Effect of Seed Soaking Treatment with Some Antioxidants and Chitosan on Germination Parameters of Three Maize Genotypes (*Zea mays* L).

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Abstract

This research was completed in May 2022 at the Agronomy Department Laboratory of Seed Testing, Faculty of Agriculture, Mansoura University, Egypt, to examine the effects of soaking treatments with varied amounts of antioxidants and chitosan (control treatment, soaking in distilled water, ascorbic acid (AA) at the rates of 100, 150 and 200 ppm, salicylic acid (SA) at the rates of 100, 150 and 200 ppm and chitosan at the rates of 0.25, 0.50 and 0.75% on germination parameters of some maize genotypes (SC-2031, synthetic cultivar Giza-2 and TWC-324). Four replications of a factorial experiment using a randomised complete block design (RCBD) were used in the lab experiment. The best results of germination parameter were recorded from sown SC-2031 hybrid, followed by sown synthetic cultivar Giza-2 and then sown TWC-324 hybrid. Soaking maize seeds at 200 ppm of AA resulted in the best results of germination parameter, followed by soaking maize seeds at 150 or 100 ppm of AA or 100 ppm of SA. The descending order of other soaking seed treatments was as follows; SA (150 ppm) > salicylic acid (200 ppm) > distilled water > without (untreated "control treatment") > chitosan (0.75%) > chitosan (0.50 %). To maximize the germination parameters of maize, it could be recommended to soak maize SC-2031 hybrid seeds at 200 ppm of (AA) or 100 ppm of (SA) for 12 h.

Keywords: Zea mays, genotypes, ascorbic acid, salicylic acid, chitosan, germination.

تأثير نقع البذور ببعض مضادات الأكسدة والشيتوزان على إنبات ثلاثة تراكيب وراثية من الذرة الشامية

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المستخلص

أجري هذا البحث لدراسة تأثير معاملة النقع ببعض مضادات الأكسدة والشيتوزان بمستويات مختلفة على صفات الإنبات لبعض التراكيب الوراثية للذرة الشامية تحت الظروف المعملية. لتحقيق هذا الهدف أجريت تجربة مختبرية في قسم المحاصيل/ كلية الزراعة/ جامعة المنصورة خلال شهر ايار من عام 2022م. أجريت تجربة عاملية بعاملين بتصميم القطاعات كاملة العشوائية (RCBD) بأربعة تكررات. شمل العامل الأول ثلاثة تراكيب وراثية مختلفة من الذرة الشامية (هجين فردى أبيض هاى تك-2031 والصنف التركيبى الأبيض جيزة-2 وهجين ثلاثي أبيض-324). أما العامل الثانى فقد شمل على إحدى عشر معاملة لنقع تقاوي الذرة الشامية فى بعض مضادات الأكسدة والشيتوزان بمستويات مختلفة على النحو التالي: بدون نقع (بدون معاملة "معاملة المقارنة")، النقع فى الماء المقطر، النقع في حامض الأسكوربيك بتركيز 100، 150 و200 جزء في المليون، حامض الساليسيليك بتركيز 100، 150 و200 جزء في المليون والشيتوزان بتركيز 0.25، 0.50 و0.75%. نتجت أفضل القيم لصفات الإنبات من زراعة هجين فردى أبيض هاى تك-2031، يليه الصنف التركيبى الأبيض جيزة-2 ثم أخيراً هجين ثلاثى أبيض-324. أدى نقع تقاوى الذرة الشامية في حامض الأسكوربيك بمعدل 200 ملغم لتر$^{-1}$ قبل بدء اختبار الإنبات للحصول على أفضل القيم لصفات الإنبات يليه نقع تقاوى الذرة الشامية في حمض الأسكوربيك بمعدلات 150 أو 100 ملغم لتر$^{-1}$ أو حامض الساليسيليك بمعدل 100 جزء في المليون. من أجل تعظيم صفات الإنبات لتقاوي الذرة الشامية. يوصى بنقع تقاوي الهجين الفردى أبيض هاى تك-2031 في حامض الأسكوربيك بمعدل 200 جزء في المليون أو حمض الساليسيليك بمعدل 100 جزء في المليون لمدة 12 ساعة.

الكلمات المفتاحية: الذرة الشامية، تراكيب وراثية، فيتامين C، حامض الساليسليك، الشيتوزان، الأنبات.

Introduction

It is commonly acknowledged that cereal grains are essential to the diet of millions of people worldwide. The most significant cereal grain in the world, after wheat and rice, is maize (*Zea mays* L.), which supplies nourishment to both people and animals. Given that it comprises around 72% carbohydrate, 10% protein, 4.8% oil, 5.8% fibre, and 3% sugar, maize grain has a high nutritional value (Rafiq *et al.,* 2010). The grains are used in industry for various purposes, such as the production of textiles and plastics. To close the gap between maize production and consumption, a substantial focus should be made on raising maize productivity through the increased cultivated

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area, improved germination and seedling characteristics, and maximised yield per unit area. Due to the growing importance of maize in the seed business, public and commercial seed corporations are now focusing more on producing maize hybrids. As a result of ongoing research, numerous hybrids have also been created. The essential need for any variety or hybrid is the timely availability of good seeds. Several scientists have created new "Seed Enhancing Methods" to produce seeds of greater quality. By enhancing the technical quality of seeds, this approach aims to maximise the application of seed treatment products. Effective seed treatment techniques may dramatically boost output while improving seed quality. Farmers' preserved seeds have a decreased germination rate, but there is a noticeable improvement after treatment. It suggests that farmers may be able to upgrade to using treated preserved seeds.

Rapid germination and seed germination are crucial steps in the establishment of seedlings. With many field crops, such as maize, seed-soaking techniques have been utilised to boost germination, raise germination uniformity, improve seedling establishment, and accelerate vegetative development (Farooq *et al.,* 2008).

Choosing the high-yielding ability genotypes is essential to raise the parameters of maize germination and seedlings. For this reason, this study aims to evaluate different maize genotypes for scooping light on the best genotype that can be used under the environmental conditions similar to the study area. Tabakovic *et al.* (2020) revealed that the highest total germination (93.7%) was recorded for ZP 677 hybrid compared to other studied hybrids, ZP 505 and ZP 684. Yagiz and Konuskan (2020) indicated that seed germination properties like germination ratio, index, and time varied significantly depending on maize hybrids. Al-Omairi and Al-Hilfy (2021) found that some germination characteristics significantly differed in maize cultivars (Baghdad 3 and Buhoth 5018). Omar *et al.* (2022) indicated that the hybrids SC (F1), DC (F1), and TC (F1) have exceptional germination characteristics, with a 100% germination rate on the fifth day. As a result, the findings unambiguously demonstrate that hybrid seeds germinate more frequently and grow more vigorously than their parent seeds.

One of the most significant antioxidants is ascorbic acid (AA), crucial for cellular functions such as cell division, expansion, metabolic activity when germination began, cell detoxification, protection from reactive oxygen species, and cell survival. Ascorbic acid is frequently used as a pre-sowing treatment because it enhances performance and stands establishment under various external conditions, such as excessive salinity (Afzal *et al.,* 2005). Al-Rawi *et al.* (2020) deduced that soaking maize seeds in ascorbic acid at 600 ppm for 18 hours before germination improved germination parameters. Cetinkaya *et al.* (2021) revealed that ascorbic acid application at 100 ppm positively affects the physiological and biochemical parameters in maize seeds. Kadhim and Hamza (2021) concluded that soaking maize seeds in ascorbic acid (100 mg l^{-1}) for 18 hours improved germination properties.

Salicylic acid (SA) is a hormone-like compound that regulates various physiological processes, including ion absorption and transport, suppression of ethylene production, transpiration, photosynthesis, growth, nitrate metabolism, and stress tolerance. Consequently, using SA increases plants' ability to withstand a variety of biotic and abiotic stressors, including cold, salt, drought, heat, fungus, bacteria, and viruses (Khan et al., 2010). Nimir *et al.* (2015) noted that priming seeds with salicylic acid improved germination traits. Sallam, Sallam and Ibrahim (2015) showed that seed priming with 0.6 g L salicylic acid gave the highest germination speed and germination percentage of teosinte.

As a result of chitin's deacetylation, chitosan is a naturally occurring polymer. Shellfish byproducts from food processing may be easily converted to chitin. Due to its fungicidal activities and induction of defence mechanisms in plant tissues, chitosan, a high molecular polymer that is nontoxic and bioactive, has become a valuable and well-liked substance. Chitosan creates a semi-permeable coating that controls gas exchange, lowers the rate of respiration and transpiration, and delays the ripening processes (Terry and Joyce, 2004). According to Al-Omairi and Al-Hilfy (2021), soaking maize seeds in chitosan-containing solutions at both doses (100 and 500 mg L^{-1}) did not produce any discernible results compared to selenium treatments.

This study may be used to prove that applied sciences are highly significant in life because of their various applications in the present and the past (Abido and Zsombik, 2018, Abido and Zsombik, 2019 Abido *et al.,* 2021). Hence, this research was created to investigate the impact of soaking treatments with some antioxidants and chitosan at various levels on the germination parameters of some maize genotypes under laboratory conditions.

Materials and Methods

Experimental design and treatments

In May 2022, this study was completed in the Faculty of Agriculture, Agronomy Department Laboratory of Seed Testing, Mansoura University, Egypt. Studying the impact of soaking certain antioxidants and chitosan at different concentrations on germination characteristics of specific maize genotypes was the goal of this laboratory inquiry.

Four replications of a factorial experiment using a randomised complete block design (RCBD) were used in the lab experiment. There were two variables in the investigation. The first factor comprised three maize genotypes: Single Cross 2031 (SC-2031), Synthetic cultivar Giza-2 and Three Way Cross 324 (TWC-324). The studied maize white Single Cross Hybrid seeds, HYTECH-2031 (SC-2031), is produced and obtained from Misr Hytech Seed International Company. In comparison, the seeds of studied maize genotypes synthetic white cultivar Giza-2 and Three Way Cross white-24 (TWC-324) were created and collected from Egypt's Agricultural Research Center's (ARC) Maize Division, Experimental Farm of Gemmeiza Agriculture Research Station. The second factor included eleven soaking treatments of maize hybrids seeds in some antioxidants and chitosan at various levels as follows: (untreated " control treatment"), distilled water, ascorbic acid (AA) at the rates of 100, 150 and 200 ppm, salicylic acid (SA) at the rates of 100, 150 and 200 ppm chitosan at the rates of 0.25, 0.50 and 0.75%. The soaking treatments with ascorbic acid (AA), salicylic acid (SA) and chitosan were done for 12 hours.

Ascorbic acid (AA) and salicylic acid (SA) as antioxidants, as well as chitosan, were produced by El-Nasr Pharmaceutical Chemicals Co., Egypt. It was purchased from the El-Gomhouria Company for Trade in Pharmaceutical, Chemical, & Medical. A suitable quantity of poly-(1.4-B-D-glucopyranosamine and 2-amino-2-deoxy-(1-4)-B-D-glucopyranose was dissolved in a 5% acetic acid solution to create chitosan powder.

400 random seeds for each treatment were planted in sterile Petri plates on top of filter paper (14 cm diameter). According to the regulations of the International Seed Testing Association, the Laboratory for Seed Testing in the Agronomy Department, Faculty of Agriculture, Mansoura University, Egypt, during the first week of May 2022, will evaluate four Petri dishes that each contain 25 grains as if they were one Petri dish containing 100 seeds (ISTA, 1996). Every day, dishes were checked, and distilled water was added as necessary. After the radical has pierced the coleorhiza and the seed has grown to a length of about 2 to 3 mm, it is said to have physiologically germinated. The number of seeds that germinated on the fourth day was the initial count of the germinated seeds. Once the germination test was complete, the number of seeds germinating every 24 hours was tallied (7 days). According to ISTA (1996), seeds were divided into three categories: viable (abnormal, dead, or diseased seeds), hard (no imbibitions or swelling), and germinated (radical 2 mm long).

Germination parameters:

1. Germination percentage (GP%). The following equation, provided by ISTA, was used to estimate it (1996):

$$\mathrm{GP}\ (\%) = \frac{\text{Number of normal seedlings}}{\text{Number of total grains}} \times 100$$

2. Speed germination index (SGI). It was calculated by the following formula (ISTA, 1996):

$$\mathrm{SGI} = \frac{\text{No. of germinated grains}}{\text{Days to first count}} + \frac{\cdots\cdots}{\cdots\cdots} + \frac{\text{No. of germinated grains}}{\text{Days to final count}}$$

3. Germination index (GI). It was calculated according to the following equation (Karim *et al.* 1992):

$$\mathrm{GI} = \frac{\text{Germination percentage in each treatment}}{\text{Germination percentage in control treatment}}$$

4. Co-efficient of germination (CG). It was calculated using the following formula according to Copeland (1976):

$$\mathrm{CG} = \frac{100\ (A_1 + A_2 + \dots\dots + A_n)}{A_1\ T_1 + A_2\ T_2 + \dots\dots\ A_n\ T_n}$$

Where;

A is the number of germinations;

T = The number of days that make up A; n is several days until the final tally.

5. The average germination time (MGT). It was determined using the equation provided by Ellis and Roberts (1981):

$$\mathrm{MGT} = \frac{\sum \mathrm{Dn}}{\sum \mathrm{n}}$$

Where (n) represents the number of grains germinating on a given day, and (D) represents the number of days since germination began.

6. Germination's energy (EG). It was calculated using the ratio of the number of seeds germinating at the first count (4 days after sowing) to the total number of seeds examined (Ruan *et al.,* 2002).
7. Abnormal seedlings (%). According to ISTA (1996), it was measured and represented as the proportion of aberrant seedlings after 7 days.
8. Hard seeds (%). According to ISTA (1996), it was measured and represented as the percentage of firm seeds after 7 days.
9. Rotten seeds (%). According to ISTA (1996), it was measured and expressed as a percentage of rotting seeds after 7 days.

Statical analysis

For the factorial experiment in a randomised complete block design (RCBD) with four replications as reported by Gomez and Gomez (1984), data were statistically analysed using the analysis of variance (ANOVA) technique using the "MSTAT-C" computer software package (1984). To analyse the differences between treatment means at the 5% level of probability, Snedecor and Cochran (1980) claim that the least significant difference (LSD).

Results and Discussion

Genotypes performance

From obtained results in Table 1, it could be noticed that the studied maize genotypes (SC-2031, Giza-2 and TWC-324) significantly differed in germination parameters. The highest means of GP (%), SGI, GI, CG, MGT and EG and lowest values of abnormal seedlings percentage (%), hard seeds percentage (%) and rotten seeds percentage (%) were recorded from sown SC-2031 hybrid (Table 1). However, the lowest values of GP (%), SGI, GI, CG, MGT, EG, and hard seeds (%) were produced from sown TWC-324 hybrid. The differences among studied maize genotypes in germination parameters may be due to vertical genetic differences. Kandil *et al.* (2019 a) illustrated that Giza 176 hybrid surpassed other genotypes (Giza 168 and Giza 167) in final germination percentage, followed by Giza 167 hybrid, which came in the second rank. These results are in good agreement with those reported by Konuskan *et al.* (2021), Tabakovic *et al.* (2020), Yagiz and Konuskan (2020), Al-Omairi and Al-Hilfy (2021) and Omar *et al.* (2022).

Effect of soaking treatment with some antioxidants and chitosan at various levels

The obtained results of this study indicated that there was a significant difference in germination parameters.

Soaking maize seeds in ascorbic acid at the rate of 200 ppm before starting the germination test resulted in the highest means of GP (%), SGI, GI, CG, MGT, EG and lowest values of abnormal seedlings (%), hard seeds (%) and rotten seeds (%). However, soaking in AA at 150 or 100 ppm or SA at the rate of 100 ppm ranked after soaking AA at 200 ppm (Table 1). The descending order of other soaking seed treatments was as follows; salicylic acid (150 ppm) > salicylic acid (200 ppm) > distilled water > without (untreated "control treatment") > chitosan (0.75 %) > chitosan (0.50%). Whilst the lowest GP (%), SGI, GI, CG, MGT, EG, and highest values of abnormal seedlings (%), hard seeds (%) and rotten seeds (%)resulted from soaking at 0.25% of chitosan. Ascorbic acid is an essential metabolite in many cellular activities, including cell division. This may explain why soaking maize seeds in AA before beginning the germination test had a positive effect. Ascorbic acid is also used by both zygotic and somatic embryos during the early phases of germination (Arrigoni *et al.,* 1997). Moreover, salicylic acid may have contributed to the beneficial impact by considerably enhancing the activity of germination-related enzymes (Eastmond and Graham, 2001). However, Al-Omairi and Al-Hilfy (2021) observed that soaking maize seeds in chitosan-containing solutions at either dose of 100 mg L^{-1} or 500 mg l^{-1} did not produce any discernible effects. These results are in good accordance with those reported by Al-Rawi *et al.* (2020) and Kadhim and Hamza (2021). On the other hand, Lizarraga-Paulin *et al.* (2013) showed that the final germination percentage increased significantly when seeds were soaked in chitosan solutions at a concentration of 20 ppm.

Effect of interaction

The results of this investigation indicated a significant effect due to the interaction between maize genotypes and soaking treatment with some antioxidants and chitosan at various levels on germination parameters. The statistical analysis of obtained data showed that the highest means of GP % (Fig. 1), SG (Fig. 2), GI (Fig. 3), CG (Fig. 4), MGT (Fig. 5), EG (Fig. 6) and lowest values of abnormal seedlings % (Fig. 7), hard seeds % (Fig. 8) and rotten seeds % (Fig. 9) were resulted from soaking maize SC-2031 hybrid seeds in AA at the rate of 200 ppm or SA at the rate of 100 ppm. Following the previously mentioned interaction treatments in order of priority are the following two methods: soaking maize SC-2031 hybrid seeds in AA at a rate of 150 ppm or SA at a rate of 150 ppm and then soaking them in AA at a rate of 100 ppm or SA at a rate of 200 ppm. On the contrary, the lowest means of GP (%), SGI, GI, CG, MGT, EG and highest values of abnormal seedlings (%), hard seeds (%) and rotten seeds (%) were obtained when soaking maize TWC-324 hybrid seeds in 0.25 % of chitosan for 12 h.

Table 1. Effect of seed soaking treatment with some Antioxidants, chitosan and maize genotype on germination parameters.

Characters / Treatments	GP (%)	SGI	GI	CG	MGT	EG	Abnormal seedlings percentage (%)	Hard grains percentage (%)	Rotten grains percentage (%)
Genotypes performance									
SC-2031	73.81	4.81	1.225	24.16	19.63	73.45	1.455	17.36	0.091
Giza-2	73.72	4.61	1.176	24.04	18.56	72.54	5.364	19.63	2.364
TWC-324	60.90	3.88	1.042	20.68	15.65	60.72	2.273	30.90	0.909
F. test	*	*	*	*	*	*	*	*	*
LSD at 5 %	2.30	0.14	0.058	1.32	0.60	2.46	1.623	2.03	1.040
Effect of soaking treatment with some antioxidants and chitosan at various levels									
Without	61.00	4.05	1.000	24.52	16.25	64.66	5.667	11.33	1.000
Distilled water	69.33	4.14	1.141	24.91	16.58	66.00	2.667	6.33	0.667
Ascorbic acid (100 ppm)	96.33	6.12	1.593	24.95	24.57	97.00	2.333	1.33	0.000
Ascorbic acid (150 ppm)	96.33	6.15	1.596	24.95	24.66	97.66	1.333	1.00	0.000
Ascorbic acid (200 ppm)	97.66	6.17	1.618	24.97	24.75	98.33	0.333	0.00	0.000
Salicylic acid (100 ppm)	96.00	6.13	1.590	24.97	24.59	98.00	2.333	0.66	0.333
Salicylic acid (150 ppm)	91.00	5.84	1.504	24.93	23.41	92.66	2.667	1.66	0.333
Salicylic acid (200 ppm)	83.00	5.46	1.372	24.93	22.16	84.00	2.667	3.33	0.333
Chitosan (0.25 %)	8.66	0.52	0.140	13.81	2.25	7.00	7.000	91.66	4.667
Chitosan (0.50 %)	16.66	1.11	0.274	17.02	4.83	14.00	3.333	82.66	3.667
Chitosan (0.75 %)	48.33	3.10	0.794	22.60	13.41	38.66	3.000	49.00	2.333
F. test	*	*	*	*	*	*	*	*	*
LSD at 5 %	4.42	0.27	0.110	2.54	1.16	4.71	3.107	3.90	1.992
Effect of interaction *(F. test):*									
A × B	*	*	*	*	*	*	*	*	*

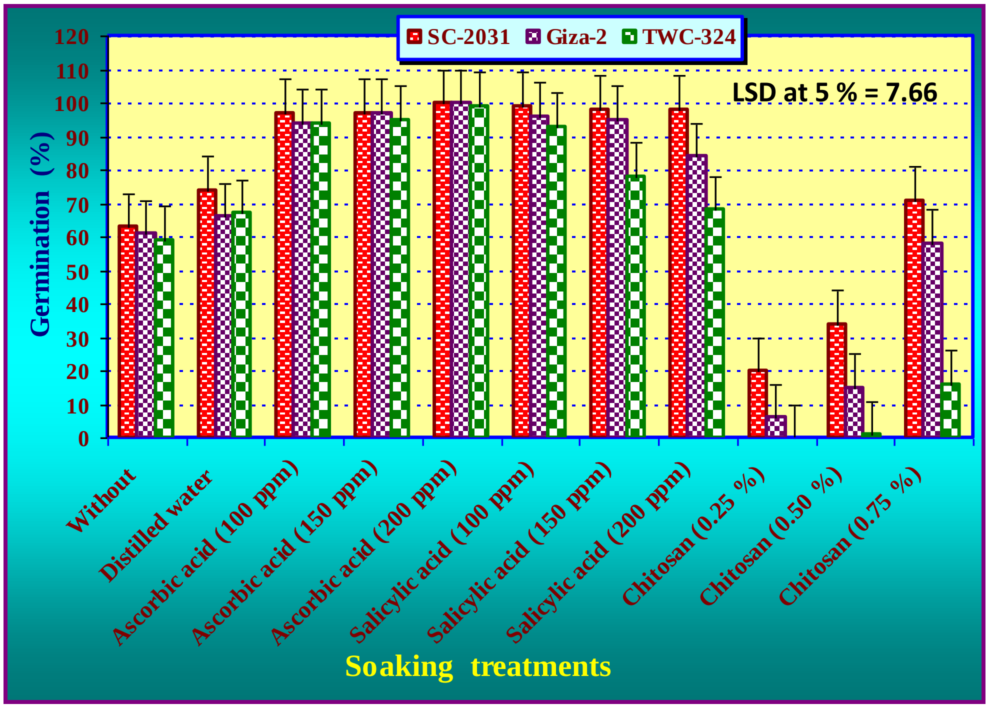

Figure 1. Maize germination percentage (%) is influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

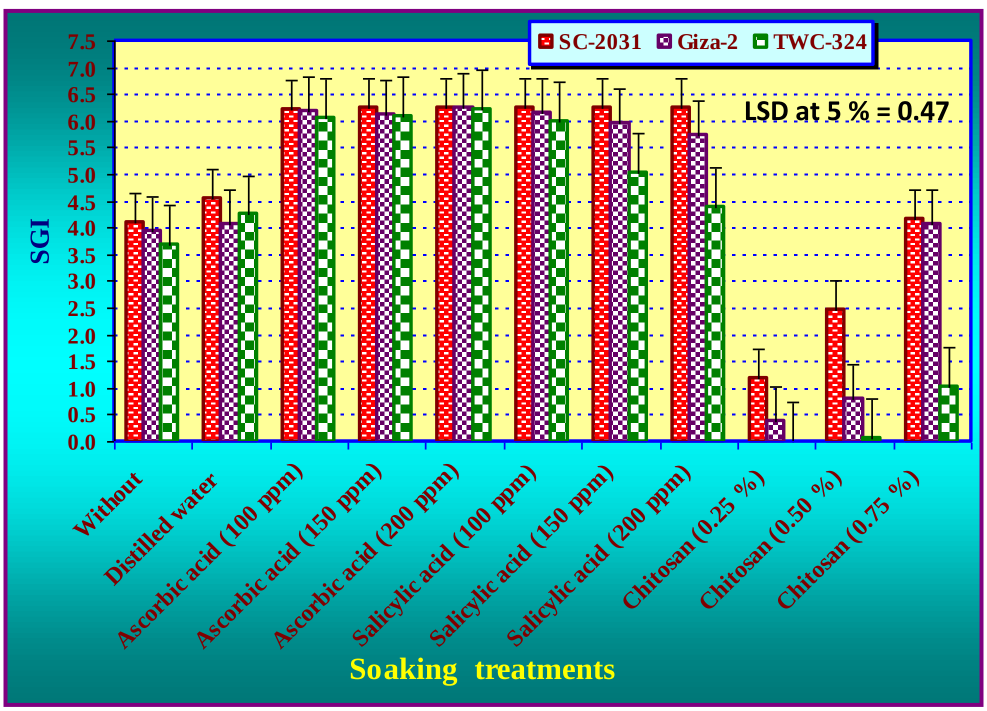

Figure 2. Maize peed germination index (SGI) is influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

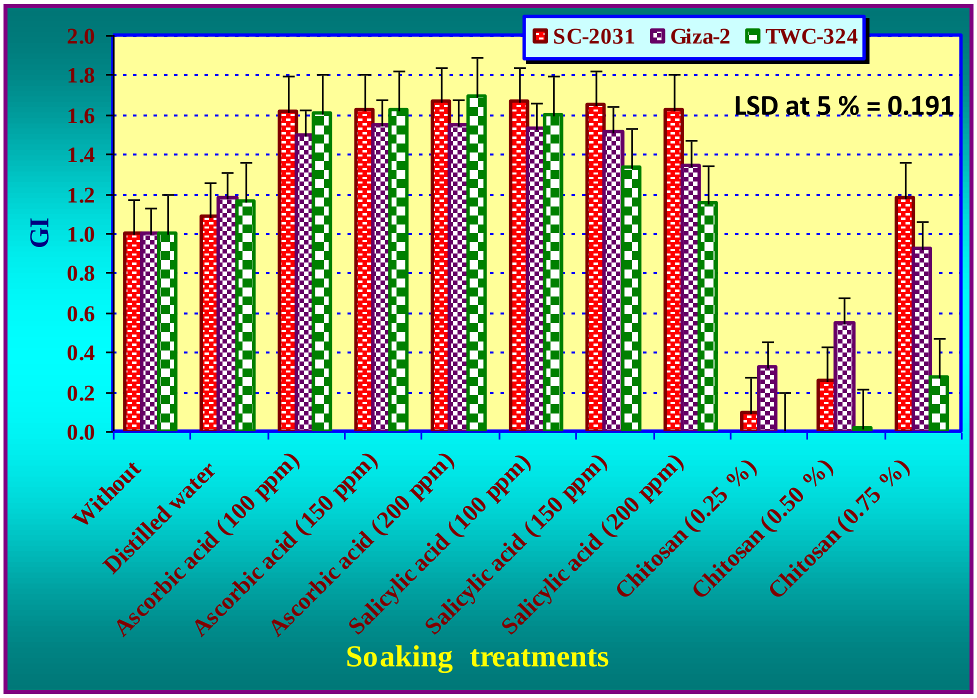

Figure 3. Maize germination index (GI) is influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

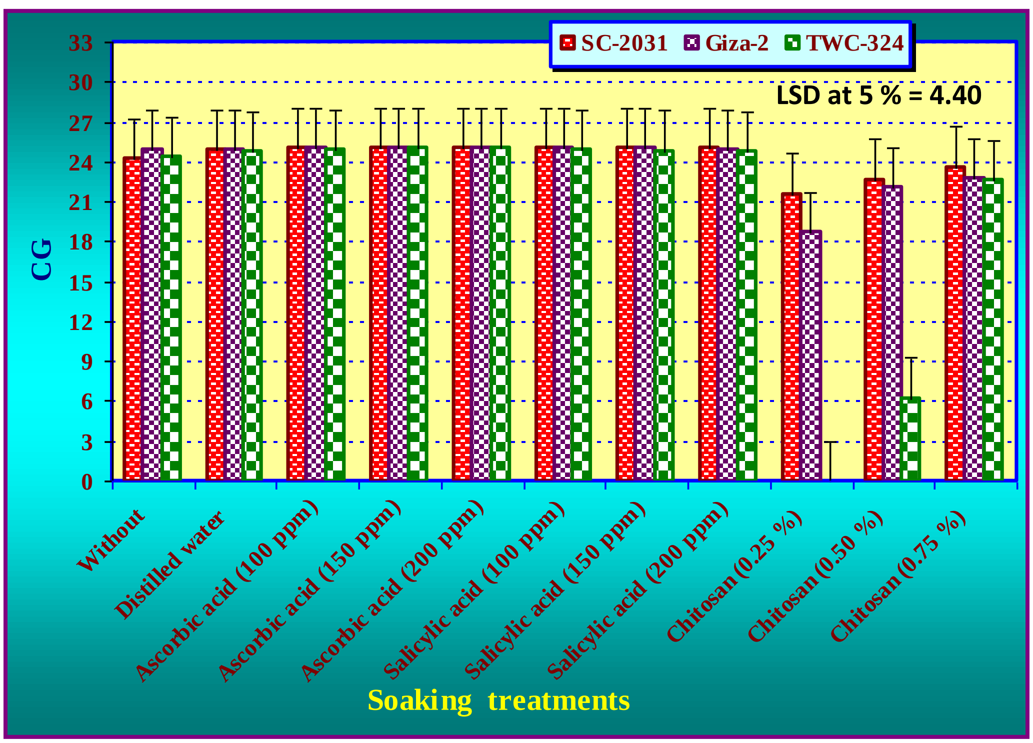

Figure 4. Maize co-efficient of germination (CG) is influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

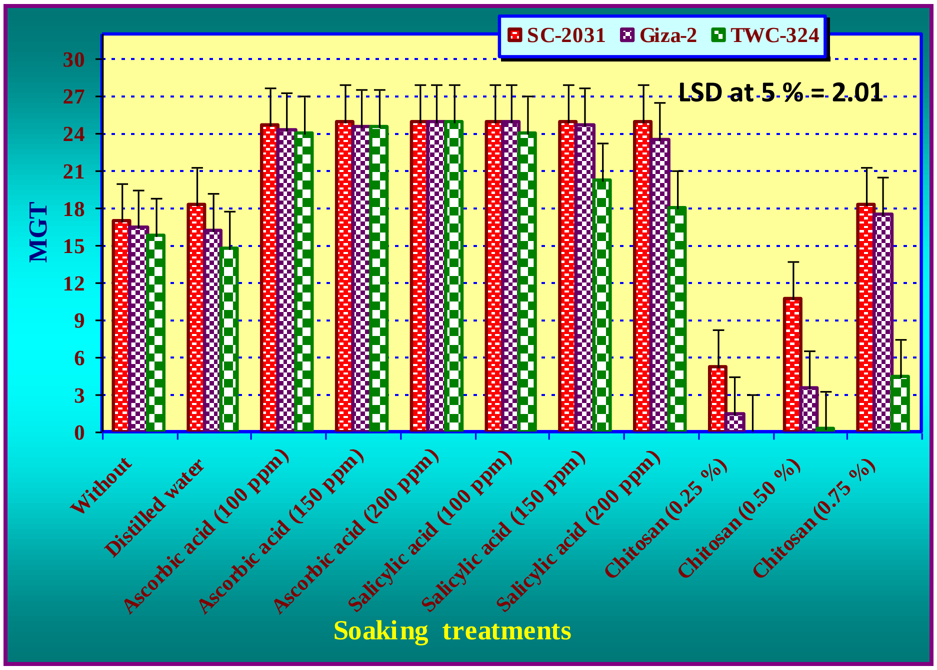

Figure 5. Maize means germination time (MGT) is influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

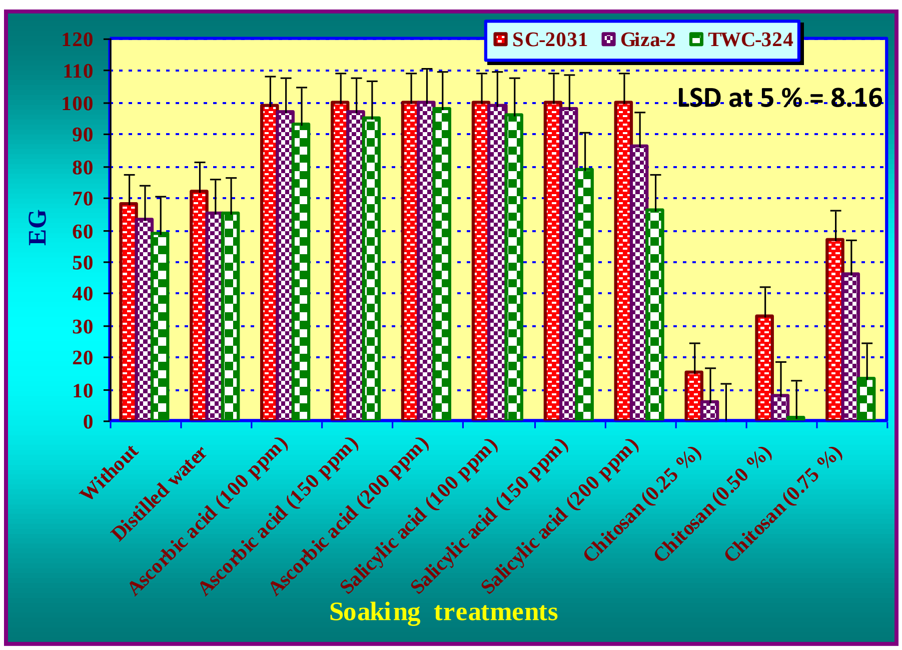

Figure 6. Maize energy of germination (EG) is influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

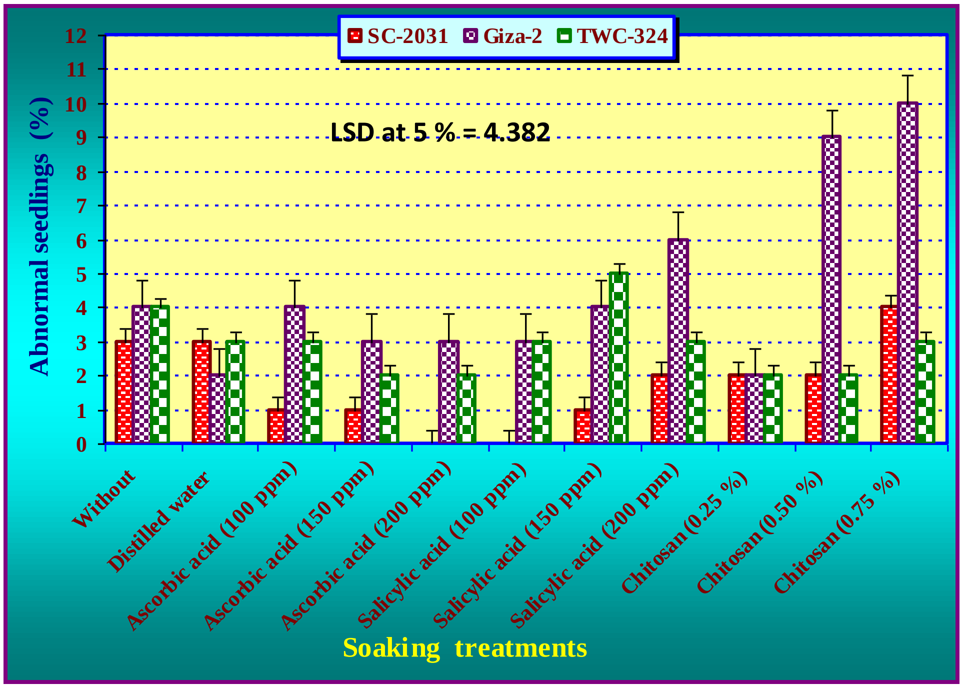

Figure 7. Maize abnormal seedlings (%) as influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

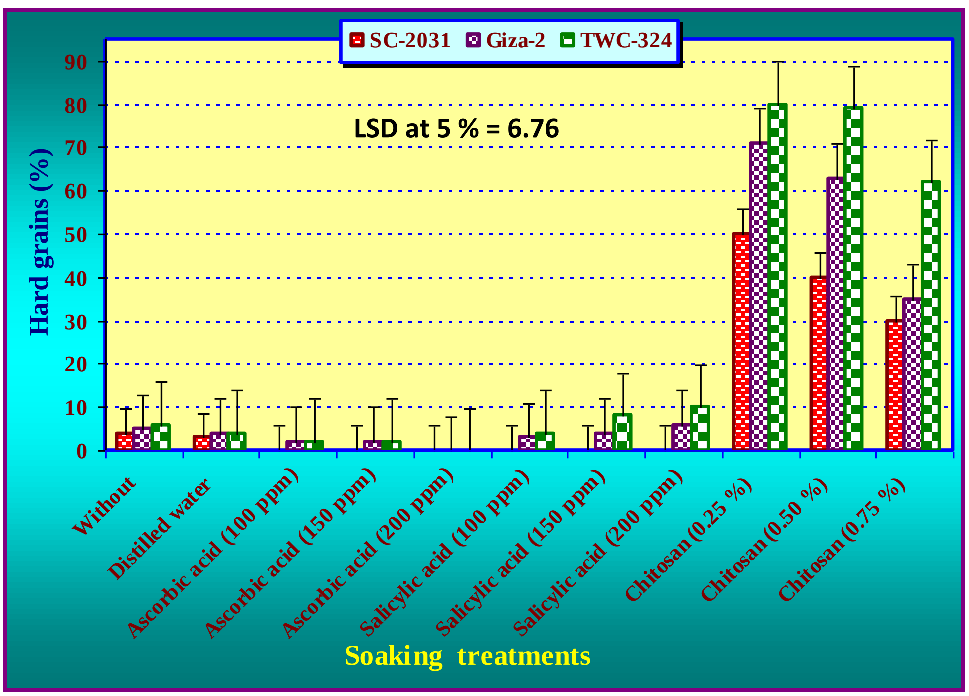

Figure 8. Maize hard grains (%) of maize as affected by the interaction between hybrids and soaking treatment with some antioxidants and chitosan at various levels.

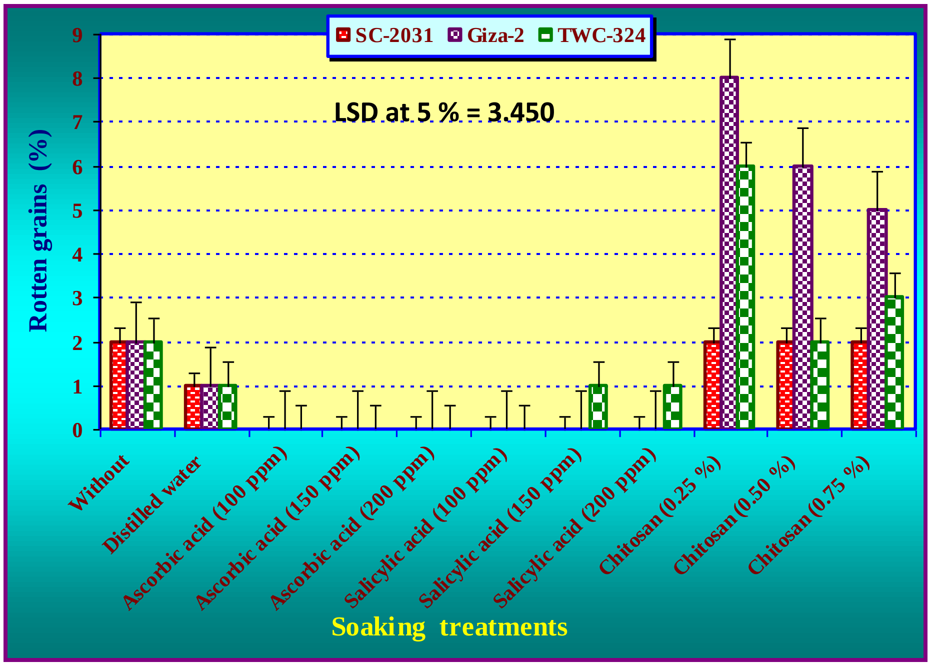

Figure 9. Maize rotten grains percentage (%) is influenced by the interaction of hybrids and soaking treatment with certain antioxidants and chitosan at different levels.

Conclusion

This study was accomplished to study the effect of soaking treatment with some antioxidants and chitosan at various levels (control treatment, distilled water, 100, 150 and 200 ppm of ascorbic acid (AA), 100, 150 and 200 ppm of salicylic acid (SA) and 0.25, 0.50 and 0.75 % of chitosan on germination parameters of some maize genotypes (SC-2031, synthetic cultivar Giza-2 and TWC-324). It could be noticed that for maximizing germination parameters of maize, it could be recommended that soak maize SC-2031 hybrid seeds in ascorbic acid (AA) at the rate of 200 ppm or salicylic acid (SA) at the rate of 100 ppm for 12 hours.

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