Effect of Soaking Maize Seeds with Selenium and Chitosan on Improving Germination, Vigour and Viability of Seed and Seedling

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Abstract. An experiment was carried out in the Laboratory of Seed Technology, College of Agricultural Engineering Sciences, University of Baghdad, Iraq, to studying the stimulation of maize seeds by soaking with selenium and chitosan to improve the vigour and viability of seeds. By using factorial experiment according to the Complete Random Design (CRD) with three replications. The seeds of two synthetic cultivars of maize (Baghdad 3 and Buhoth 5018) were soaked in selenium solutions at two concentrations (2 and 5 mg L⁻¹), and chitosan (100 and 500 mg L⁻¹) for 10 hours, as well as the two control treatments, which were dried seeds (without soaking) and the seeds soaked in distilled water. Results are shown that low concentration of 2 mg L⁻¹ give highest mean were recorded in the percentage of germination in the first and final counts, the mean time germination, length of root and the plumule, the seedling vigour index, germination speed, and dry weight of the seedling. Seeds soaking with the solutions containing chitosan at both concentrations did not give significant results compared to the selenium treatments, both cultivars differed significantly in some germination characteristics and the vigour and viability of the seed: (length of root and the plumule, dry weight of the seedling). The results also showed a significant effect of the interaction between seed soaking treatments and cultivar on all studied traits. We conclude from this study that treatments of stimulating seeds by soaking them with distilled water and solutions of selenium and chitosan positively affected germination and its characteristics, so we recommend using low concentrations of selenium and chitosan to improve seed germination, vigour, and viability of the seedling.

1. Introduction

Maize (Zea mays L.) is an important cereal crop for its various nutritional and manufacturing uses. It is planted in Iraq in two seasons, Autumn season, it is dominant and occupies most of the areas designated for this crop, more suitable for a climatic conditions, and the spring is still not prevalent and is planted in very few areas[1], because of the unsuitability of the climatic conditions, especially the low soil temperatures at the germination stage, which causes a decrease in the percentage of field emergence of the crop seeds[2]. Then giving low plant density, weak and heterogeneous field establishment, this in turn depends on the prevailing environment of the cultivated genotype [3]. The seeds differ in rate of emergence and growth, and this reflected in weak rate of growth for seedling and the resulting plant [4]. Appropriate solutions must be found, including the use of seed priming technology, which means soaking the seeds with chemical and organic materials, without fetal development (root and plumule) [5].

Selenium has an important role in improving seed germination and seedling growth by positively affecting the plasma membrane and many metabolic processes in the cell [6] ; [7]. It was found that maize seeds soaking in a solution containing sodium selenate Na₂SeO₄ at a concentration of 0.075 mM for 24
hours led to a significant increase in germination percentage in the final count, germination index, and germination vigour index [8]. Sorghum seeds responded to soaking with different concentrations of selenium (0, 3, 6 and 12 mg L\(^{-1}\)) for 6 hours. The best results were at low concentrations of sodium selenate (3 and 6 mg L\(^{-1}\)), which improved length of plumule, length of root, fresh and dry weight. For initiative and these characteristics began to decrease at a concentration of 12 mg L\(^{-1}\) [9].

Chitosan is an effective biostimulant for promoting plant growth, alters the permeability of the plasma membrane in seeds. The decline of malondialdehyde (MDA) content and the increase of the concentrations of soluble sugars and proline, peroxidase (POD) activity, and catalase (CAT) activity, priming with 0.50% chitosan for about 60–64 h seemed to have the best effects, seed priming with chitosan may improve the speed of germination of maize seed and benefit for seedling growth under low temperature stress [10]. Seeds soaking with chitosan solutions at a concentration of (20 gm L\(^{-1}\)) had positive effects, as it led to a significant increase in the final germination percentage [11]. Also, soaked the seeds with chitosan by 75% increased root length, increased the number of root branches and a significant increase in the dry weight of the seedling, also reduction in malondialdehyde content (MDA) [12]. Because of role of chitosan in reducing fat oxidation through chelation with ions or incorporation with lipids [13].

The genetic difference between cultivars affects the chemical and hormonal content, which consequently affects the physiological performance in the germination and growth stages, and this was confirmed by a number of studies that indicated that the viability of maize seeds and other crops is greatly affected by the genetic factor [14]. Nada, [15] found a difference in the performance of the synthetic cultivars of maize (Baghdad 3, Al Maha, Sarah and Fajr 1), as Baghdad 3 cultivar excelled in giving the highest means the percentage of germination, root length, vigour index, percentage of normal seedlings, length of plumule and the dry weight of the seedling. In agreement with the findings of Al-Fahd [16] in the different performance of maize seedlings resulting from the cultivation of seeds of different genotypes (Al-Maha, Sarah, Baghdad 3 and Fajr 1) under standard laboratory germination conditions, fresh and dry weight of the seedling.

The aim of this study was to know the response of the seeds of two cultivars of maize, for soaking with selenium and chitosan and its reflection in improving the germination characteristics, vigour and viability of the seed and seedling.

2. Materials and methods
This experiment was carried out in the Laboratory of the Seed Technology - College of Agricultural Engineering Sciences - University of Baghdad – Iraq, to study the effect of seed soaking with selenium and chitosan on two cultivars of maize on viability tests and seed vigour in laboratory germination. The Complete Randomized Design (CRD) was used for the factorial experiments with three replications. Selenium solutions at two concentrations (2 and 5 mg L\(^{-1}\)), and chitosan (100 and 500 mg L\(^{-1}\)), as well as the two control treatments, which were dried seeds (without soaking) and the seeds soaked in distilled water, the seeds were placed in the solutions for 10 hours at room temperature 25 C. Then the seeds were washed with distilled water, 20 sound mature seeds were taken for each duplicate and placed alternately between two layers of filter paper and put with nylon bags to maintain moisture and placed in the germinator in a position that was grow vertically as much as possible under temperature (25°C ±5), relative humidity 80%, and lighting by ordinary neon, which is originally available in the germinator [17]. And all the tools were sterilized with alcohol well to avoid infection with fungi.

A high solubility standard solution of sodium hydrogen selenite (NaHSeO\(_3\)) was prepared by dissolving 1.91 g in a liter of distilled water to obtain a concentration of 1000 mg L\(^{-1}\)and the dilution equation was used. Soaking concentrations (2 and 5) mg L\(^{-1}\) were prepared according to the dilution law following:

\[
\text{volume taken from the standard solution} = \frac{\text{Required Concentration} \times \text{Required Volume}}{\text{Standard solution concentration}}
\]

Chitosan CHT produced by (Sigma-Aldrich) Company, USA, and the required concentrations were prepared by dissolving (500 and 100 mg L\(^{-1}\)) in 0.5% concentrated glacial acetic acid and completing the volume to a liter and leaving the mixture at laboratory temperature to the next day, centrifuge was used at a speed of 1000 cycles for 5 minutes to get rid of the insoluble residues. The pH of solution was adjusted to 6.0 using 1% NaOH. until it was ready to apply the treatments [18].
2.1. Studied traits:

2.1. The percentage of germination in the first and final count:
Only natural seedlings were counted after four days for the first count and after seven days for the final count, then the results were converted into percentages according to the following law:
Germination percentage = (number of natural seedlings / number of total seeds) \times 100 \[19\].

2.2. Mean Germination Time (day)
According to the mean germination time of natural seedlings according to the following equation:
\[ \text{MGT} = \frac{\sum (D_i \times n_i)}{\sum n_i} \]
where:
- \( n_i \) is the number of seeds germinating per day
- \( D_i \) Number of days counted from the start of the test or examination \[20\].

2.3. Germination speed (seedling. Day\(^{-1}\)):
The number of natural seedlings in the germination test was calculated daily from sowing and for 7 days from sowing, then the germination speed was calculated as in the following equation:
\[ \text{Gs} = \frac{\sum n_i}{\sum D_i} \]
where:
- \( n_i \) Since is the number of seedlings emerging per day.
- \( D_i \) Number of days after planting \[21\].

2.4. Length of Root and plumule (cm):
Ten normal seedlings were taken after the examination period (seven days), then the root and plumule was separated from its point of contact with the seed.

2.5. Seedling vigour index:
It was calculated according to the following equation:
Seedling vigour index = percentage of germination \times (root length + length of plumule) \[22\].

2.6. Dry weight of the seed (mg):
Ten natural seedlings were taken after the end of the examination period (seven days), then each of the root and shoot were separated from their point of contact with the seed and placed in a perforated paper bag and dried at a temperature of 80° C for 24 hours. Their number \[23\]; \[24\].

Statistical analysis
The data was analyzed statistically by using the program (GenStat Release 10.3 DE), and extracting the value of the least significant difference (L.S.D) to compare the mean of the traits at the 5% level \[25\] .

3. Results and discussion

3.1. The percentage of germination in the first and final count

Table 1 shows that there were no significant differences between cultivars in the percentage of germination in the first and final counts, while the treatments differed significantly. Regarding the first count, soaking the seeds with distilled water gave a significant increase of 66.80% compared to the dry seeds (without soaking), because of the positive role of soaking with water in the synthesis of new tissues, which were obtained from the metabolic processes of degrading enzymes in the tissues of germinating seeds \[26\]. The seeds responded to low concentration of selenium and chitosan, while increasing the concentration negatively affected this trait. 2 mg L\(^{-1}\) Se gave the highest mean (84.20%). The reason for this may be attributed to the role of selenium in improving seed germination and seedling growth by positively affecting the plasma membrane and several metabolic processes in the cell \[6\]; \[7\]. Also Selenium regulates the membrane transport mechanism and activates phytohormones involved in cell division and hypertrophy \[27\]. Chitosan at a concentration of 500 mg L\(^{-1}\) gave the lowest germination percentage in the first count (17.50%).

As for the percentage of germination in the final count, all seed soaking treatments excelled and they did not differ significantly. 2 mg L\(^{-1}\) Se gave higher mean of (95.83%). Due to the role of seed stimulating treatments through soaking them with water, selenium solutions and chitosan in improving seed germination and seedling growth by positively affecting the activity of amylase enzyme and accelerating
the breakdown of carbohydrates. The survival or continuity of seedling growth is positively correlated with amylase activity [28]. The reason for the outstanding performance of the soak-stimulated seeds may be attributed to the integrity of the cell membranes, since during the slow hydration process, the cell membranes tend to reorganize to restore their original structures. Metabolic activities are expected to increase significantly in seeds after soaking [29]. While the comparison treatment, dried seeds (without soaking) gave an mean lower 85.00%. This is consistent with what was found by Ghenikova et al.[30] and Nawaz et al.[8] who showed a significant increase in selenium-saturated maize seeds in the final germination ratio.

The interaction between the two factors had a significant effect for first and final count, as the cultivars followed the same direction of the response, differed in the amount of response. The increase in the concentration of selenium or chitosan affected the germination of the seeds of the cultivar Buhoth 5018 more than Baghdad 3. The combination 2 mg L⁻¹ Se x Baghdad 3 gave the highest mean germination percentage in the first count (85.00%), while the combination 500 mg L⁻¹ chitosan x Baghdad 3 gave the lowest mean was 15.00%. The combination 2 mg L⁻¹ Se x Buhoth 5018 gave the highest mean germination percentage in the final count 96.67%, while the comparison treatment recorded dry seeds, the lowest mean reaching 85.00% for both cultivars.

Table 1. The effect of soaking maize seeds with selenium and chitosan on germination ratio at first and final count (%)

| Seed soaking treatments (mg L⁻¹) | Cultivars | first count | | final count | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Baghdad 3 | Buhoth 5018 | Mean | Baghdad 3 | Buhoth 5018 | Mean | | |
| Control | 20.00 | 30.00 | 25.00 | 85.00 | 85.00 | 85.00 | | |
| distilled water | 38.30 | 45.00 | 41.70 | 90.00 | 90.00 | 90.00 | | |
| Selenium 2 | 85.00 | 83.30 | 84.20 | 95.00 | 96.67 | | | |
| 5 | 73.30 | 61.70 | 67.50 | 95.00 | 93.33 | | | |
| Chitosan 100 | 18.30 | 25.00 | 21.70 | 88.33 | 91.67 | | | |
| 500 | 15.00 | 20.00 | 17.50 | 90.00 | 90.00 | | | |
| LSD 5% | 12.79 | 12.79 | 9.05 | 8.88 | | | | | |
| Mean | 41.70 | 44.20 | 90.00 | 90.56 | 91.11 | | | | |
| LSD 5% | n.s | n.s | | | | | | | |

3.2. Mean germination time (day⁻¹)

Table 2 shows that there were no significant differences between the cultivars in the mean germination time. While treatments differed significantly. Soaking the seeds with distilled water reduced the germination time by 8.67% compared to dry seeds, increasing the concentration negatively affected this trait, and the treatment of soaking the seeds with selenium solution at a concentration of 2 mg L⁻¹ was superior reduced the time required for seed germination, as it recorded (3.95 day), because of highest mean in the percentage of germination in the first and final counts (Table 1). Due the role of selenium in regulating the membrane transport mechanism and activating plant hormones involved in cell division and its magnification [27]. While the seeds soaked in chitosan at a concentration of 500 mg L⁻¹ took the longest time to germinate, which was (5.79 day), this is because of the fact that they recorded the lowest mean percentage of germination in the first count (Table 1). In impeding water absorption, because the adhesion of chitosan to the surface of the seed, which caused its germination delay [31]. The interaction between the two factors
had a significant effect in this trait. Increasing the concentration of selenium or chitosan affected the germination time of the seeds of the cultivar Buhoth 5018 more than that of the Baghdad cultivar 3. The treatment of soaking the seeds with selenium at a concentration of 2 mg L⁻¹ for the Baghdad 3 cultivar excelled in reducing the time of seedlings. The germination time was 3.83 days, while the seeds soaked in chitosan at a concentration of 500 mg L⁻¹ of the cultivar Buhoth 5018 took the longest time for germination which was 5.90 days.

Table 2. The effect of soaking maize seeds with selenium and chitosan on Mean Germination Time (day)

| Seed soaking treatments (mg L⁻¹) | Cultivars           | Mean  |
|---------------------------------|---------------------|-------|
|                                 | Baghdad 3           | Buhooth 5018 |       |
| Control                         | 5.53                | 5.30   | 5.42  |
| distilled water                 | 5.10                | 4.80   | 4.95  |
| Selenium                        |                     |        |       |
| 2                               | 3.83                | 4.07   | 3.95  |
| 5                               | 4.23                | 4.53   | 4.38  |
| Chitosan                        |                     |        |       |
| 100                             | 5.57                | 5.20   | 5.39  |
| 500                             | 5.67                | 5.90   | 5.79  |
| LSD 5%                          | 0.33                |        | 0.23  |
| Mean                            | 4.99                | 4.97   |       |

3.3. germination speed (seedling. day⁻¹)

Table 3 shows that there were no significant differences between the cultivars in the germination speed, while the treatments differed significantly. The treatment of soaking seeds with distilled water led to a significant increase of 15.38% compared to dry seeds. 2 mg L⁻¹ Se was superior recorded (25.60 seedling. day⁻¹). Because of the fact that this treatment was superior in the percentage of germination in the first count and it took fewer days to complete germination (Table1). Chitosan at a of 500 mg L⁻¹ gave the lowest mean (15.63 seedling.day⁻¹), which did not differ significantly from the treatment of soaking the seeds with chitosan 100 mg L⁻¹ and comparison treatment without soaking. The combination of soaking the seeds with selenium at a concentration of 2 mg L⁻¹ for the Baghdad 3 cultivar recorded the highest mean of 26.57 seedling day⁻¹. Whereas, the soaking treatment with chitosan at a concentration of 500 mg L⁻¹ for the Buhooth 5018 cultivar recorded the lowest mean for this trait amounting to 14.80 seedling.day⁻¹.

Table 3. The effect of soaking maize seeds with selenium and chitosan on Germination speed (seedling day⁻¹)

| Seed soaking treatments (mg L⁻¹) | Cultivars           | Mean  |
|---------------------------------|---------------------|-------|
|                                 | Baghdad 3           | Buhooth 5018 |       |
| Control                         | 16.17               | 16.73  | 16.45 |
| distilled water                 | 18.57               | 19.40  | 18.98 |
| Selenium                        |                     |        |       |
| 2                               | 26.57               | 24.63  | 25.60 |
| 5                               | 23.20               | 21.77  | 22.48 |
| Chitosan                        |                     |        |       |
| 100                             | 16.47               | 18.23  | 17.35 |
| 500                             | 16.47               | 14.80  | 15.63 |
| LSD 5%                          | 2.7                 |        | 1.91  |
| Mean                            | 19.57               | 19.26  |       |

LSD 5% n.s
3.4. length of root and plumule

It is noticed from the results of Table 4 that there is a significant difference between the cultivars in the of root and plumule length, where the cultivar Baghdad 3 recorded the highest mean (14.17 and 10.87 cm) for two traits respectively. Because of the genetic difference which affected the chemical and hormonal content and thus, it has an effect on the physiological performance in the germination and growth stage, and this was confirmed by a number of studies that indicated that the viability of maize seeds is significantly affected by genetic factor (Al-Fahd, 2017 and Nada, 2018). As for the treatments of seed soaking, they differed significantly. Soaking the seeds with distilled water led to a significant increase in root and plumule lengths (7.56% and 23.14%) respectively compared to dry seeds without soaking for each trait, due to the positive role of water in the synthesis of new tissues that it is obtained from the metabolic processes of degrading enzymes in the tissues of germinating seeds (Bewley and Black, 1978) [26]. Soaking in selenium 2 mg L⁻¹ recorded the highest mean root and plumule lengths (18.89 and 13.07 cm) respectively, due to the fact that this treatment was superior in the percentage of germination in the first and final numbers (Table 1) and took the least time to complete germination (Table 2), allowing It has a longer time to form root and plumule growth until the end of the examination period and this is consistent agree with who found by Subraman Yam et al., (2019) [32], while the treatment of seed soaking with chitosan at a concentration of 500 mg L⁻¹ recorded the lowest root length (11.28 cm), and the comparison treatment recorded the lowest mean the length of the plumule (8.47 cm).

The interaction between the two factors had a significant effect on root and plumule lengths. The increase in selenium or chitosan concentration affected the root and plumule length of Baghdad 3 cultivar more than Buhoth 5018. Soaking the seeds with selenium 2 mg L⁻¹ gave the highest mean was (18.97 and 13.32 cm) for both traits, respectively. Comparison treatment of Buhoth 5018 recorded the lowest mean for these two traits, which is (11.18 and 7.82 cm) for root and plumule lengths respectively.

Table 4. The effect of soaking maize seeds with selenium and chitosan on length of root and plumule (cm)

| Seed soaking treatments (mg L⁻¹) | length of root | length of plumule |
|--------------------------------|----------------|-------------------|
| Cultivars                      | Mean           | Cultivars         | Mean              |
| Baghdad 3                     |                | Buhoth 5018       |                  |
| Control                        | 12.38          | 11.88             | 11.78             | 9.12 | 7.82 | 8.47 |
| distilled water                | 12.77          | 12.57             | 12.67             | 10.35 | 10.52 | 10.4 |
| Selenium                      | 18.97          | 18.82             | 18.89             | 13.32 | 12.83 | 13.0 |
| 5                              | 17.97          | 17.13             | 17.55             | 12.37 | 12.13 | 12.2 |
| 100                            | 11.63          | 11.30             | 11.47             | 10.28 | 9.82 | 10.0 |
| Chitosan                       | 11.33          | 11.22             | 11.28             | 9.80 | 9.52 | 9.66 |
| 500                            | 14.17          | 13.70             | 14.87             | 10.87 | 10.44 | 10.87 |
| LSD 5%                         | 0.82           | 0.58              | 0.81              | 0.57 |
| Mean                           | 0.34           | 0.33              |
3.5. Seedling vigour index

The results of Table 5 showed that there were significant differences between the cultivars in the seedling vigour index, while the treatments differed significantly. The treatment of soaking the seeds with distilled water led to a significant increase of 23.88% compared to dry seeds (without soaking), and the seeds responded to the low concentration of selenium and chitosan. 2 mg L$^{-1}$ Se was superior in recording the highest mean (3062), because of the fact that this treatment was superior in the percentage of germination in the final count and the length of root and plumule (Table 1 and 4), and this is consistent agree with Nawaz et al. (2021) [8] who found, Soaking maize seeds in selenium gave the highest mean of seedling vigour index. The comparison treatment without inclusion recorded the lowest mean for this trait (1679).

The interaction between the two factors had a significant effect on the seedling vigour index, as the combination of 2 mg L$^{-1}$ x Baghdad 3 gave the highest mean of (3067), while the comparison treatment of Buhoth 5018 recorded the lowest mean for this trait amounting to (1615).

Table 5. The effect of soaking maize seeds with selenium and chitosan on the seedling vigour index

| Seed soaking treatments (mg L$^{-1}$) | Cultivars | Mean |
|-------------------------------------|-----------|------|
|                                     | Baghdad 3 |      |
| Control                             | 1742      | 1615 |
| distilled water                     | 2082      | 2079 |
| Selenium 2                         | 3067      | 3058 |
| Selenium 5                         | 2880      | 2732 |
| Chitosan 100                       | 1938      | 1932 |
| Chitosan 500                       | 1901      | 1865 |
| LSD 5%                              | 225.50    | 159.50 |
| Mean                                | 2268      | 2214 |

3.6. Dry weight of seedling (mg)

In table 6 the dry weight of the seedling differences significant in both cultivars, Buhoth 5018 recorded the highest mean dry weight of the seedling reached (90.61 mg), while the Baghdad 3 recorded (86.88 mg), and this is consistent with what was found [15];[16], from significant differences in germination characteristics, vigour and viability of the seed between cultivars because of genetic difference. All treatments were superior and did not differ significantly among them in this trait, while they differed significantly with the control treatment, which is dry seeds. The treatment of soaking the seeds with selenium solution at a concentration of 2 mg L$^{-1}$ was superior in recording the highest mean dry weight of the seedling reached (100.78 mg). Due of the fact that this treatment was superior in the percentage of germination in the first and final counts and took the least time to complete germination (Table 1 and 2), which allowed it a longer time to form root growths and vegetative until the end of the examination period (Table 4) and this is consistent with what was found by Nie et al., [33], while the comparison treatment without soaking recorded the lowest dry weight of the seedling (75.78 mg), due the role of seed stimulating treatments through soaking with water and solutions selenium and chitosan in improving seed germination and seedling growth by positively affecting the activity of the amylase enzyme and breaking down carbohydrates also accelerates in the stimulated seeds, as the survival or continuity of seedling growth is positively correlated with the activity of amylase [28].
The combination of soaking the seeds with selenium at a concentration of 2 mg L\(^{-1}\) for the cultivar Buhoth 5018 gave the highest mean of 103.15 mg, while the comparison treatment of Baghdad 3 cultivar recorded the lowest mean for this trait amounting to 71.77 mg.

Table 6. The effect of soaking maize seeds with selenium and chitosan on dry weight of seedling (mg)

| Seed soaking treatments (mg L\(^{-1}\)) | Cultivars | Mean |
|---------------------------------------|-----------|------|
|                                       | Baghdad 3 | Buhoth 5018 |
| Control                               | 71.77     | 79.78 |
| distilled water                       | 84.69     | 87.65 |
| Selenium                              | 2         | 98.42 |
|                                       | 89.86     | 103.15 |
|                                       | 90.50     | 100.78 |
| Chitosan                              | 100       | 88.52 |
|                                       | 87.99     | 89.82 |
|                                       | 88.90     |      |
| LSD 5%                                |           | 7.29 |
| Mean                                  | 86.88     | 90.61 |

4. Conclusion

We conclude from this study that treatments of stimulating seeds by soaking them with distilled water and solutions of selenium and chitosan positively affected germination and its characteristics, so we recommend using low concentrations of selenium and chitosan to improve seed germination, vigour, and viability of the seedling.

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