 15 (15DAPt) days after pollination. Plants harvested at full maturity were used as control set. Each genotype was sawn in two rows 0.75 m apart, five plants per row and 0.3 m between plants. Rows were overplanted (three kernels per hill) and thinned to one plant per hill at 5-7 leaf stage. Only border plants were cut, providing the same environmental conditions to the all plants measured, and also only border control plants were measured. Cut-off plants were put into the shed house, away from direct sunlight.

A total of 15 traits were tested on both cut-off and control plants. Morphological traits were: anthesis-silking interval (ASI) in days, ear (EH, cm) and plant height (PH, cm), total number of leaves (NL), number of leaves above the uppermost ear (LAE), length (LL, cm) and thickness (LT, cm) of the ear leaf. Average chlorophyll content (CL, SPAD units) was measured by a SPAD (Minolta) chlorophyll meter, and duration of chlorophyll content (CD) in days from silking till SPAD values reached units beneath 10. Ear and kernel traits were: number of ears per plant (EP), seed set (SS, %) - visual estimate of the percent of pollinated kernels on the ear, grain filling (GF, %) - visual estimate of the percent of filled kernels from the total of pollinated kernels, number of kernels per ear (KE), grain yield per plant (GP, g) and 100 kernel weight (KW, g).

Statistical analysis was done by threre-way analysis of variance (ANOVA) for genotypes combined over sets and years for each trait. Significances of differences among years, genotypes and treatments were determined by LSD test at 0.05 probability level. Pearson's correlation coefficients were calculated for CL, CD, EP, SS, GF, KE, GP and KW for three cut-off treatments and control in order to estimate prediction ability of the treatments on the final values of control plants.

# **Results and Discussion**

Highly significant differences between years (p < 0.001) were found for ASI, EP, ES, EH, PH, NL, LT, CD, SS, KE and GP, and significant (p < 0.01) for CL and KW (data not shown). These was expected, since climate conditions in 2014 and 2015 were drastically different at Zemun Polje. Namely, 2014 was highly favorable in comparison with 2015, as sum of precipitation was higher for 56.3%. The largest difference was observed in July, during maize pollination, with precipitation of 187.4 mm in 2014 and only 7.2 mm in 2015. On the other hand, more rainfall occurred in August (time of grain filling) in 2015 than in 2014 (56 and 41 mm, respectively). In south-east Europe the first part of maize vegetation is most often

sufficient in rainfall, but about two to three weeks before or at the flowering time drought frequently occurs. The consequence is poor development of root system in the first part of vegetation that remains inefficient to compensate drought stress for rest of vegetation. The second peak of drought occurs during grain filling, when uptake of soil assimilates by maize plants is poor (Anders *et al.*, 2014). Our experiment was designed to simulate this common situation - by cutting off the plants at the first internodes assimilates from the soil became unavailable, thus imitating poor development of the root system.

Genotypic differences were highly significant (p<0.001) for all traits except KW (non-significant), which is in accordance with review given by Borrás and Vitantnonio-Mazzini (2017). Year × genotype interaction was significant for ASI, EH, CD, EP, SS, GF and KE. Differences among sets were non-significant for all the morphological traits, indicating they were fully developed before time of stress (Abrecht, 1999). Induced stress provoked significant differences in all other traits (p<0.001 and for CL p<0.01). Minimal, maximal and average values for these traits are given in Tab. 1. The trait of particular importance is KE and the average values were 37.73 at 5DAPt, 115.14 at 10DAPt and 175.20 at 15DAPt. From breeding standpoint these KE are sufficient for planting the next generation. In the previous pilot experiment with gene bank material, kernels obtained in this way were viable and had good emergency (data not published). The minimum values for SS, GF, KE, GP and KW were 0.00 for B73 at 5DAPt in 2015. This line has passed ear formation (V10) successfully, but the severe drought stress during pollination caused the absence of seed set.

Table 1. Minimal, maximal and average absolute values of the analyzed traits

Trait	Treatment (DAP)					Checks
	Range	5	10	15	Trend	•
Chlorophyll content	Min	18.20	21.96	19.20	Λ	19.34
CL (SPAD units)	Max	33.98	37.35	35.94	Λ	39.32
	Aver.	27.19	28.63	26.41	Λ	28.77
Chlorophyll duration	Min	29.50	34.00	31.00	Λ	35.58
CD (days)	Max	101.50	101.25	99.75	$\downarrow$	96.59
	Aver.	71.68	68.92	63.20	$\downarrow$	58.43
Ears per plant	Min	0.50	1.00	0.75	Λ	0.81
EP (number)	Max	1.33	2.00	2.00	<b>↑</b>	1.81
	Aver.	1.04	1.29	1.28	Λ	1.36
Seed set	Min	0.00	10.00	8.50	Λ	11.28
SS (%)	Max	87.50	88.75	94.00	<b>↑</b>	95.08
	Aver.	39.42	59.85	64.41	<b>↑</b>	63.39
Grain filling	Min	0.00	10.00	30.00	<b>↑</b>	43.44
GF (%)	Max	78.75	88.75	93.75	$\uparrow$	97.58
	Aver.	26.84	59.03	74.24	<b>↑</b>	82.42
No.of kernels per ear	Min	0.00	10.00	12.00	1	22.13
KE (number)	Max	124.25	250.50	494.50	$\uparrow$	484.67
	Aver.	37.75	115.18	175.20	<b>↑</b>	240.99
Grain yield per plant	Min	0.00	2.43	2.25	Λ	5.83
GP(g)	Max	13.92	24.44	58.10	$\uparrow$	164.13
	Aver.	3.52	13.00	21.03	<b>↑</b>	71.15
Kernel weight	Min	0.00	3.63	7.33	<b>↑</b>	16.89
KW (g)	Max	13.33	68.60	25.36	Λ	36.92
	Aver.	6.31	14.27	14.49	<b>↑</b>	27.07

Λ - maximum value of the particular parameter is at 10 DAP; ↓ - decreasing value trend of the trait from 5 towards 15 DAP; ↑ - increasing value trend of the trait from 5 towards 15 DAP All three values (min, max and av.) for CL were highest at 10DAPt, although still smaller than for control plants. This was unexpected, since plants cut-off at 10DAPt had five days less for photosynthesis than those at 15DAPt. Probably photosynthesis continued for some time on the cut-off plants of this treatment. These results are in accordance with Borrás *et al.* (2004), who found that maize kernel growth is highly source-dependent when tested in drastic reduction of assimilates during grain feeling. Minimum CD values showed the same trend, but maximum and average values decreased from 5DAPt to 15DAPt, and for all three treatments values were higher than for checks. As a response to stress, leaf tissue probably shrunk more than in checks, so the relative chlorophyll concentration in the leaves was higher. For EP erratic values and trends were obtained, presumably due to differences between the genotypes tested. For all other traits mostly increasing trends were obtained and in almost all instances values of the treatments were smaller than those of the checks.

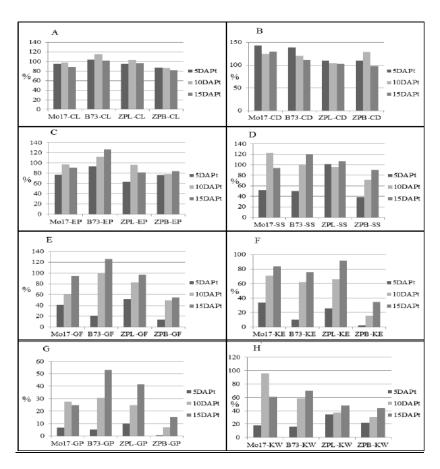


Figure 1. Values for CL (A), CD (B), EP (C), SS (D), GF (E), KE (F), GP (G) and KW (H) are presented in percent of the control for four lines and three cut-off treatments.

LSD values at 0.05 level for genotypes over years were calculated (data not shown). For all analyzed traits, ZPL was significantly better or at the level of Mo17, indicating that it is an improved Lancaster line regarding drought tolerance. On the other hand, B73 and ZPB had, in most cases, non-significant differences between trait averages, except for GF at 15DAPt and KE at 10DAPt where B73 was superior, but only overall both seasons. Therefore, ZPB showed better stability of drought tolerance over B73, but it is not significantly improved for this trait considering both years of research.

If pollination occurs 3-8 days after first silk exposure maximal seed set is expected (Carcova et al., 2000; Anderson et al., 2004). According to this, plants would be adequately pollinated already at the first treatment in our experiment (5DAPt). The results for SS, however, showed that pollination was not sufficient. Namely, SS was less than 33.00% for three lines and 76.25% for ZPL. SS in our experiment raised with number of DAP. This trait, however, remained constant in the review presented by Borrás and Vitantnonio-Mazzini (2017). Regarding GF the experiment was efficient, since there was a growing trend from 5DAPt to 15DAPt. The least values were obtained for ZPB. In maize and other cereals there are three phases of grain filling: a lag phase, a linear phase and a phase of slowing down approaching the physiological maturity (Johnson and Tanner, 1972). The first phase lasts 15-18 DAP and during this time very little dry weight is accumulated. In our experiment normal grain filling was stopped at 5DAPt and 10DAPt since the lag phase was not finished. KE was particularly low at 5DAPt for all lines and again ZPB was the poorest one. The most jeopardized trait was GP with maximum value of 53.2% in comparison with the control for B73 at 15DAPt. KW showed increasing trend from 5DAPt to 15DAPt, except for Mo17 at 10DAPt, and smaller values for all treatments were obtained for ZPL and ZPB. Middle leaves have essential role in photosynthesis and deriving grain yield (Siahkouhian et al., 2013). Defoliation in mentioned research produced many immature and small kernels on ear tips. Similar phenomenon occurred in our experiment. Premature death of leaves induced by cutting off the stalks resulted in yield losses and plants most probably remobilized stored carbohydrates from the leaves and/or stalks to the developing ears, but yield potential was already lost. Death of all plant tissues, occurring about 15 days after cutting off the plants, stopped any further remobilization of stored carbohydrates into the ears.

Pearson's correlation coefficients between treatments and checks were significant only for 15DAPt and checks for KE (0.681; p<0.01) and GP (0.543; p<0.05), and we could assume that at 15DAPt our experiment became feasible for predicting final grain yield under uncontrolled environmental conditions.

### **Conclusions**

In the literature we could not find cutting off plants as a way of source-sink manipulation in plants. High plant density is often used during inbreeding or double haploid breeding for obtaining new, drought tolerant maize inbred lines. An improved breeding scheme for increased drought tolerance could be proposed. The new approach would include self-pollination of border plants on high density progenies, cutting off selfed plants at 15 DAP and evaluating their kernel properties. Besides for SS and GF, open-pollinated progeny could be used for estimating other important traits (ASI, stay green, tolerance to plant and stalk lodging, diseases and pests). From the chosen progenies kernels from selfed and cut-off plants should be used for next generation of breeding. One of the advantages of such approach might be shortening of vegetative period of late materials, thus allowing more generations per year.

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