International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-9, Issue-12; Dec, 2022
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.912.51

Impact of ultraviolet C radiation and ozone application on the physiological quality of organic maize seeds

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Received: 25 Nov 2022,

Receive in revised form: 16 Dec 2022,

Accepted: 22 Dec 2022,

Available online: 31 Dec 2022

***Keywords*—Seed treatment, germination, physiological quality, Zea mays L.**

***Abstract*—*Non-thermal energy sources have been utilized to control diseases and boost the physiological quality of maize seeds. The aim of this paper was to assess the impact of UV-c exposure and ozone application, associated or not, on organic maize seed germination and seedling vigor. Three cultivars (BRS Caimbé, BRS 1060 and BR 451), three exposure levels to ozone (15, 30 and 60 minutes) in the absence and presence of ultraviolet radiation (UV-c), were used in a completely randomized design with a factorial scheme of 3 x 3 x 2 + 3 (additional treatments) experiment. Evaluations of electrical conductivity, emergence in soil, and germination (at 4th and 7th day) on paper roll were done. Daily measurements of the emerged seedlings were taken up to the 14th day after emergence. The measurements included total emergence (EC), coefficient of variation in emergence time (CVt) in percentage, coefficient of emergence velocity (CVe) in percentage, emergence rate index (ERI) in days, and emergence synchronization index (Z). The tested genotypes caused differences in all studied variables. Increasing the percentage with a 30-minute exposure to ozone in the presence of UV-C radiation resulted in a validated enhance in the first germination reading. The association of O_3 and UV-c techniques can be used to treat organic maize seeds without significantly affecting their physiological quality.*

I. INTRODUCTION

Maize is one of the most economic and significant agricultural commodities in the world due to its importance in providing the food security of humans and animals and in the industrial sector. In Brazil, the 2021/2022 corn harvest is expected to produce 117.2 million tons of grains, a 34.6% increase over the previous growing season, with the predicted grain harvest for the 2022/2023 at 125.87 million tons (CONAB, 2022).

Seed treatment methods are used to enhance the performance of seeds to produce high-quality crops. These methods can be classified into three main categories: biological, chemical, and physical. Utilizing beneficial microorganisms, biological seed treatments enhance seed health and performance. Chemical treatments employ synthetic inputs such as fungicides and insecticides, whereas physical seed treatments involve physical processes such as heating to improve seed performance (Rifna et al., 2019). Seed treatment methods can help to enhance the expression of the physiological quality of seeds, providing a high level of field performance while minimizing their environmental impact (krzyzanowski et al., 2020).

Among the most common treatment technics, heat is routinely used in organic and conventional agriculture as a tool with high effectiveness and efficiency for controlling pathogens in corn seeds (Coutinho et al., 2007), and other commercially significant crops (Da Silva et al., 2021). However, novel non-thermal energy sources have recently received attention in the seed industry (Ziuzina et al. 2019; Rifna et al. 2019). In this context, we can mention the use of ozone as a physiological seed conditioner (Pandiselvam et al., 2020; De Alencar et al., 2021), that has been demonstrated to improve vigor in corn seeds in recent studies (Monteiro et al., 2021; Rosa et al., 2021).

Ozone gas promotes a range of consequences on maize seeds. Ozone can be absorbed by the seed coat, where it can be transformed into other compounds that inhibit germination and emergence. Additionally, ozone can react with other molecules in the seed, such as proteins and lipids, resulting in chemical changes that can impact the viability of the seed and seedling vigor (Violleau et al., 2008). In addition, ozone can influence the growth and development of the seedling after it emerges from the seed. The gas can injure plant cells and tissues, resulting in restricted development and growth. It can also impair a plant's capacity to photosynthesize and generate energy, resulting in lower yields (Choquette et al., 2020). Overall, the effects of ozone on maize seeds and seedlings are complex and can vary based on ozone concentration and exposure period. It is essential to regulate ozone levels to preserve maize seeds physiological quality and ensure optimum crop harvests (Pandiselvam et al., 2019).

UV radiation is another technological strategy for using energy to improve the quality of seed lots, especially the c type. UV is a non-ionizing radiation and is classified into three categories: A, B, and C (UV-c presents wavelengths in the range of 100 to 280nm). Plants are not adapted to recognize and defend against UVc, and its use in certain conditions in seeds causes germination potential or vigor loss due to deterioration (krzyzanowski et al., 2020). There are, nevertheless, some studies that show the benefits of seed conditioning and priming (Sadeghianfar et al., 2019; Farooq et al., 2019). Despite a wide range of studies on ozone and ultraviolet radiation applications, there are gaps in the research and the findings that combine the techniques.

The purpose of this study was to examine the effects of ozone application and UVc radiation, either associated or not, on the germination and vigor of organic maize seeds.

II. MATERIAL AND METHODS

Seeds of three distinct maize cultivars of Embrapa were evaluated, including a single-cross hybrid (BRS 1060) and two common varieties, BRS Caimbé and BR 451 (a high-Quality Protein Maize cultivar). Pure seeds of each genotype were used for the experiment. The experimental design was completely randomized in a factorial scheme of 3 x 3 x 2 + 3 (additional treatments), with a total of 21 treatments, including three cultivars (BRS Caimbé, BRS 1060, and BR 451), three exposure times to ozone (15, 30, and 60 minutes) in the absence and presence of ultraviolet radiation, and three additional treatments (3 cultivars without UV-c and O3) using three replicates. Each experimental plot comprised a total of 50 seeds.

The seeds were exposed to ozone in the gaseous phase for the treatment's length of time (levels). For seed conditioning and treatment, a 100-liter treatment chamber was used. A low-cost cold plasma generator, with a capacity of 2 grams per hour, was employed for the synthesis of ozone. After treating the seeds with O_3, the seeds were arranged in a single layer and exposed to UVc radiation. As source, a mercury UV-C lamp (Philips) emitting an irradiance of 0.3 W m^{-2} at 10 cm was utilized.

Following the treatments, the seeds were stored in a cold chamber (10ºC and 40% RH) for subsequent evaluations of germination in a paper roll, electrical conductivity, and sowing emergence. The percentage of germination was determined in conformity with the Rules for Seed Analysis (Brasil, 2009), counting as normal those seedlings with fully developed primordial components. Two measurements were taken, the first at four days and the second at seven days, as germination was complete.

For the electrical conductivity test, by mass method, three repetitions of 50 seeds from each treatment were utilized. The seeds were submerged in 75 ml of deionized water for 24 hours at a temperature of 25 degrees Celsius. The data were reported in µS cm^{-1} g^{-1} of seeds.

The seeds were sowed in soil comprising agricultural topsoil in order to measure the field emergence and growth of seedlings to determine the vigor of the treated seed lot. After sowing, daily measurements of the emerging seedlings were taken for fourteen days.

Total emergence percentage, in percentage (Em), emergence rate index (EI) in seedling days, coefficient of variation of emergence time (CVt) in seedling day^{-1}, coefficient of velocity of emergence (CVe) in percentage and emergence synchronization index (Z), were evaluated.

After checking the assumptions of normality and homoscedasticity, the collected data were subjected to the

F test and an analysis of variance was performed. If significant, the averages were submitted to Tukey's average test using the R package Germinationmetrics (Aravind et al., 2021).

III. RESULTS AND DISCUSSION

All analyzed variables varied depending on the genotypes evaluated (Tables 1). For the First germination reading (1G), however, a response was observed for application of the ozone levels and for the combination of all tested factors (cultivars x Ozone x UV-c). While UV-c radiation had an influence on the emergence of seedlings (Em) and Emergence rate Index (ERI).

Table 1 – Summary of the analysis of variance for the first germination reading (1G) germination (G), abnormal seedlings (AS), dead seeds (DS), electrical conductivity (EC), total emergence (Em), emergence rate index (ERI), coefficient of variation of emergence time (CVt), emergence speed coefficient (CVe) and emergence synchronization index (Z) of seeds of maize genotypes exposed to ozone and UV-c radiation.

Source of variation	GL	Mean squares									
		1G	G	AS	DS	EC	Em	ERI	CVt	CVe	Z
Cultivars(C)	2	5499.0**	3840.5**	840.3**	1093.6**	395.3**	2592.5**	20.5**	74.7**	1.3**	0.2 **
Ozone (O)	2	151.4*	253	34.7	28.7	1.5	6.7	0.1	8.7	0.2	0.02
Ultraviolet-C (U)	1	1.85	66.67	35.85	4.74	0.45	224.1**	1.22*	1.75	0.03	0.02
C x O	4	109.63	143.85	21.74	5.22	12.28	9.63	0.11	14.06	0.08	0.02
C x U	2	164.15	124	19.85	12.52	4.15	39.19	0.32	8.62	0.03	0.02
O x U	2	2.37	87.11	35.19	0.96	1.5	17.85	0.12	0.3	0.01	0
C x O x U	4	115.41*	370.22	22.19	36.41	6.67	18.3	0.09	14.49	0.12	0.02
(Fatorial)	17	708.26**	513.29**	121.86**	143.69**	51.84**	332.26**	2.60 **	17.68*	0.22 **	0.04 **
Add. Vs. Factorial	1	25.41	15.28	26.46	1.52	0.95	1.28	0	24.87	0.09	0
Fact. Add.	2	765.78**	499.11**	99.11**	185.33**	90.05**	625.33**	4.66 **	10.59	0.16	0.0**
(treatment)	20	679.87**	486.97**	114.82**	140.74**	53.11**	345.02**	2.67**	17.33*	0.21**	0.0**
CV (%)		8.13	8.04	8.44	43.99	13.43	5.76	6.1	31.9	1.44	11.84

**, * = significant at 5% and 1% probability, respectively.

The final and early germination test results for the hybrid BRS 1060 were higher than those of the tested varieties (Table 2). In this reference, BRS Caimbé variety had the lowest vigor values, as evidenced by the measured metrics, particularly the highest electrical conductivity value, the lowest emergence speed, and the final emergence in the soil (Table 2). It is widely demonstrated the influence of genotypes, and genetic background, over the seed physiological quality indicator (Mortele et al., 2012), with variable capacity of tests on measure the vigor of maize lots (Milivojević et al., 2021).

Table 2 - Means of first reading of the germination test (1G), Germination (G), abnormal seedlings (AS), dead seeds (DS), electrical conductivity (EC), total emergence (Em), emergence rate index (ERI), coefficient of variation of emergence time (CVt), emergence speed coefficient (CVe) and emergence synchronization index (Z) of seeds of maize genotypes submitted to different exposure levels of ozone and ultraviolet C.

Cultivars	1G	G	AS	DS	EC	Em	ERI	CVt	CVe	Z
	%				$\mu S.cm^{-1}.g^{-1}$	%	days	%		-
BRS 1060	93.67 a	97.89 a	1.56 b	0.56 b	13.79 c	97.89 a	8.03 a	7.38 b	16.33 a	0.85 a
BR 451	66.56 b	72.22 b	14.00 a	14.00 a	17.87 b	80.44 b	6.46 b	10.44 a	15.92 b	0.68 b
BRS Caimbé	61.00 c	73.22 b	12.67 a	14.11 a	23.14 a	74.89 c	5.99 c	11.24 a	15.83 b	0.64 b
Mean	73.74	81.11	9.41	9.56	18.27	84.41	6.83	9.69	16.03	0.72

Means followed by the same letter do not differ from each other in the columns by Tukey's test (p<0.05)

In addition to vigor, the effect of seed size and shape on electrical conductivity values has been reported (Dubal et al., 2020), which can influence the observed results once the roundness of the seeds (phenotype) has a strong relationship with the genotypes. Although the indicator based on seedlings, such as emergence in the soil, has an irrefutable association with the vigor of seed lots and the field performance (krzyzanowski et al., 2020).

The evaluation of seed vigor can be performed, among other biometric measures, through the first reading. It was verified that the application of ozone gas, combined or not with the UVc, promoted the highest germination value on the fourth day of reading in relation to the time of 15 minutes (Figure 1).

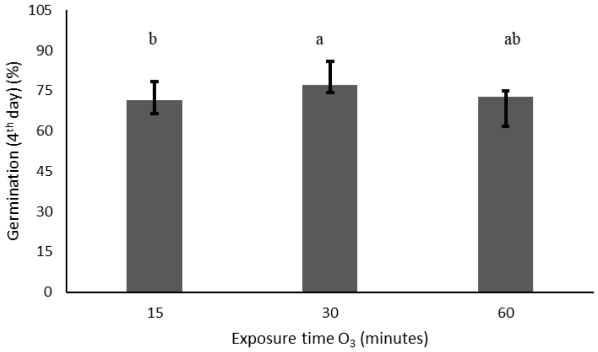

Fig.1. First reading averages of the germination test (1G) of seeds subjected to different ozone times.

Means followed by the same letter do not differ from each other in the columns by Tukey's test ($p<0.05$)

These values are consistent with those found in other studies. In our case, the ozone application data (table 5) included the effects of ultraviolet radiation, as well as the various ozone application times (levels) on corn seeds and the mean of the evaluated cultivars. Rose et al. (2021) have showed positive results from the use of ozone for germination and vigor of corn seeds, increasing by up to 1.8 times the germination percentage of corn seeds with low initial germination and up to 1.2 times the seedling length when ozone was used at a concentration of 10 mg L^{-1} with exposure times of 30 to 120 min. Monteiro et al. (2021) in addition to observing a significant effect on the percentage of germination of corn seeds (lot with low vigor), an increase in seedling length and germination speed index was also observed, with a time of exposure to ozone of 30 minutes. These authors also demonstrated that maize seed electrical conductivity values decreased when exposed to ozone for 30 to 120 minutes compared to a control treatment without ozone.

The use of type c ultraviolet radiation promoted stress to the seeds and consequent expression of their vigor observed in the emerged seedlings (Table 4).

Table 4 - Mean seedling final emergence (EC) and emergence rate index (ERI) for seeds maize treated with ozone and UVc

UV-c Radiation	EC	ERI
Presence	82,37 b	6,67 b
Absence	86,44 a	6,97 a
CV (%)	5.76	6.10

Means followed by the same letter do not differ from each other in the columns by Tukey's test ($p<0.05$)

The presence of radiation decreased the final emergence values of the seedlings and the emergence rate index, generating, however, a higher index of emergence speed in our study. Exposure to UVc radiation can cause stress to plants, as it can damage their DNA and other cellular components (Rajashekara et al., 2021). Plants have developed several mechanisms to protect themselves from the harmful effects of UV radiation. The effects of cadmium stress, excessive soil salinity, and ultraviolet C irradiation on the level of somatic DNA mutations in Arabidopsis thaliana revealed that somatic DNA mutations substantially increased when the plant was exposed to cadmium stress, but decreased when the treatment was UVc (Kiselev et al., 2018). The effects of UVc radiation on plants vary depending on the type of plant and the intensity and duration of exposure. In general, excessive exposure to UVC radiation can lead to reduced growth, deformities, and even death in plants.

In treated maize seeds, there was a strong correlation between germination and evaluated emergence metrics (figure 1). The maize seed germination and seedling emergence are two essential, but distinct developmental stages. Germination involves the breakdown of the seed's stored food reserves by water imbibition and the emergence of the seedling from the seed coat (Bewley et al., 2013). On the other hand, emergence is the process by which the seedling breaks through the surface of the soil and begins to grow upwards. Emergence tests and correlated metrics are essential to predict this phenomenon and the vigor of seed lots (krzyzanowski et al., 2020). In this sense, it is possible for a seed to germinate but not emerge, for example if it is sown too deeply in the soil or if it is unable to break through the soil or grow for some other reason (Hamman et al., 2002). Overall, the correlation between first germination reading and emergence in maize seeds was stronger than the final germination index (Figure 2).

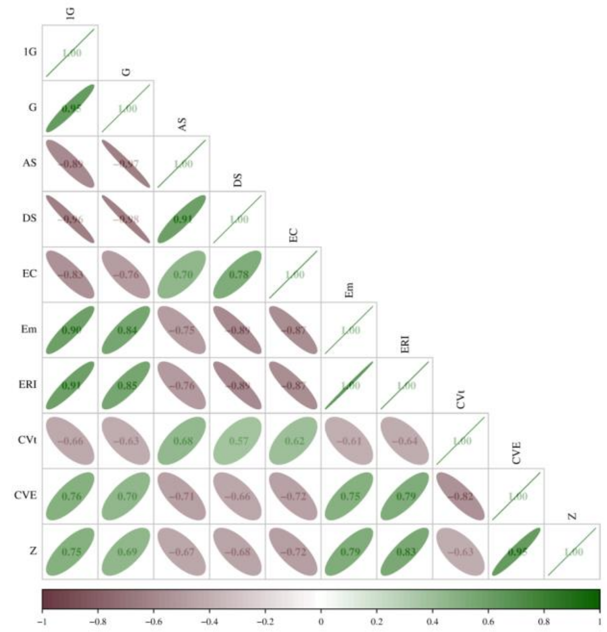

Fig.2 – Pearson correlation germination test (1G), Germination (G), abnormal seedlings (AS), dead seeds (DS), electrical conductivity (EC), total emergence (Em), emergence rate index (ERI), coefficient of variation of emergence time (CVt), emergence speed coefficient (CVe) and emergence synchronization index (Z) of seeds of maize genotypes submitted to different exposure levels of ozone and ultraviolet C.

Germination is required for a seed to effectively emergence, although other conditions can also affect a seedling's capacity to emerge from the soil. Through the action of pests and pathogenic microorganisms, restrictive phytosanitary conditions may inhibit the development of crops. Thus, the capacity to germinate rapidly promotes a better stand in the field, allowing the plant to escape such unfavorable conditions.

Also, the emergence synchronization index (U) presented high correlation to the total emergence (Em), emergence rate index (ERI). A good and uniform stand is essential for a successful corn crop. A good stand refers to the number of plants per unit area, and a uniform stand refers to the evenness of plant spacing and growth within that area.

Having a good stand is important because it ensures that the crop has the maximum number of plants per unit area, which can lead to higher yields. Having a uniform emergence provides better stand (less dominated plants) which ensures that the plants have the space and resources they need to grow and develop properly. When plants are spaced evenly and have similar growth rates, they are less likely to compete with each other for light, water, and nutrients.

IV. CONCLUSION

The use of ozone associated with the application of type C ultraviolet radiation affects the first germination reading in a differentiated way. Exposure times are decisive for this variable, observing a higher level of vigor for the 30-minute exposure time.

Radiation generates small effects on seedling emergence (final emergence of seedlings and emergence rate index).

The combination of O3 and UVc techniques can be used to treat organic maize seeds without significantly affecting their physiological quality.

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