

Diogo Dembocurski^{1,3} (✉), Elaine Cristina Disner¹, Adilson Ricken Schuelter^{1,3}, Isabel Regina Prazeres de Souza², Silvia Renata Machado Coelho³ and Divair Christ³

¹ Faculdade Educacional de Medianeira, Medianeira, PR, Brasil
E-mail: adilson_schuelter@yahoo.com.br, diogo_dembo14@outlook.com, elaine_disner@hotmail.com

² Embrapa Milho e Sorgo, Sete Lagoas, MG, Brasil
E-mail: isabel.prazeres@embrapa.br

³ Programa de Pós-graduação em Engenharia Agrícola (PGEAGRI), Universidade Estadual do Oeste do Paraná, Cascavel, PR, Brasil
E-mail: silvia.coelho@unioeste.br, divair.christ@unioeste.br

✉ Corresponding author

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USE OF UNICONAZOLE IN GROWTH REGULATION AND BIOCHEMICAL CHANGES IN MAIZE

Abstract – The current paper evaluated the incubation time, and doses of Uniconazole (UCZ) applied to maize seeds to verify the plants' response at different phenological stages. MSG1001 hybrid seeds were treated with UCZ (0, 50, and 100 mg.kg⁻¹ of seed) with different incubation times (IT – 1 min and 12 h). In the lab experiment, the treated seeds were displaced in rolls of the previously moistened Germitest paper and incubated for a week in a BOD chamber at 25°C with a 12-hour photoperiod. In the greenhouse experiments, sowing was carried out in pots with sand, and supplementary irrigation was applied for ten days. In the field experiment, sowing was performed with 0.5 m spacing between rows in densities of 75,000 and 65,000 plants.ha⁻¹. In the BOD and the greenhouse, the design of both experiments was entirely randomized in a double-factor scheme (IT x UCZ). A randomized block design in a triple-factor scheme (IT x UCZ x Population) was used in the field experiment. Seed treatment with UCZ did not promote germination inhibition. The concentration of 100 mg UCZ kg⁻¹ of seed in the 12-hour IT resulted in a more significant accumulation of root mass in seedlings after one week in the BOD. In the greenhouse, plants in the V1 stage presented longer shoot lengths for the treatments that received UCZ. Incubation time influenced root length, and IT = 12 h at a concentration of 50 mg UCZ kg⁻¹ of seed was more efficient than IT = 1 min. Application of 100 mg UCZ kg⁻¹ of seed was more effective for accumulating chlorophylls A and B in IT = 1 min. In the field, seeds treated with UCZ changed plants for female flowering and the number of green leaves below the ear. The population of 75,000 plants.ha⁻¹ was more productive, with UCZ contributing to the hybrid's yield. Incubation of the seeds with 50 or 100 mg UCZ kg⁻¹ of seed in IT = 1 min contributed to more productivity than IT = 12 h. The grains' starch content increased with the application of UCZ at a dose of 100 mg kg⁻¹ of seed, and the starch percentage was modified according to population density and incubation time.

Keywords: *Zea mays*, growth regulator, triazole, starch, chlorophylls A and B

USO DO UNICONAZOLE NA REGULAÇÃO DO CRESCIMENTO E MODIFICAÇÕES BIOQUÍMICAS EM MILHO

Resumo - O presente trabalho teve como objetivo avaliar o tempo de incubação e doses de Uniconazole (UCZ), aplicadas em sementes de milho, visando verificar a resposta das plantas em diferentes estádios fenológicos. Sementes do híbrido MSG1001 foram tratadas com UCZ (0; 50; 100 mg.kg⁻¹ de semente) sob diferentes tempos de incubação (TI – 1 min e 12 h). No experimento em laboratório, as sementes tratadas foram dispostas em rolos de papel Germitest previamente umedecido, e incubadas por uma semana em BOD a 25°C com fotoperíodo de 12 h. No experimento conduzido em casa de vegetação a semeadura foi realizada em vasos contendo areia, e irrigação suplementar foi realizada no período de 10 dias. No experimento de campo, a semeadura foi realizada empregando-se espaçamento de 0,5 m entre fileiras nas densidades de 75 e 65 mil plantas.ha⁻¹. O delineamento dos experimentos em BOD e em casa de vegetação foi inteiramente casualizado em esquema fatorial duplo (TI x UCZ). No experimento de campo foi empregado o delineamento de blocos casualizados em esquema fatorial triplo (TI x UCZ x População). O tratamento de sementes com UCZ não promoveu a inibição da germinação. Na concentração de 100 mg UCZ kg⁻¹ de sementes no TI de 12 h resultou em maior acúmulo de massa de raiz em plântulas após uma semana em BOD. Em casa de vegetação, plantas no estágio V1, apresentaram maior comprimento da parte aérea para os tratamentos que receberam UCZ. O tempo de incubação apresentou influência sobre o comprimento radicular sendo que TI = 12 h na concentração de 50 mg UCZ.kg⁻¹ de sementes foi mais eficiente que TI = 1 min. A aplicação de 100 mg UCZ kg⁻¹ de sementes foi mais efetiva para acumulação das clorofilas A e B no TI = 1 min. Em campo, sementes tratadas com UCZ resultaram em alteração em plantas para florescimento feminino e número de folhas verdes abaixo da espiga. População de 75 mil plantas ha⁻¹ foram mais produtivas, com o UCZ contribuindo para o rendimento do híbrido. Incubação das sementes nas concentrações de 50 ou 100 mg UCZ kg⁻¹ semente no TI = 1 min contribuiu mais para a produtividade do que em TI = 12 h. O teor de amido dos grãos apresentou incremento com a aplicação de UCZ na dose de 100 mg kg⁻¹ de sementes e, a percentagem de amido foi modificada conforme a densidade populacional e o tempo de incubação.

Palavras-chave: *Zea mays*, regulador de crescimento, triazol, amido, clorofilas A e B

According to the United Nations (2019), the world population is expected to reach 9.7 billion by 2050, corresponding to 20.6%, with 70% of the total concentrated in urban centers. In this context, increasing the production of food and meat products is of fundamental importance, which according to FAO (2017) should be 3 billion tons year⁻¹ and 463 million tons year⁻¹ to meet the demand, respectively.

With the ongoing climatic changes, it is estimated that global agricultural production will be reduced by 17% (Assad et al., 2019). However, Levis et al. (2018) highlight that the reduction in crop yields will occur primarily in parts of tropical and subtropical Africa and South America due to increased plant respiration and decreased soil moisture, both reflections of the increased temperatures. There is thus a significant paradox: the need to increase agricultural production in more adverse edaphoclimatic conditions for the crops.

Brazil is the third-largest maize producer globally, with three sowing seasons, where the *Safrinha* (Second maize crop) concentrates approximately 76% of the cultivated area (Acompanhamento da Safra Brasileira de Grãos, 2021). The *Safrinha* name was introduced in the 1970s due to the low yields of crops with sowing between January and April (Gerage & Bianco, 1990), subjected to unfavorable conditions especially, water availability (Gonçalves et al., 2002). *Safrinha* maize, including Paraná, is cultivated at a large scale and driven by technologies that contribute

to the crop's productivity (Contini et al., 2019). However, it is considered high risk due to frequent droughts in the period that coincides with the vegetative and grain filling stages and a decrease in the temperature from April onwards. In this context, different strategies have been adopted to reduce the harm caused by water deficit, including choosing more tolerant hybrids and products that increase the plants' ability to adapt to adverse conditions (Zhang et al., 2008).

In this context, some research results show that plants treated with Uniconazole (UCZ - C₁₅H₁₈ClN₃O; (E)-1-(4-Chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)pent-1-en-3-ol), subjected to water deficit, presented a reduction in the production losses in the maize crop (Ahmad et al., 2018, 2019). Furthermore, in different species of treated plants, changes were verified in the grains' chemical composition, such as starch (Liu et al., 2019; Huang et al., 2015).

UCZ is a synthetic organic compound of the triazole group, capable of promoting changes in cell metabolism by inhibiting the synthesis of gibberellins. These changes affect the cytokinins synthesis and photosynthetic pigments and delay the leaf senescence (Ahmad et al., 2019; Yan et al., 2015). Thus, UCZ reduces the growth and promotes changes in the plants' architecture (Schlutenhofer et al., 2011).

The current paper aimed at evaluating the effect of UCZ at different concentrations and seed exposure times on the growth and development of maize plants, as well as on the plants' grain yield and chemical composition

components under different population densities.

Material and Methods

Characterization of the experimental area

The field experiment was implemented in the 2019/2020 harvest on a farm located in the municipality of Serranópolis do Iguaçu, PR, Brazil, at an altitude of 233 m, coordinates 25°23'30.5" South and 54°07'45.8" West. Soil classification is Distroferric Red Latosol (Santos et al., 2006), with 70% clay content (class 3 - very clayey), determined by soil analysis. According to Koppen and Geiger's classification, the climate of this region is CFA (humid subtropical climate: humid summer, given unstable tropical masses), with mean annual temperature and rainfall values of 19°C and 1,923 mm. During the experimental period, from February to July, the mean temperature varied from 18°C to 24°C, and rainfall was 512 mm with uneven distribution, with 395 mm occurring after the plants' water bubble grain stage (R2).

The laboratory and greenhouse experiments were carried out at Faculdade Educacional de Medianeira (UDC Medianeira), Medianeira, PR, Brazil.

Maize cultivar and treatment of seeds

Seeds of the MSG1001 single-hybrid maize (MaisGenes Sementes LTDA, Toledo, PR, Brazil), with early cycle and semi-dented grain type, were subjected to treatment with Uniconazole (commercial product with 10%

of active ingredient) at concentrations of 0 mg (control, use of deionized water), 50 mg and 100 mg UCZ kg⁻¹ of seed, incubated for 12 h (Ahmad et al., 2018, 2019) or 1 min. After the incubation period, the seeds were triple washed with distilled water, placed in open Petri dishes, and dried in a temperature-controlled room at 25°C for 1 h.

Seed germination and seedling growth in a BOD incubator

Germination of the seeds subjected to the UCZ treatments was conducted in Germitest paper previously moistened with distilled water. After sowing, the rolls were maintained for a week in a BOD (Biochemical Oxygen Demand) chamber under a 12 h photoperiod with the temperature regulated at 25°C.

The experimental design was completely randomized in a double-factor scheme (Uniconazole Concentration x Incubation Time), with four replications. The experimental unit consisted of four paper rolls containing twenty-five seeds per roll. At the end of the incubation period, the following variables were evaluated: germination percentage (%G), seedling height (SH), shoot fresh mass (SFM), root fresh mass (RFM), total fresh mass (TFM), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM).

Initial growth of plants conducted in a greenhouse

Seeds of hybrid MSG1001, subjected to the treatments with UCZ, were sown at a depth of 4 cm in pots with a capacity of 8.7 dm³ filled with washed sand. The irrigation schedule was twice a day, totaling 30 mm daily. The greenhouse means temperature was 28°C.

With six replications, the experimental design was randomized in a double-factor scheme (UCZ Concentration x Incubation Time). The experimental unit consisted of three plants per pot.

The evaluations were carried out at the first leaf stage with visible collar (V1) for the following characteristics: shoot height measured from the ground to the insertion of the first leaf (SIFL); shoot height of the plant (SHP); the number of roots (NR); root length (RL), measured from the lower part of the plant's neck to the end of the fasciculated root system; fresh shoot mass (FRM); shoot dry mass (SDM), carried out in a drying oven at 65°C; fresh root mass (FRM); total fresh mass (TFM); and stem diameter (SD) measured at the base of the aerial part of the plant using a digital pachymeter.

The leaf tissue was collected from plants in the V1 stage grown in the greenhouse for chloroplast pigments (chlorophyll A, chlorophyll B, and carotenoids) determination. Twenty leaf discs with 8 mm in diameter and an area of 50.26 mm² were collected per treatment, five for each of the four replications. The five-leaf discs

from each repetition were immediately immersed in absolute ethanol and incubated in a water bath at 25°C for 24 h. The quantification of chlorophylls A and B and carotenoids was performed in a spectrophotometer (Tecnal Metash, model: UV-5100 UV/VIS SPECTROPHOTOMETER) with the absorbance values of 664.2 nm, 648.6 nm, and 470 nm, using the equations developed by Lichtenthaler (1987).

Growth and development of maize plants in field conditions

The hybrid MSG1001 seeds were treated with UCZ and sown in an area with a history of no-tillage without the need for soil acidity correction, with two seeds per hole. Planting fertilization consisted of 30 kg of N, 45 kg of P₂O₅, and 45 kg of K₂O, and nitrogen topdressing was carried out at stages V3 and V7 with 30 kg ha⁻¹ and 60 kg ha⁻¹ of N, respectively. Finally, thinning was carried out with plants in the V2 stage, adjusting for populations of 65,000 or 75,000 plants.ha⁻¹.

The experimental design was randomized blocks with three replications in a triple-factor scheme: Uniconazole Concentration (0, 50 and 100 mg kg⁻¹ of seed); Incubation Time (1 min and 12 h); Population (65,000 and 75,000 plants.ha⁻¹). The experimental plot consisted of four 4 m rows spaced 0.5 m, where the two central rows were the functional area.

As needed, crop management consisted of manual weeding and chemical control of the main pests. After topdressing nitrogen fertilization at

the V7 stage, 30 mm supplemental irrigation was used. The accrued precipitation index was 512 mm during the experiment period, with uneven rainfall distribution, indicating 104.5 mm up to stage R1 with a mean temperature of 23.7°C.

The evaluations were initiated five days after emergence (DAE) with the periodic determination of the plants' height up to the tassell emission stage (VT). When at least 50% of plants in a plot reached flowering, the number of days for female (NDF) and male (NDM) flowering was determined, followed by the conversion into degrees-days.

The contents of chloroplast pigments (chlorophyll A, chlorophyll B, and carotenoids) were determined by initially collecting leaf discs from the plants at the stage of 7 developed leaves (V7), verified at 30 DAE. Then, after full flowering, discs were collected from the leaf of the first ear at 63, 79, 93, 108, and 153 DAE to evaluate the foliar senescence of the maize plants. The procedure for collecting and determining the pigments was similar to the one described in the previous item.

After physiological maturation, manual harvesting was carried out, as well as evaluations of ear length (EARLEN), ear circumference (EARCIR), ear diameter (ED) and cob diameter (CD), productivity (PROD), thousand-grain mass (TGM) and grain moisture at harvest (GMH). After drying the grains to 13% moisture, the centesimal composition was determined for starch (ST), ethereal extract (EE), crude fiber (CF), mineral material (MM), and crude

protein (CP) by the Infrared Spectroscopy non-destructive method (Qiu et al., 2019, with an FT-NIR Spectrometer, Tango model, Brucker Company).

Statistical analysis

The data obtained were submitted to the error normality and homogeneity analysis, followed by the analyses of variance and comparison of means, both with a 5% significance level. The parametric analyses were performed using the SISVAR (Ferreira, 2011) and SPSS (IBM Corporation, 2020) computational applications. The nonparametric Kruskal-Wallis and Friedman tests were applied using the Action Stat 3.7 program.

Results and Discussion

Germination and initial development of the plants

The experiment under controlled conditions (Table 1) verified that the UCZ concentrations applied with different incubation times did not influence the germination rate (%G), with an overall mean of 98.75%. This non-interference of UCZ in the germination percentage is very important for the crop's settling. Differently, the seedling height (SH), shoot fresh mass (SFM), and total fresh mass (TFM) characteristics changed with incubation time (IT). Furthermore, the seeds' exposure to different concentrations for 1 min resulted in higher mean values when compared

to the 12-hour incubation treatments.

The studies conducted by Liao et al. (2014) showed that seeds treated with Uniconazole presented changes in the α -amylase activity, being stimulated only at the end of germination without interfering with germination percentage. A significant difference was also detected for %G (Table 1). However, the longer exposure time to UCZ probably allowed for more excellent absorption by the seed, and a reduction was verified in the SH, SFM, and TFM variables. This reduction can be related to hormone balance since triazoles inhibit gibberellin biosynthesis and lead to abscisic acid and cytokinin increments (Soumya et al., 2017; Liu et al., 2015). As a result, the growth of the seedlings is delayed by the UCZ levels applied and does not prevent the germination process.

For the root dry mass (RDM) variable, the UCZ x IT interaction was significant (Table 1). The test of means (Table 2) verified that seed incubation for 12 h at a concentration of 100 mg UCZ kg⁻¹ of seed resulted in seedlings with more root dry mass concerning the other treatments, control and 50 mg UCZ kg⁻¹ of seed. According to Kieber and Schaller (2018), cytokinins are a group of phytohormones synthesized by the root meristem cells and shoots. They are translocated by the xylem and phloem, influencing different aspects of plant development, including shoot growth and plant response to biotic and abiotic stresses. Therefore, it is suggested that the increased root mass due to seed incubation for 12 h at 100 mg UCZ kg⁻¹ may be associated

with an increase in the number of sites for the phytohormone synthesis. This result in a more significant initial start of the plants due to its stimulation of cell division into meristems and vascular development (Osugi & Sakakibara, 2015).

In the experiment carried out in the greenhouse (Table 3), in seedlings at the V1 stage, it was verified that the incubation time of seeds with UCZ exerted an influence on root length (RL), root fresh mass (RFM), and total fresh mass (TFM). The 12 h time increased the magnitude of these variables concerning the 1 min time. The concentration factor exerted an influence on shoot height (SHP) and root length (RL) without modifying the mean number of roots (NR) and the fresh (SFM, RFM, and TFM) and dry (SDM) mass variables. The SHP variable presented a significantly higher value when compared to the control when the seeds were treated in a solution of 100 mg UCZ kg⁻¹; however, it did not differ from the treatments with 50 mg UCZ kg⁻¹ of seed.

Concerning the mean length of the root system (RS), the IT x UCZ interaction was significant with the 12 h treatment, being superior to the 1 min treatment, at the concentration of 50 mg UCZ kg⁻¹ of seed, which can represent the main contribution of this regulator for initial plant growth (Table 4). In the 1 min time, the UCZ treatments were significantly superior to the control, although they did not differ. However, it is suggested that the increase in root dry mass, detected in the experiment conducted

Table 1. Summary of the analysis of variance and test of means of the experiment carried out in a BOD Incubator to evaluate the effect of the Uniconazole (UCZ) concentration, using two incubation times (ITs) in treatment of the seeds, for the variables evaluated in seedlings: germination percentage (%G), seedling height (SH), shoot fresh mass (SFM), root fresh mass (RFM), total fresh mass (TFM), shoot dry mass (SDM), root dry mass (RDM), and total dry mass (TDM).

FV	DF	Mean Squares for the variables									
		%G	SH	SFM	RFM	TFM	SDM	RDM	TDM		
Incubation Time (IT)	1	1.50ns	79.913**	0.361**	1.246ns	2.948**	0.0006ns	0.0017ns	0.0044ns		
Uniconazole (UCZ)	2	6.0ns	18.967ns	0.017ns	1.218ns	1.447ns	0.0002ns	0.0052ns	0.0076ns		
TI x UCZ	2	6.0ns	1.721ns	0.021ns	0.089ns	0.082ns	0.0005ns	0.015*	0.021ns		
Residual	18	2.944	10.171	0.071	0.364	0.523	0.0007	0.0039	0.0067		
CV (%)		1.74	13,39	23.39	19.91	17.32	11.93	9.07	8.86		
Overall Mean		98.75	23.819	1.146	3.030	4.177	0.232	0.695	0.927		
Mean Values for the IT											
Factor											
Time – 1 min		98.5a	25.644a	1.269a	3.258a	4.527a	0.237a	0.704a	0.941a		
Time – 12 h		99.0a	21.99b	1.024b	2.802a	3.826b	0.227a	0.686a	0.014a		
Mean Values for the UCZ											
Factor											
Control		99,25a	24.86a	1.170a	2.883a	4.053a	0.231a	0.678a	0.910a		
50 mg UCZ kg ⁻¹ of seed		97,75a	22.05a	1.092a	2.735a	3.827a	0.227a	0.683a	0.910a		
100 mg UCZ kg ⁻¹ of seed		99,25a	24.54a	1.177a	2.743a	4.650a	0.238a	0.725a	0.963a		

* Significance at 5% probability; ** Significance at 1% probability; ^{ns} Not significant; ^v Mean values followed by the same letter in the column do not differ statistically by Tukey's test at 5% probability

Table 2. Comparison of means by Tukey's test corresponding to the data obtained from seedlings from the experiment conducted in a BOD incubator, for the Dry Root Mass (DRM) variable, of the seed treatments involving two incubation times and UCZ concentrations.

Incubation Time	UCZ Concentration (mg kg ⁻¹ of seed)			
	Control	50	100	Mean
1 min	0.710Aa	0.719Aa	0.682Aa	0.704
12 h	0.646Ab	0.646Ab	0.767Aa	0.686
Mean	0.678	0.683	0.725	-

^{1/} The mean values followed by the same upper-case letter in the column and in the row do not differ statistically by Tukey's test at 5% probability.

in a BOD incubator (Tables 1 and 2), is the first effect promoted by UCZ in maize seedlings, contributing to stimulating shoot growth.

A deeper root system (Ali et al., 2016) and a few lateral roots (Zhan et al., 2015) are traditional selection criteria that can be adopted to identify genotypes with tolerance to water deficit. In a study conducted by Li et al. (2015), it was verified that the root architecture of maize lines presents high heritability and that tropical germplasm showed greater tolerance to water deficit. However, changes in the environmental conditions or the use of biostimulants can result in alterations in the root system pattern. In the soybean crop, Yan et al. (2013) verified that treatment of soybean seeds with UCZ stimulated root growth and influenced the plants' mineral nutrition, showing that this compound affects the nitrogen transfer mechanism to support further growth. Liao et al. (2014) verified that using UCZ in maize reduced the α -amylase activity in the initial germination stage, followed by its increase, culminating in the formation of more

uniform plants with an increase in the root-shoot ratio, in the number of roots and the chlorophyll content.

For analysis of variance of the chlorophyll "A" and "B" levels, employing leaf discs of plants at stage V1 (Table 3), a significant difference was detected by the F test for IT x UCZ. By comparing the mean values using Tukey's test (Table 4), it was found that incubation with 100 mg UCZ kg⁻¹ of seed for 1 min resulted in higher chlorophyll A (12.75 $\mu\text{g}\cdot\text{cm}^2$) and B (3.47 $\mu\text{g}\cdot\text{cm}^2$) values when compared to those obtained at 12 h: 10.22 $\mu\text{g}\cdot\text{cm}^2$ and 2.82 $\mu\text{g}\cdot\text{cm}^2$, respectively. In this study and Liao et al. (2014), the chlorophyll content in plants originating from seeds treated with UCZ can be related to the increase in cytokinin biosynthesis by the roots and transported to the aerial parts of the plant. In this way, seed treatment with UCZ may positively contribute to settling the maize crop stand, making it more competitive (Sbrussi & Zucarelli, 2014). In addition, the rapid development of the seedlings' root system

Table 3. Summary of the variance analysis and means comparison by Tukey's test of a factorial experiment conducted in a greenhouse to evaluate the effect of Uniconazole (UCZ) concentration on shoot height (SHP), the first leaf insertion height (FLIH), root length (RL), stem diameter (SD), number of roots (NR), shoot fresh mass (SFM), root fresh mass (RFM), total mass fresh (TFM), shoot dry mass (SDM), and chlorophyll "A" (CHLORA) and chlorophyll "B" (CHLORB) contents, using two incubation times (ITs).

FV	DF	Mean Squares for the variables										
		SHP	FLIH	RL	SD	NR	SFM	RFM	TFM	SDM	CHLROA	CHLORB
Incubation Time (IT)	1	1.348ns	0.579ns	30.55**	0.128ns	0.308ns	0.0003ns	1.340**	1.301**	0.0003ns	4.909ns	0.424ns
Uniconazole (UCZ)	2	9.497**	0.235ns	25.07**	0.009ns	3.02ns	0.035ns	0.044ns	0.016ns	0.0051ns	0.36ns	0.051ns
TI x UCZ	2	1.719ns	0.127ns	105.25*	0.135ns	2.70ns	0.0129ns	0.278ns	0.386ns	0.0009ns	4.969**	0.267*
Residual	30	2.340	0.230	5.85	0.058	2.103	0.0115	0.121	0.166	0.0018	1.424	0.114
CV (%)		14.79	10.89	13.68	6.98	12.67	10.22	9.07	16.15	9.14	10.61	10.87
Overall Mean		10.34	4.40	17.68	3.459	11.44	1.052	1.477	2.525	0.47	11.24	3.09
Mean Values for the IT Factor												
Time – 1 min		10.15a	4.28a	16.76b	3.518a	11.53a	1.048a	1.228b	2.335b	0.467a	11.69a	3.22a
Time – 12 h		10.53a	4.53a	18.60a	3.399a	11.35a	1.055a	1.667a	2.715a	0.474a	10.79a	2.96a
Mean Values for the UCZ Factor												
Control		9.69b	4.56a	14.77b	3.431a	10.97a	1.032a	1.537a	2.560a	0.469a	11.13a	3.13a
50 mg UCZ kg ⁻¹ of seed		9.97ab	4.34a	19.08ab	3.458a	11.97a	1.010a	1.477a	2.487a	0.450a	11.10a	3.00a
100 mg UCZ kg ⁻¹ of seed		11.35a	4.31a	19.18a	3.487a	11.38a	1.113a	1.415a	2.528a	0.492a	11.49a	3.14a

* Significance at 5% probability; ** Significance at 1% probability; ns Not significant; ^{1/} Mean values followed by the same letter in the column do not differ statistically as per the F test at 5% probability; ^{2/} Mean values followed by the same letter in the column do not differ statistically by Tukey's test at 5% probability

Table 4. Comparison of means by Tukey's test corresponding to the data obtained from seedlings from the experiment conducted in a greenhouse for the root length (RT), chlorophyll "A" content (CHLORA), and chlorophyll "B" (CHLORB) content variables for the seed treatments involving two incubation times and UCZ concentrations.

Variables	Incubation Time	UCZ Concentration (mg.kg ⁻¹)			Mean
		Control	50	100	
RT	1 min	12.05Ab	17.62Ba	20.61Aa	16.760
	12 h	17.50Aa	20.55Aa	17.76Aa	18.603
	Mean	14,775	19.085	19.185	-
CHLORA	1 min	10.83Aa	10.71Aa	12.75Aa	11.43
	12 h	11.44Aa	11.50Aa	10.22Ba	11.05
	Mean	11.13	11.10	11.49	-
CHLORB	12 h	3.09Aa	3.11Aa	3.47Aa	3.22
	Mean	3.17Aa	2.89Aa	2.82Ba	2.96
	Mean	3.13	3.00	3.14	-

^{1/} The mean values followed by the same upper-case letter in the column and in the row do not differ statistically by Tukey's test at 5% probability.

promotes an increased soil exploration capacity (Marcos Filho, 2015).

Growth and development of plants in field conditions

The plant growth from seeds subjected to different UCZ concentrations in two incubation times (ITs) was subjected to the analysis of variance for repeated measures in time. Initially, the absence of sphericity was detected by the Mauchly's test ($W = 0.053$ and $p < 0.01$), thus, the data were corrected by Greenhouse-Geisser ($\epsilon = 0.592$). Significance of the effect of incubation time [$F(2.962;91.814) = 56.092$ and $p < 0.01$] on the plant height variable was verified, without significance for the IT x Time [$F(2.962;91.814) = 0.909$ and $p > 0.05$],

Population (P) x Time [$F(2.962;91.814) = 0.033$ and $p < 0.05$] and UCZ x Time [$F(5.924; 91.814) = 0.856$ and $p > 0.05$] interactions, indicating that the treatments employed did not differentially influence plant growth in relation to cultivation time. However, by comparing marginal mean values estimated by the Pairwise method (Bonferroni correction), plants at 5 DAE (first evaluation) from seeds that were inoculated with 100 mg UCZ kg⁻¹ of seed presented a lower shoot height ($9.028 \text{ cm} \pm 0.325$) than the control ($10.351 \text{ cm} \pm 0.325$). However, they differed from those from seeds that received 50 mg UCZ kg⁻¹ of seed ($9.317 \text{ cm} \pm 0.325$).

Schluttenhofer et al. (2011) found that foliar application of UCZ (1.0 mg L⁻¹ solution) resulted in height reduction without affecting

maize grain yield. In a study by Ahmad et al. (2018), the maize plant size reduction was verified from UCZ doses above 75 mg Kg⁻¹ of seed. However, in the current paper, the Uniconazole doses of 50 and 100 mg Kg⁻¹ of seed promoted a slight reduction of the plant size at the beginning of plant development, disappearing quickly with their settling in the field. The inhibitory effect on plant growth was also verified in wheat (Yong-Chao et al., 2015). The reduction of plant growth can lead to a decrease in productive potential; however, it can favor increases in productivity depending on the dose. In this sense, Ahmad et al. (2018) obtained an increase in maize productivity in the treatment of seeds with 25 mg UCZ kg⁻¹ of seed or 25 mg UCZ L⁻¹ of solution.

No effect of the treatments was verified on the emission of male or female inflorescences, whose requirements in growing degree units (GDUs) were 767.51 and 805.46, respectively (data not shown). However, the difference between the thermal sum accumulated for female and male flowering (DFM) for plants from the incubation of seeds with 50 mg UCZ kg⁻¹ (IT = 1 min) and 100 mg UCZ kg⁻¹ (IT = 12 h) presented a lower DFM compared with the other treatments with the population of 75,000 plants. ha⁻¹. As DFM is one of the parameters employed to identify maize plants tolerant to water stress (Durães et al., 2004), it can be suggested that the reduction in magnitude may indicate one of the effects of applying UCZ.

The average number of green leaves below the ear at 57 DAE of a population of 75

thousand plants ha⁻¹ was evaluated using the Friedman test ($\chi^2 = 10.15$; GL = 5; p-value = 0.07). Further, comparing the medians by the Dunn's test (5% probability) showed that plants of the seed treatment of 100 mg UCZ kg⁻¹ seed (TI = 12 h) had a higher mean number of green leaves (8.5±0.38) than the control (0 mg UCZ kg⁻¹ seeds and TI = 12 h), with 7.7±0.38. Studies by Ahmad et al. (2019) suggest that UCZ may play a protective role against the degradation of pigments involved in photosynthesis. The authors detected the activation of enzymes in the antioxidative process, which leads to a delay in senescence of the lower leaves. Furthermore, studies involving the use of UCZ in maize under water deficit conditions showed that the plants presented a delay in the leaf senescence process (Fang et al., 2018; Yong-Chao et al., 2015), observed through the quantification of chloroplast pigments in leaves (Takamiya et al., 2000). In the current paper, the determinations of pigments carried out in the ear leaf, in the presence of sphericity by Mauchly's test for total chlorophyll (W = 0.615 and p > 0.05) and carotenoids (W = 0.761 and p > 0.05), only the effects of time and population were verified. These results show that the IT and UCZ factors did not promote changes in the pigment degradation process in the ear leaf. However, by comparing estimated marginal mean values (data not shown) using the Pairwise method (Bonferroni correction), maize plants in a population of 65,000 plants. ha⁻¹ presented higher levels of total chlorophyll and carotenoids at 6, 22 and 51 DAE concerning

those from the population of 75,000 plants.ha⁻¹.

According to Dhama and Cazzonelli (2020), carotenoids play a vital role in the plants, such as the accumulation of lutein, β -carotene, violaxanthin, and neoxanthin in the thylakoid membrane. Also, carotenoids are responsible for capturing and transferring energy to chlorophyll "A" during photosynthesis. They are associated with incident luminosity and protective function on photosystems (Kurz et al., 2008). Thus, the accumulation of zeaxanthin and foliar antheroxanthin under high light intensity and high temperatures maintains membrane integrity in thylakoids and, consequently, in chloroplasts (Dhama & Cazzonelli, 2020). In a study conducted by Ren et al. (2017), they verified that, with the increase in maize population density, there was a reduction in the chlorophyll content, while there was an initial decrease for carotenoids, followed by an increase in the content of this pigment. Thus, the higher chlorophyll content in the population of 65,000 plants.ha⁻¹ than that of 75,000 plants.ha⁻¹ may be associated with the greater effectiveness of carotenoids, despite the greater flow of incident light energy on the leaves for the population with lower plant density, suggesting that these accessory pigments prevent photo-oxidation of the chlorophylls (Dhama & Cazzonelli, 2020).

Production and components of yield

The analysis of variance for agronomic traits related to production (Table 5) presented a significant difference for ear length (EARLEN) and cob diameter (CD), by the F test, respectively,

at 5% and 1% significance, for the Incubation Time (IT) factor. By comparing means, regardless of the UCZ concentration, IT = 12 h resulted in greater ear length and diameter than IT = 1 min.

The productivity variable (Table 5) presented a significant difference for the Population (P), the main factor, and the P x UCZ and IT x UCZ interactions. In average terms, the productivity of the MSG1001 hybrid cultivated at a population density of 75,000 plants.ha⁻¹ produced 5,471 kg.ha⁻¹, while in the population of 65,000 plants.ha⁻¹, it reduced grain production: 5,021kg.ha⁻¹. Several studies have shown that population density can define maize crop yield (Haarhoff & Swanepoel et al., 2018; Boiago et al., 2017; Sangoi, 2001). Also, production maximization depends on genetic factors and environmental factors, such as water availability, soil fertility, sowing season, and plant arrangement (Sangoi, 2001). In the current study, we obtained 20 ears.plot⁻¹ and 17 ears.plot⁻¹, respectively, for the populations of 75,000 (30 plants.plot⁻¹) and 65,000 plants.ha⁻¹ (26 plants plot⁻¹). These results shows that limiting environmental factors such as water deficit and high temperatures may have contributed to increasing intraspecific competition, resulting in the presence of plants with ears without grains. However, the causes for the emergence of this type of plant are unknown, although they can be associated with environmental conditions before flowering, as the differentiation process of female inflorescences begins at stage V3

Table 5. Summary of the analysis of variance and Tukey's test at 5% probability of the triple-factor experiment carried out in the field to evaluate the effect of Uniconazole (UCZ) concentration, using two incubation times (ITs) for the ear length (EARLEN), ear circumference (EARCIR), ear diameter (ED), cob diameter (CD), productivity (PROD), thousand-grain mass (TGM) and maize moisture at harvest (CMH) of maize hybrid MSG1001, obtained from plants grown under different population densities (P).

FV	Mean Squares for the variables									
	DF	EARLEN	EARCIR	ED	CD	PROD	TGM	CMH		
Block	2	0.731ns	0.086ns	0.009ns	0.001ns	289680.472ns	14.106ns	0.121ns		
Population (P)	1	0.003ns	0.093ns	0.009ns	0.0002ns	1824149.092*	69.444ns	0.340ns		
Incubation Time (IT)	1	0.922*	0.245ns	0.025ns	0.017**	117865.613ns	39.062ns	0.267ns		
Uniconazole (UCZ)	2	0.029ns	0.029ns	0.003ns	0.005ns	285302.839ns	255.512ns	1.081ns		
P x IT	1	0.115ns	0.004ns	0.0004ns	0.003ns	8723.541ns	34.028ns	1.102ns		
P x UCZ	2	0.369ns	0.073ns	0.007ns	0.002ns	1173800.490*	15.668ns	1.414ns		
IT x UCZ	2	0.377ns	0.045ns	0.004ns	0.002ns	2032904.998**	195.443ns	0.031ns		
P x IT x UCZ	2	0.412ns	0.0120ns	0.0122ns	0.002ns	10903.106ns	117.231ns	1.080ns		
Residual	22	0.227	0.082	0.008	0.002ns	308595.227	152.174	0.958		
CV (%)		3.39	1.90	1.90	1.63	10.59	3.98	4.26		
Overall Mean		14.05	15.14	4.82	2.76	5246.79	309.65	22.96		
Mean Values for the P ^{1/} Factor										
Population: 75,000		14.06a	15.09a	4.80a	2.76a	5471.89a	308.26a	23.06a		
Population: 65,000		14.04a	15.19a	4.84a	2.76a	5021.69b	311.04a	22.87a		
Mean Values for the IT ^{1/} Factor										
Time – 1 min		13.89b	15.06a	4.79a	2.74b	5304.01a	308.61a	22.88a		
Time – 12 h		14.21a	15.22a	4.84a	2.78a	5189.57a	310.69a	23.05a		
Mean Values for the UCZ ^{2/} Factor										
Control		14.02a	15.18a	4.83a	2.78a	5049.23a	305.62a	23.26a		
50 mg UCZ kg ⁻¹ of seed		14.10a	15.17a	4.83a	2.77a	5234.29a	314.69a	22.97a		
100 mg UCZ kg ⁻¹ of seed		14.02a	15.08a	4.80a	2.74a	5406.85a	308.64a	22.66a		

* Significance at 5% probability; ** Significance at 1% probability, ^{ns} Not significant;

¹ The mean values followed by the same letter in the column do not differ statistically by t test at 5% probability

(Magalhães & Durães, 2006).

With the splitting of the P x UCZ interaction for PROD (Table 6), it was verified that seeds treated with UCZ at doses of 50 and 100 mg kg⁻¹ of seed resulted in higher grain production in the population of 75,000 plants ha⁻¹. The IT x UCZ splitting (Table 7) presented a significant difference for grain production at IT = 12 h, with 100 mg UCZ kg⁻¹ of seed being superior concerning the control and not differing from the UCZ concentration of 50 mg kg⁻¹ of seed. For the incubation times, evaluation of the UCZ concentration showed that, for IT = 1 min, the hybrid's mean productivity was higher with

a 12 h incubation time. However, for IT = 12 h, and using 100 mg UCZ kg⁻¹ of seed resulted in a higher mean productivity. These results show that using UCZ can contribute to productivity more than the influence of the incubation time on this variable.

Uniconazole and chemical composition of the grains

For the starch content (Table 8), a significant difference was verified by the F test for the following main factors: Population (P), Incubation Time (IT), and Uniconazole Dose. It is verified that the starch percentage was

Table 6. Comparison of means by Tukey's test at 5% significance corresponding to the productivity variable (Kg ha⁻¹) for different population densities and UCZ concentrations.

Population ^{1/}	UCZ Concentration (mg kg ⁻¹ of seed)			
	Control	50	100	Mean
75,000 plants.ha ⁻¹	4987.76Aa	5741.07Aa	5686.85Aa	5471.89
65,000 plants.ha ⁻¹	5210.69Aa	4727.51Ba	5126.86Ba	5021.69
Mean	5099.23	5234.29	5406.85	-

¹ The mean values followed by the same upper-case letter in the column and in the row do not differ statistically by Tukey's test at 5% probability.

Table 7. Comparison of means by Tukey's test at 5% significance for the productivity variable (Kg ha⁻¹), involving two incubation times and different UCZ concentrations

Incubation Time ^{1/}	UCZ Concentration (mg kg ⁻¹ of seed)			
	Control	50	100	Mean
1 min	5614.81Aa	5171.14Aa	5126.08Ba	5304.01
12 h	4583.65Bb	5297.45Aab	5687.62Aa	5189.57
Mean	5099.23	5234.29	5406.85	-

NOTE: ¹ The mean values followed by the same upper case letter in the column and in the row do not differ statistically by Tukey's test at 5% probability.

higher in grains from plants belonging to the population containing 65,000 plants.ha⁻¹. The seed treatment with IT = 12 h resulted in plants that produced grains with higher starch content than with IT = 1 min. Using 100 mg UCZ kg⁻¹ of seed promoted an increase in the starch content concerning to the control, with no statistical difference from the dose of 50 mg kg⁻¹ of seed. These results agree with those found in the literature, where UCZ has increased the starch content in grains from different plant species (Huang et al., 2015; Liu et al., 2015, 2019).

The crude fiber (CF) percentage, which by definition corresponds to the content of cellulose, hemicellulose, pectic substances, lignin, and other compounds resistant to enzymatic digestion (Dhingra et al., 2012), only presented a significant difference by the F test for the IT x UCZ interaction (Table 8).

For the crude fiber variable, it was verified that in control (Table 9), IT = 1 min was superior to IT = 12 h. However, no significant difference was detected in incubation time concerning the different UCZ concentrations. This result allows raising the hypothesis that incubation time exerted an influence on CF, although not associated with the effect of UCZ.

Concerning the EE and MM variables (Table 8), a population effect was detected with grains from 75,000 plants.ha⁻¹ presenting higher mean values when compared to those from 65,000 plants.ha⁻¹. In addition, plant grains from seeds inoculated with UCZ reduced the ether extract, revealing that this regulator may have a

deleterious effect on the accumulation of lipids in the grain. Berardo et al. (2009), evaluating maize germplasm by the near-infrared (NIR) spectroscopy technique, detected that ether stratum varied from 5.26% to 7.17%, similar to the value determined in the current study. In this context, when compared to the increase promoted by UCZ for the starch percentage, the low lipid content in maize grains can be considered insignificant.

UCZ has been used in tropical fruit species, such as avocado and mango, aiming at floral induction and fruit set (Brogio et al., 2018; Lima et al., 2016), but there is no record for use in maize. However, research results have revealed that UCZ exerts a beneficial effect on the modification of plant metabolism that results in increased grain production under water deficit conditions (Ahmad et al., 2018, 2019). Thus, the use of UCZ in maize can be one of the alternatives to help mitigate the negative effects of droughts, which are frequently observed in maize-growing areas in Brazil. In this context, seed treatment with UCZ, promoting an increase in the mean length of the roots at the beginning of plant development, can result in increased seedling survival, allowing for rapid settling of the crop, especially in soils with greater loss of surface moisture.

Alternatives capable of meeting the food demand due to population growth associated with the climate changes that have led to a reduction in crop productivity, the use of UCZ in seed treatment may present socio-economic relevance.

Table 8. Summary of the analysis of variance and Tukey's test at 5% probability of the three-factor experiment carried out in the field to evaluate the effect of Uniconazole (UCZ) concentration and using two incubation times (ITs) on starch (STARCH), ether extract (EE), crude fiber (CF), mineral material (MM) and crude protein (CP) contents from the MSG1001 hybrid maize grains obtained from plants cultivated under different population densities (P).

FV	Mean Squares for the variables					
	DF	STARCH	EE	CF	MM	CP
Block	2	0.018ns	0.020ns	0.004ns	0.001	0.022ns
Population (P)	1	4.913**	0.289*	0.015ns	0.020*	0.177ns
Incubation Time (IT)	1	0.624*	0.030ns	0.020ns	0.003	0.039ns
Uniconazole (UCZ)	2	0.470*	0.036*	0.000058ns	0.002	0.021ns
P x IT	1	0.185ns	0.003ns	0.012ns	0.0008	0.042ns
P x UCZ	2	0.322ns	0.043ns	0.000083ns	0.002	0.059ns
IT x UCZ	2	0.363ns	0.120ns	0.034*	0.007	0.035ns
P x IT x UCZ	2	0.359ns	0.040ns	0.003ns	0.001	0.128ns
Residual	22	0.124	0.042	0.009	0.002	0.051
CV (%)		0.55	6.20	6.29	3.6	2.09
Overall Mean		63.66	3.31	1.50	1.29	10.83
Mean Values for the P ^{1/} Factor						
Population: 75,000		63.88b	3.39a	1.52a	1.32a	10.90a
Population: 65,000		64.03a	3.21b	1.48a	1.27b	10.76a
Mean Values for the IT ^{1/} Factor						
Time – 1 min		63.52b	3.34a	1.52a	1.30a	10.87a
Time – 12 h		63.79a	3.28a	1.48a	1.28a	10.80a
Mean Values for the UCZ ^{2/} Factor						
Control		63.50b	3.37a	1.50a	1.31a	10.79a
50 mg UCZ kg ⁻¹ of seed		63.59ab	3.29b	1.50a	1.29a	10.86a
100 mg UCZ kg ⁻¹ of seed		63.88a	3.26b	1.51a	1.28a	10.86a

* Significance at 5% probability; ** Significance at 1% probability; ns Not significant; ¹ Mean values followed by the same letter in the column do not differ statistically as per the t test at 5% probability

Table 9. Comparison of means by Tukey's test at 5% significance for the crude fiber variable (CP), for the treatments involving two incubation times and different UCZ concentrations.

Incubation Time ^{1/}	UCZ Concentration (mg.kg ⁻¹)			Mean
	Control	50	100	
1 min	1.58Aa	1.47Aa	1.52Aa	1.52
12 h	1.42Ba	1.53Aa	1.49Aa	1.48
Mean	1.50	1.50	1.51	-

¹ The mean values followed by the same upper-case letter in the column and in the row do not differ statistically by Tukey's and the t tests, respectively, at 5% probability.

In this context, the increase in the starch content in the grain verified in the current study can impact different industry sectors, including those related to animal feeding.

Conclusions

Maize seeds treated with 100 mg UCZ kg⁻¹ of seed for 12 hours promote an increase in root mass in seedlings in a BOD chamber without reducing the germination percentage.

Maize plants in the V1 stage, which result from applying UCZ to seeds, present higher shoot height, and incubation for 12 h with 50 mg UCZ kg⁻¹ of seed is more efficient for increasing the root length. In addition, using 100 mg UCZ kg⁻¹ of seed in the 1-min incubation time increases the accumulation of chlorophylls "A" and "B."

Seed treatment with UCZ does not result in any modification of female and male flowering. However, it influences the number of green leaves below the ear, indicating a possible delay in the leaf senescence process.

The population of 75,000 plants ha⁻¹ was

more productive, with UCZ exerting an influence on the hybrid's yield. However, incubating the seeds with 50 or 100 mg UCZ kg⁻¹ of seed for 1 min contributed more productivity than IT = 12 h.

The grains' starch content increases when applying UCZ at the dose of 100 mg kg⁻¹ of seed. In addition, the starch percentage can be modified according to population density and incubation time.

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