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Effect of Stimulate of Sorghum Seeds with Banana Peel Extract and Citric Acid on Seeds Viability and Vigour

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Abstract. Sorghum seeds suffer from a low germination ratio, so a factorial experiment was carried out in the Seed Technology Laboratory, Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad during 2022 according to a Completely Randomized Design with four replications to study the effect of stimulating seeds with aqueous extract of banana peels with a concentration of (0, 15, 25 and 35%) and citric acid at concentrations (0, 50, 100 and 200 mg L⁻¹) on viability and vigour of seed properties. Seeds that soaked with banana peel extract at a concentration of 25% outperformed in first count (79.8%), final count (85.0%), radicle length (13.2 cm), plumule length (11.6 cm), and seedling vigour index (2109), noting that they didn't differ significantly with a concentration of 35%. Concentration of 35% outperformed in seedling dry weight (0.015 mg), cold test (46.2%) and accelerated aging (65.0%). Citric acid at a concentration of 200 mg L^{-1} was superior at the first count (84.3%), final count (87.5%), radicle length (13.9 cm), plumule length (11.5 cm), seedling dry weight (0.014 mg), and seedling vigour index (2227). Concentration of 35% + 100mg L^{-1} of banana peel extract and citric acid was significantly superior at final count (94.0%), radicle length (15.8 cm), plumule length (13.6 cm), seedling dry weight (0.017 mg), seedling vigour index (2761), accelerated ageing (75.0%), It can be concluded that banana peel extract is rich with nutrients and mineral elements that stimulate germination and seedling growth, and that citric acid provides an appropriate pH that increases the activity of the enzymes responsible for germination process, so it can be recommended that using a concentration of $35\% + 100 \text{ mg } \text{L}^{-1}$ of the aqueous extract of banana peels and citric acid together to enhance germination in sorghum.

Keywords. Soaking seeds, Germination, First count, Speed of germination, Accelerated age, Cold test, Seedling vigour.

1. Introduction

Sorghum cultivation faces the problem of poor seed germination due to genetic or environmental reasons, or both [1]. Previously conducted studies relied on multiple methods for seed treatment before planting, including wetting and soaking the seeds to reach the beginning of germination by soaking them with plant extracts or nutrient solutions which stimulates germination, growth, and the manifestation of the potential energy in the seeds [2] in a wide environmental condition such as biotic and abiotic stresses [3]. Accordingly, the technology of seed stimulation was developed for the purpose of improving the performance of the seed during the process of germination and emergence

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and making it homogeneous [4,5]. The process of stimulating the seeds with solutions helps the seeds to soak and swell as well as accelerate the production of ATP and increase the activity of enzymes and the production of simple sugars that help the embryo of the seed to grow and the emergence of the radicle and the plumule during the first stage of germination [6]. One of the most important means that helps the plant to complete its life cycle is to treat the seeds before planting with different materials and compounds [7]. It is clear from the results obtained that the use of seed soaking technology can be one of the important solutions to improve growth [8,9]. Recent studies tended to use some agricultural applications, including soaking seeds with some types of plant extracts, the most important of which are aqueous extracts in which water is the solvent for the compounds and elements present in the material to be extracted [10]. Seeds can be stimulated by plant extracts as they contain natural compounds that encourage germination and growth [11] in addition to being cheap, easy to use and environmentally friendly. Plant extract have a positive role in increasing the germination ratio [12]. Banana peels contain a high ratio of nutrients that encourage growth, especially nitrogen and potassium [13]. Citric acid is a highly soluble organic acid in water. It is one of the most important non-enzymatic antioxidants, it has an important role in mitigating and tolerating plants to biotic and abiotic stresses. It contributes to the building of the plant cell and the formation of its compounds such as fats, proteins and various carbohydrates that the plant makes during its growth stages from germination. This study aimed to improve the germination of sorghum seeds.

2. Materials and Methods

A factorial experiment was carried out with two factors (4×4) at the Seed Technology Laboratory, Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad in the year 2002, according to a Completely Randomized Design with four replications. The first factor was soaking the seeds with aqueous extract of banana peels at a concentration of (15, 25 and 35%) in addition to the control treatment (soaking with distilled water only). The second factor is soaking the seeds with citric acid at a concentration of (50, 100 and 200 mg L^{-1}), in addition to the control treatment (soaking with distilled water only). The period of soaking the seeds was 12 hours [14], at room temperature $25^{\circ}C \pm 2$ on blotting paper in Petri dishes. The aqueous extract of banana peels (100 $g L^{-1}$) was prepared by chopping the peels coarsely and soaking them in distilled water and leaving them until fermentation complete at room temperature. Then the extract was filtered to separate the solid impurities using filter paper (Whatman No.1) and Buechner funnel to obtain the aqueous extract in liquid form, which was used to prepare the required concentrations. The first concentration was prepared by taking 15 ml of aqueous extract and completing the volume to 100 ml of distilled water, and so on for the rest of the concentrations. Citric acid concentrate ($C_6G_8O_7$, molecular weight 192.12, purity 99% from Avonchem Company) was prepared by dissolving 50 g of citric acid in one liter of distilled water, and so on for the rest of the concentrations. Data were analysed statistically according to the analysis of variance. Means were compared using least significant difference test at the level of probability 0.05 using the GenStat program for the following studied characteristics:

2.1. Germination Ratio at First and Final Count (%) at Standard Germination Test

200 pure seeds were taken and distributed in four replications for each treatment. Seeds were sown alternately in paper towels, wrapped and placed in plastic bags in the germinator vertically at a temperature of 25 ± 5 °C and a humidity of 70%. Only normal seedlings were counted after four days for the first count and after ten days for the final count, then the results were converted into percentages [15].

2.2. Radicle and Plumule Length (cm) at Standard Germination Test

Ten normal seedlings were taken after the end of the germination period (ten days), then the radicle was separated from its point of contact with the seed, and the plumule was separated from the point of its connection with the middle embryonic hypocotyl [16], then the length of the radicle and plumule was measured separately using a ruler.

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2.3. Seedling Dry Weight (mg) at Standard Germination Test

The same steps followed in the standard germination test were applied, then 10 normal seedlings were taken after the end of the test period (ten days), then the radicle was separated from its point of contact with the seed, and the plumule was separated from its point of contact with the middle embryonic hypocotyl. They were placed in a perforated paper bag and dried at 70°C for 24 hours [16,17]. Seedling dry weight was calculated by dividing the weight of all dry seedlings divided by their number.

2.4. Seedling Vigour Index at Standard Germination Test

It's calculated as followed [18]:

Seedling vigour index = final count \times (radicle length + plumule length)

2.5. Germination Ratio (%) at Accelerated Aging Test

A sample of pure seeds was taken from each experimental unit, then placed in a small wire basket inside a box containing water without the water touching the seeds. The box was placed in a germinator and subjected to stress for 72 hours at a temperature of 43 ± 1 °C and a humidity of 100%. Then the seeds were taken, provided that their moisture after acceleration was 20-30%, and they were subjected to a standard germination test. Only one count was taken after 7 days of changing the germination temperature according to the conditions of the standard germination test to determine the number of normal seedlings only. The results were then converted into percentages and recorded as the ratio of germination by the accelerated age test [15,17].

2.6. Germination Ratio (%) at Cold Test

Soil was taken from the field in which the experiment was carried out, cleaned of plant residues, and mixed with sand at a ratio of 1:1 for the purpose of covering the seeds in this test. 200 pure seeds were sown in four replications using paper towels that were moistened with cold water at a temperature of 10 °C. The seeds were covered with a light layer of moist soil, then transferred and placed in the germinator at a temperature of $10^{\circ}C \pm 0.5$ for 7 days, then the temperature of the germinator was changed to $25^{\circ}C \pm 5$ for another four days. Normal seedlings were calculated only after the end of the examination period (11 days), and then the results were converted into percentages [15,17].

3. Results and Discussion

3.1. Germination Ratio at First Count (%) at Standard Germination Test

Results indicated that there are significant differences due to the influence of the two study factors and the interaction between them (Table 1). The concentration 25% of the extract of banana peels outperformed with highest mean of 79.8%, and it did not differ significantly from the concentration of 35%. While the concentration of 15% recorded lowest mean of 73.0%. The concentration, which gave the lowest mean of 68.3%. The treatment ($35\% + 200 \text{ mg L}^{-1}$) of banana peel extract and citric acid outperformed with the highest mean (89.0%), and it did not differ significantly from several treatments, and the lowest mean was for the treatment ($15\% + 0 \text{ mg L}^{-1}$) with mean of 63.0%. The reason for the superiority of the treatment of soaking with aqueous extract of banana peels may be due to the role of the acid in activating the enzymes inside the seeds and reducing the effect of abscisic acid, which inhibits germination-stimulating enzymes such as isoperoxidase poly phenol oxidase and catalase [20]. Citric acid is a non-enzymatic antioxidant that works to protect the cell from external influences and works to increase the absorption of beneficial ions such as potassium and prevent the accumulation of toxic ions [21].

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Concentration of aqueous	Citric acid concentration (mg L ⁻¹)					
extract of banana peels (%)	0	50	100	200	Mean	
0	66.5	72.0	82.0	83.5	76.0	
15	63.0	79.0	68.5	81.5	73.0	
25	77.0	82.5	76.0	83.5	79.8	
35	67.0	73.0	86.0	89.0	78.8	
LSD 0.05	10.0				5.0	
Mean	68.3	76.6	78.1	84.3		
LSD 0.05		5.0				

Table 1. Effect of stimulating seeds with banana peel extract and citric acid and their interaction on
germination ratio at first count (%) in sorghum.

LSD 0.05, means were compared using least significant difference test at probability level of 0.05.

3.2. Germination Ratio at Final Count (%) at Standard Germination Test

Results indicated a significant effect of soaking with banana peel extract and citric acid and the interaction between them on the final count (Table 2). The concentration 25% of the extract of banana peels outperformed with highest mean of 85%, and it did not differ significantly from the concentration of 35%. While the concentration of 15% recorded lowest mean of 77.2%. The concentration of 200 mg L⁻¹ of citric acid gave the highest mean (87.5%), and it did not differ significantly from the concentration of 100 mg L⁻¹, compared to the 0% concentration, which gave the lowest mean of 73.6%. The treatment (35% + 100 mg L⁻¹) of banana peel extract and citric acid outperformed with the highest mean of 94.0%, and it did not differ significantly from several treatments, and the lowest mean was for the treatment (15% + 0 mg L⁻¹) with mean of 68.0 %. This character was similar in its behavior to the characteristic of the first count (Table 1).

Table 2. Effect of stimulating seeds with banana peel extract and citric acid and their interaction on germination ratio at final count (%) in sorghum.

Concentration of aqueous	Citric acid concentration (mg L ⁻¹)					
extract of banana peels (%)	0	50	100	200	Mean	
0	70.0	80.5	86.0	85.5	80.5	
15	68.0	83.5	74.5	83.0	77.2	
25	82.0	88.0	80.5	89.5	85.0	
35	74.5	76.5	94.0	92.0	84.2	
LSD 0.05	9.0 4.					
Mean	73.6	82.1	83.8	87.5		
LSD 0.05	4.5					

LSD $_{0.05}$, means were compared using least significant difference test at probability level of 0.05.

3.3. Radicle Length (cm) at Standard Germination Test

Treatment of soaking with banana peel extract at a concentration of 25% outperformed with the highest mean of 13.2 cm (Table 3). It did not differ significantly from the concentration of 35%, while the concentration of 15% recorded the lowest mean of 11.7 cm. Soaking treatment with citric acid at a concentration of 200 mg L⁻¹ outperformed with mean of 13.9 cm, and did not differ significantly from the concentration of 100 mg L⁻¹, while the control treatment (soaking with distilled water only) gave the lowest mean (10.3 cm). The treatment (35% + 100 mg L⁻¹) of banana peel extract and citric acid outperformed with the highest mean (15.8 cm), while the lowest mean was to the treatment (15% + 0 mg L⁻¹) with mean of 7.2 cm. The response of the seeds to soaking with the aqueous extract of banana peels and citric acid may be due to the fact that banana peels contain a group of important elements, including phosphorus, calcium and magnesium, which are important for building normal roots and shoots. As well as the role of citric acid, one of the organic acids that contribute to increasing the germination ratio of seeds and increasing the surface area of the roots and thus the absorption of nutrients. These results agree with [22,23], or it may be the reason for its superiority in the first and

final count of standard laboratory germination, and this was confirmed by the results of [24], which indicated that the treatments that outperformed in the radicle length characteristic were originally superior in the ratio of normal seedlings in the first and final count, which took a longer time to grow. **Table 3.** Effect of stimulating seeds with banana peel extract and citric acid and their interaction on radicle length (cm) in sorghum.

Concentration of aqueous	Citric acid concentration (mg L ⁻¹				
extract of banana peels (%)	0	50	100	200	Mean
0	9.1	13.2	12.0	14.3	12.1
15	7.2	13.5	12.3	13.6	11.7
25	11.8	13.5	12.9	14.6	13.2
35	13.2	10.5	15.8	13.2	13.1
LSD 0.05		0.8			
Mean	10.3	12.7	13.2	13.9	
LSD 0.05		0.8			

LSD 0.05, means were compared using least significant difference test at probability level of 0.05.

3.4. Plumule Length (cm) at Standard Germination Test

Results indicated a significant effect of soaking with banana peel extract and citric acid and the interaction between them on the final count (Table 4). The treatment of soaking with banana peel extract at a concentration of 35% outperformed with the highest mean of 11.6 cm, It did not differ significantly from the concentration of 25%, while the concentration of 15% recorded the lowest mean of 9.9 cm. Soaking treatment with citric acid at a concentration of 100 mg L⁻¹ outperformed with mean of 11.5 cm, and did not differ significantly from the concentration of 200 and 50 mg L⁻¹, while the control treatment (soaking with distilled water only) gave the lowest mean (8.7 cm). The treatment (35% + 100 mg L⁻¹) of banana peel extract and citric acid outperformed with the highest mean (13.6 cm), and it did not differ significantly with some other treatments. While the lowest mean (5.6 cm) was to the treatment (15% + 0 mg L⁻¹). The reason may be due to the fact that banana peels contain carbohydrates, proteins, and unsaturated fatty acids, especially nutrients [25], that are essential for cell building and growth. These results agree with [26].

Table 4. Effect of stimulating seeds with banana peel extract and citric acid and their interaction on plumule length (cm) in sorghum.

Concentration of aqueous	Citric acid concentration (mg L ⁻¹)					
extract of banana peels (%)	0	50	100	200	Mean	
0	7.1	10.9	11.2	11.4	10.1	
15	5.6	11.4	10.9	11.6	9.9	
25	10.7	12.7	10.4	11.8	11.4	
35	11.3	10.0	13.6	11.4	11.6	
LSD 0.05	1.8				0.9	
Mean	8.7	11.2	11.5	11.5		
LSD 0.05	0.9					

LSD 0.05, means were compared using least significant difference test at probability level of 0.05.

3.5. Seedling Dry Weight (mg) at Standard Germination Test

Results showed that there are significant differences in the mean dry weight of seedlings due to the effect of seed soaking treatment with the two study factors and their interaction (Table 5). The treatment of soaking the seeds with banana peel extract at a concentration of 35% was significantly outperformed the rest of the concentrations and gave the highest mean (0.015 mg), while the treatment of soaking with distilled water only (0%) gave the lowest mean (0.013 mg). Soaking treatments with citric acid at a concentration of 50, 100 and 200 mg L⁻¹ did not differ significantly between them and gave mean of 0.014 mg, but they were significantly outperformed control treatment (0 mg L⁻¹) with mean of 0.013 mg. The treatment of banana peel extract with citric acid (35% + 100 mg L⁻¹) excelled and gave the highest mean (0.017 mg), while the lowest mean (0.011 mg) for the treatment (15% + 0

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mg L^{-1}). This may be due to the superiority of this treatment in the speed of laboratory germination ratio of the seedling in its early stages compared to other treatments that are late in its germination, which produces active and strong seedlings that lead to an increase in dry weight, or, the superiority of the dry weight of the seedling may be attributed to its superiority in the two characteristics of radicle and plumule length (Tables 1, 3 and 4).

Table 5. Effect of stimulating seeds with banana peel extract and citric acid and their interaction on seedling dry weight (mg) in sorghum.

Concentration of aqueous	Citric acid concentration (mg L ⁻¹)					
extract of banana peels (%)	0	50	100	200	Mean	
0	0.012	0.013	0.013	0.015	0.013	
15	0.011	0.015	0.014	0.014	0.014	
25	0.014	0.014	0.014	0.013	0.014	
35	0.016	0.015	0.017	0014	0.015	
LSD 0.05	0.002 0.					
Mean	0.013	0.014	0.014	0.014		
LSD 0.05	0.001					

LSD 0.05, means were compared using least significant difference test at probability level of 0.05.

3.6. Seedling Vigour Index at Standard Germination Test

Results showed that there are significant differences between the seed soaking treatments and their interactions in this trait (Table 6). The treatment of soaking the seeds with banana peel extract at a concentration of 35% excelled and gave the highest mean (2109), and did not differ significantly from the concentration of 25%, while the lowest mean was at a concentration of 15% (1692). Soaking treatment with citric acid at a concentration of 200 mg L⁻¹ outperformed with the highest mean of 2227, and did not differ significantly from the concentration of 100 mg L⁻¹ (2092), while the lowest mean was (1430) when soaked with distilled water only. The treatment of soaking the seeds with banana peel extract and citric acid (35% + 100 mg L⁻¹) was superior to the rest of the treatments and gave the highest mean (2761), while the lowest mean was (878) for the treatment (15% + 0 mg L⁻¹). The superiority of this treatment is attributed to its superiority in the ratio of germination in the final count, radicle and plumule length (Tables 2, 3, and 4).

Table 6. Effect of stimulating seeds with banana peel extract and citric acid and their interaction on seedling vigour index in sorghum.

Concentration of aqueous	Citric acid concentration (mg L ⁻¹)				
extract of banana peels (%)	0	50	100	200	Mean
0	1165	1954	2005	2198	1830
15	878	2073	1731	2086	1692
25	1852	2314	1870	2360	2099
35	1828	1583	2761	2265	2109
LSD 0.05		181.4			
Mean	1430	1981	2092	2227	
LSD 0.05	181.4				

LSD 0.05, means were compared using least significant difference test at probability level of 0.05.

3.7. Germination Ratio (%) at Accelerated Aging Test

There are significant differences with ratio of normal seedlings in the accelerated aging test by the effect of soaking seeds in banana peel extract (Table 7), the effect of soaking with citric acid and interaction was not significant. The concentration treatment 35% gave the highest mean (65.0%), and there was no significant difference between the concentrations of banana peel extract (15, 25, and 35%), but it was significantly superior to the treatment of soaking with distilled water (0%), which gave the lowest mean (44.5%). The accelerated aging assay simulates the environmental stress (high temperature and humidity) that leads to seed deterioration. Perhaps the effect of the banana peel

extract, with its nutrients and chemical compounds, had a role in raising the vitality of the deteriorating seeds and revealing their potential energy to give normal seedlings.

Table 7. Effect of stimulating seeds with banana peel extract and citric acid and their interaction on germination ratio (%) at accelerated aging test in sorghum.

Concentration of aqueous	Citric acid concentration (mg L ⁻¹)					
extract of banana peels (%)	0	50	100	200	Mean	
0	50.0	40.0	46.0	72.0	44.5	
15	55.0	60.0	68.0	71.0	63.5	
25	69.0	59.0	64.0	44.0	59.0	
35	64.0	59.0	75.0	62.0	65.0	
LSD 0.05		N.S				
Mean	59.5	54.5	63.2	54.8		
LSD 0.05	N.S					

LSD $_{0.05}$, means were compared using least significant difference test at probability level of 0.05.

3.8. Germination Ratio (%) at Cold Test

Results showed that there are significant differences due to the influence of the two study factors and the interaction between them in this trait (Table 8). The treatment of soaking banana peel extract with a concentration of 35% was superior to the rest of the treatments and recorded the highest mean of germination ratio at cold test (46.2%), while the treatment with a concentration of 15% recorded the lowest mean (29.5%). The soaking treatment with distilled water (0 mg L⁻¹) outperformed with mean of 46.6%, while the lowest mean was at the concentration of 50 mg L⁻¹ with 27.2%. The treatment (35% + 200 mg L⁻¹) outperformed with the highest mean (64.5%), and it did not differ significantly from the treatment (35% + 100 mg L⁻¹) and the control treatment (soaking with distilled water only) for each of the factors under study. Soaking treatment with concentration (35% + 50 mg L⁻¹) recorded the lowest mean (19.5%). The variation in the results of this characteristic may be due to the effect of the different concentrations of the two study factors due to the conditions of this examination that stimulate the microorganisms present in the soil to attack the seeds [27]. Certain concentrations and not others had a positive effect on enduring this environmental stress and contributing to the growth of embryonic axis (plumule, the radicle, and the hypocotyl) and seedling formation.

Concentration of aqueous	Citric acid concentration (mg L ⁻¹)					
extract of banana peels (%)	0	50	100	200	Mean	
0	54.0	40.5	28.0	23.0	36.4	
15	47.5	21.0	23.5	26.0	29.5	
25	40.5	28.0	33.5	24.0	31.5	
35	44.5	19.5	56.5	64.5	46.2	
LSD 0.05		8.7				
Mean	46.6	27.2	35.4	34.4		
LSD 0.05	8.7					

Table 8. Effect of stimulating seeds with banana peel extract and citric acid and their interaction on germination ratio (%) at cold test in sorghum.

LSD $_{0.05}$, means were compared using least significant difference test at probability level of 0.05.

Conclusions

It can be concluded that sorghum seeds took a positive path due to the effect of the stimulants and their concentrations. This enhances the possibility of using the aqueous extract of banana peels and citric acid as an alternative to other nutrients and growth regulators in increasing the vitality and seed vigour through the soaking process. It can be recommended soaking sorghum seeds before planting with a mixture of aqueous extract of banana peels with citric acid at a concentration of $(35\% + 100 \text{ mg L}^{-1})$ for 12 hours to improve their ability to germinate and improve seedling vigour, and the possibility of studying concentrations higher than 35% of the aqueous extract of banana peels.

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