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Variation in stomatal traits based on plant growth parameters in Corn (*Zea mays* L.)

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ABSTRACT

Stomata are the pores which surrounded by two guards cells which play an important role in regulation of plant water balance and gas exchange between plant internal tissues and the atmosphere. The research involved the examination of variations in stomatal traits: stomatal density (SD), stomatal length (SL), stomatal width (SW) and stomatal surface (SS) based on changes in the plant growth parameters in different growing media in corn. All growth parameters studied was found significant differences between combination rates in both of growing media. The stomatal parameters were changed related to the growing parameters. The increase in leaf growing parameters led to increase in SW, SL and SS values but decrease in SD. It can be said that the SS of corn plant leaf set by changing both of the SW and SL sizes, acting positively on growth conditions. It can be suggested to combine the results obtained from maize with other uninvestigated plant and growth condition to clarify mechanisms of stomatal which are undercontrol of genetic and environmental factors.

Keywords: Stomatal density, Stomatal length, Stomatal width and Stomatal surface

Abbreviations

Stomatal density (SD), stomatal length (SL), stomatal width (SW), stomatal surface (SS),leaf length (LL), leaf width (LW), fresh weight roots (FWR), fresh weight biomass (FWB), root volume (RV), fresh leaf weight (FLW), root length (RL), dry biomass weight (DBW), and dry root weight (DRW).

INTRODUCTION

Stomata are the pores which surrounded by two guard cells which play an important role in regulation of plant water balance and gas exchange between plant internal tissues and the atmosphere. Stomata are very important because they are directly responsible for water loss and carbon accumulation [1].

Generally, stomatal initiation is determined by both environmental and genetic factors [2]. Stomatal characteristics in some species have generally high heritability (i.e., less sensitive to environmental change) [3], while in the others species stomatal characteristics have being more sensitive to environmental factors [4]. Nevertheless, different effects of abiotic factors on stomatal size may depend on plant species/varieties [5, 6].

Xu and Zhou [7] reported that SL correlates with both of genome size and water conditions. Besides, SD is genetically determined as a quantitative trait, [8]. The relative importance of gene versus environment in determining SD or SL and its interspecific variation have not yet been estimated under a unified framework. A wider diversity of models for the genetic and environmental control of stoma should be considered [9]. There are some researches indicating changes in the number of stomata in various conditions such as drought [10, 11] and decrease in stomatal size [12, 13]. Although,many researches have examined stomatal responses to unfavorable conditions (such as stress) [6, 14, 15, 16, 13],there is almost no reports about response of stomata to favorable growth conditions [18].Currently, the mechanisms of stomatal response are very complex and not yet fully understood [15,

18]. Only a few model species have been used to study on the mechanisms of genetic and environmental effects on stomatal parameters. Also, variation among species has not been quantified yet [9]. Determining differences of stomatal behaviors for different species, intra species and environments will be contributed in explanation of the mechanism of stomatal responses.
This research involves the examination of variations in stomatal traits: stomatal density (SD), stomatal length (SL), stomatal width (SW) and stomatal surface (SS) in different growing media in corn plant. Stomatal parameters have been evaluated based on the plant growth parameters.

MATERIALS AND METHODS

Plant material and growth conditions

Pioneer 31D24 corn cultivars were used as plant material. Two different materials (diatomite and perlite) were used as growth media. Different ratios of these materials (5, 10, 20, 30 w/w) is mixed with soil and were filled into pots. Used diatomite and perlite were coated with olive processing waste material. Created soil combinations were considered as different growth media to investigated stomatal parameters behaviors.150, 25, 200, 25, 1, 1.5 ppm quantities of N, P, K, Mg, Zn and Mn, were added to the soil, respectively. The pot experiment was conducted with randomized complete block design with three replications. The plants grown in pots which containing only soil were used as control group. At the beginning of the experiments, four seedlings were planted in each pot. After ten days, thinning was done and only two seedlings were left in each pot.

Measurements of Traits

Growth Parameters

Seventy five days after sowing, leaf length (LL), leaf width (LW), fresh weight roots (FWR), fresh weight biomass (stem + leaf) (FWB), root volume (RV), fresh leaf weight (FLW), root length (RL), stem length (SL), dry biomass weight (DBW), and dry root weight (DRW) of the plants were determined. The mean values in each replication were used for statistical analysis.

Stomatal Parameters

Stomatal observations were carried out on 75th days after sowing (DAS) using a bright-field light microscope. The stomatal density (SD), stomatal length (SL) and stomatal width (SW) were determined from the underside of each leaf using prints made with nail varnish. SL was measured between the junctions of the guard cells at each end of the stoma as defined by two research groups [5, 19].

The SW was measured perpendicular to maximum width which represents the maximum potential opening of the stomatal pore, but not the aperture of opening that actually occurs. SD (number of stomata per mm^2) was determined as described by previous study Radoglou *et al.* [17]. Stomatal surface (SS), was obtained using equation number 1 (equation from Wang*et al.*[20], with some modifications).

$$SS = \frac{SL \times SW \times \pi}{4} \quad (1)$$

The SL and SW values are measured as micrometers (µm).

SPAD Value

While the stoma parameters investigated, the physiological aspect of the event to be ignored, SPAD values were monitored and evaluated. Chlorophyll content was determined from intact leaves using a portable chlorophyll meter SPAD-502 (Minolta, Ltd., Osaka, Japan). Reading of SPAD values were done at 75th day after treatment. Three measurements were made per plant, three leaves were chosen from each plant (lower, middle and upper leaves of plant) and three different regions of each leaf (middle and two ends of leave) were used for tests. The chlorophyll meter was used to estimate the nitrogen status of crops. The instrument measures transmission of red light at 650 nm, at which chlorophyll absorbs light, and transmission of infrared light at 940 nm, at which no absorption occurs. On the basis of these two transmissions values, the instrument calculates a SPAD value that is quite well correlated with chlorophyll content [21, 22]. Chlorophyll meter readings (SPAD values) were repeatedly taken at the center of the leaves.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using factorial randomized complete plots design with three replications, and least significant difference (LSD 0.05) was used to determine significant differences between treatment means.

RESULTS AND DISCUSSION

Growth Parameters

Belonging to different growing media, growth parameters are given in Table 1. In all studied growth parameters, significant differences between combination rates in both of growing media were found. As is evident from the growth parameters, stomatal behavior will be useful for examining different growing media. Although the increase in growth parameters was seen generally in all portion media, the increase rate for perlite 5% and diatomite 20% was remarkable compared with other combinations.

Table1. Effect of different growing media on growth parameters

(leaf length (LL), leaf width (LW), fresh weight roots (FWR), fresh weight biomass (stem + leaf) (FWB), root volume (RV), fresh leaf weight (FLW), root length (RL), stem length (SL), dry biomass weight (DBW), and dry root weight (DRW)

	LL (cm)	LW (cm)	FWR (gr)	FWB (gr)	RV (cm^3)	FLW (gr)	RL (cm)	SL (cm)	DBW (gr)	DRW (gr)
C	33.13^{d}	3.57^{b}	38.07^{e}	23.00^{c}	24.17^{d}	2.10^{c}	33.00^{bc}	64.00^{d}	3.72^{c}	5.68^{d}
P5	34.57^{d}	4.02^{b}	48.20^{d}	12.52^{d}	36.37^{c}	2.97^{b}	31.57^{cd}	66.67^{c}	4.36^{c}	7.56^{c}
P10	44.13^{c}	5.45^{a}	92.65^{a}	77.30^{b}	75.83^{b}	4.05^{a}	33.67^{b}	90.50^{a}	13.65^{b}	15.06^{a}
P20	46.83^{b}	4.75^{a}	76.62^{b}	80.22^{b}	74.45^{b}	3.22^{b}	36.33^{a}	89.00^{a}	14.65^{b}	14.16^{ab}
P30	54.00^{a}	5.43^{a}	68.08^{c}	91.37^{a}	133.33^{a}	4.60^{a}	31.33^{d}	83.80^{b}	15.75^{a}	13.50^{b}
Tot.	42.53	4.64	64.72	56.88	68.83	3.38	33.18	78.79	10.43	11.19
D5	57.27^{ab}	6.03^{a}	67.40^{d}	83.317^{a}	114.00^{c}	3.08^{b}	32.83^{b}	91.67^{a}	12.23^{b}	9.85^{c}
D10	61.23^{a}	5.60^{ab}	109.70^{b}	82.317^{a}	123.00^{b}	4.07^{a}	34.30^{b}	82.07^{c}	12.72^{b}	12.14^{b}
D20	53.00^{b}	5.47^{b}	196.72^{a}	84.27^{a}	146.67^{a}	4.38^{a}	38.27^{a}	86.70^{b}	14.19^{a}	20.84^{a}
D30	36.67^{c}	4.20^{c}	75.17^{c}	41.95^{b}	53.67^{d}	3.17^{b}	33.43^{b}	59.00^{e}	6.50^{c}	9.40^{c}
Tot.	48.26	4.97	97.41	62.97	92.30	3.36	34.37	76.69	9.87	11.58

Means with same letters are not significantly different ($p < 0.05$).

Stomatal parameters

Significant variation for stomatal parameters was observed based on growing media (Table 2). The highest SD value was observed in control plants. In parallel with increase in portion of diatomite or perlite, SD value significantly decreased except for 5% rates of both materials. The decrease in SD value in both materials in 0.5% rate is in parallel with increase in plant growth rate. Previous researches stated that stomatal density increases in unfavorable conditions (stress). These values are in line with increase in SD during stresses which were determined by Htay *et al.* [23] on bean, Kuo *et al.*[24] on cabbage, and Labate *et al.*[25] on tomato. Especially, in response to heavy metal stress, the SD increased which was agreeing with SHI and CAI [16] report. Rates of perlite and diatomite increased in parallel with the increasing width of the stoma. Although our earlier study on tobacco[26] showed that, the SL value decreased with the increase in Cr dosage, there was no significant difference in SW in both varieties. However, Zhang *et al.*[11], reported that SL increases under limited irrigation conditions, whereas SW decreases. On the contrary, corn showed a tendency to increase the width of the stoma under favorable conditions. Nevertheless, different effects of abiotic factors on stomatal size may depend on plant species/varieties [5, 6]. It can be said that the SS of corn plant leaf is set by changing both of the SW and SL, under favorable growth conditions. The SS parameters showed that there were significant differences between different portions of growing media. Generally, the increase in SS value is observed by increase in diatomite and perlite rate.

Table2. Responses of stomatal parameters

stomatal density (SD), stomatal width (SW), stomatal length (SL), stomatal surface (SS) and SPAD value to different growing media

	Stomatal density (no/mm^2)	Stomatal width (μm)	Stomatal length (μm)	Stomatal surface ($μm^2$)	SPAD value
Control	139.29^{a}	19.19^{c}	30.03^{c}	452.50^{c}	18.40^{b}
Perlite5	81.60^{d}	20.03^{bc}	31.00^{b}	487.53^{b}	24.92^{a}
Perlite 10	118.87^{b}	20.40^{ab}	32.27^{a}	516.80^{a}	26.817^{a}
Perlite 20	117.33^{b}	20.50^{ab}	29.30^{c}	471.47^{bc}	27.017^{a}
Perlite 30	107.13^{c}	21.27^{a}	32.50^{a}	542.57^{a}	26.733^{a}
Total	112.84	20.28	31.02	494.17	24.778
Control	139.30^{a}	19.13^{b}	30.03^{d}	452.50^{c}	17.40^{d}
Diatomite 5	79.60^{d}	20.33^{a}	34.43^{a}	549.77^{a}	28.28^{bc}
Diatomite 10	118.87^{b}	19.23^{b}	34.10^{a}	514.87^{ab}	25.97^{c}
Diatomite 20	112.20^{b}	20.43^{a}	31.40^{c}	503.80^{b}	29.43^{ab}
Diatomite 30	102.00^{c}	20.20^{ab}	32.96^{b}	522.57^{ab}	31.87^{a}
Total	110.39	19.88	32.58	508.70	26.59

Means with same letters are not significantly different ($p < 0.05$).

SPAD Value

SPAD value as an expresser for chlorophyll content was determined on 75th DAS. In terms of SPAD value there was a significant difference between combination rates in both of growing media(Table 2). The increase in portion of diatomite and perlite led to increase in SPAD value which is in accordance with result of other research [27]**.** Although the increase in SPAD value was seen in both media, the increase related to perlite was slight, compared with diatomite which showed a remarkable increase.

Correlations

To further understand the relationship between stomatal parameters and leaf growing parameters, a correlation analysis was performed (Table 3). Increase in leaf growth parameters is parallel with decreasing in the stomata density, while the stomata length and width is increased. Also, parallel to increase in leaf growth parameters, the SS has increased. By the way, in the research conducted on tobacco plants, the SS showed decreased in parallel to increased stress condition [26]. In tobacco reducing the SS is adjusted with reducing only the SL value, while SW dose not changed. However, in this study, increase in SS is adjusted with changes in both SL and SW values. A negative correlation was found between the SD value and SL, SW, SS, SPAD, LW (-0.606, -0.584, -0.711, -0.633, -0.519 respectively) and it is in line with results of Orcen et al[26] study on tobacco. Other researchers reported that in other stressful conditions such as drought, SD negatively correlates with SL in some *Jujube* leaves[6] and *Platanus acerifolia* leaves[28]. Also during plant evolution the same trend can be observed. Franks et al. [29] and Martinez *et al*. [30] reported that throughout an adaptation to drought, increase in SD and a decrease in cell size under water stress could occur.

Table 3.Coefficient correlations between stomatal traits and leaf growth parameters

	Leaf length (cm)	Leaf width (cm)	Fresh leaf weight (g)	Dry leaf weight (g)	Stomatal width (µm)	Stomatal length (µm)	Stomatal density (no/mm^2)	Stomatal surface (µm^2)
Leaf length	1							
Leaf width	0.915**	1						
Fresh leaf weight	0.853**	0.772**	1					
Dry leaf weight	0.903**	0.821**	0.915**	1				
Stomatal width	0.552*	0.571*	0.634*	0.645**	1			
Stomatal length	0.552*	0.644**	0.557*	0.340	0.394	1		
Stomatal density	-0.423	-0.519*	-0.290	-0.247	-0.584*	-0.606*	1	
Stomatal surface	0.642**	0.718**	0.687**	0.537*	0.763**	0.895**	-0.711**	1
SPAD value	0.781**	0.748**	0.780**	0.810**	0.841**	0.551*	-0.633*	0.787**

** Significant at p = 0.05; ** Significant at p = 0.01; ns not significant*

CONCLUSION

The stomatal parameters and SPAD value were changed related to the growing parameters. The increase in leaf growing parameters leads to increase in SW, SL, SS, and SPAD value but decrease in SD. The stomatal parameters and SPAD values are suitable indicators for growing evaluation. So, it can be suggested to combine the results obtained from maize with other uninvestigated plant and growth condition to clarify mechanisms of stomatal which are under control of genetic and environmental factors.

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