

Evaluation of the Effect of Different Priming Treatments on the Seed Germination of Maize (*Zea mays. L*) Based on In Vitro Conditions

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Abstract

Seed germination is one of the basic and vital stages in the plant life cycle which is affected by many factors. Different techniques can be used to enhance germination and crop yield like seed priming that is considered as a physiological treatment that improves seed performance and provide faster and synchronized germination. The objective of this study is to compare the efficacy of different priming methods on the seeds germination of hybrid maize (hybrid Tikrit variety) with two durations (12, 18 hours) under laboratory conditions. Seeds were subjected to hydro- priming, hormonal priming with gibberellic acid and salicylic acid, priming with liquorice extract, and the interaction among them in comparison with the control (dry seeds). A laboratory screening was conducted at the faculty of Agricultural/ Tikrit University during in March 2021 using a complete randomized design (CRD) with three replicates. The results showed that hydro- priming for 12 hours have recorded a significant increase in the level of most of the germination parameters {first and final seed germination% (86.67%, 84.00%), plumule and radical length (8.750 cm, 6.633 cm), first and final seedling vigor index (136.6, 130.8), speed of germination (1.889 seed/day), response index (-0.0412), mean of seedling fresh weight (0.7120 gm), and seed vigor (16.21)}. The maximum mean seedling dry weight was recorded to the treatment of the interaction with salicylic acid and liquorice extract, and hydro-priming (15.3000 mg) for 18 hours. While the parameter of daily seed growth rate did not give any significant differences. Results proved the effectiveness of the 12 hours hydro-priming treatment than the rest of the treatments.

Introduction

Zea mays belongs to the Gramineae family, it was considered as one of the most important crops in the world due to versatility [1]. It is a major source of food, feed and energy, ranking first in respect of total production and second regarding cultivated average after wheat [2]. This crop suffers from low productivity per unit area because of the high temperature, hot winds and improper use of soil and crop management [3].

Seed germination is one of the basic and vital stages in the plant life cycle, which is affected by many environmental and genetic factors; this process was influenced by plant

hormones [4]. Different techniques can be used to enhance germination and crop yield like seed priming that is considered as a physiological treatment that improves seed performance and provide faster and synchronized germination [5, 6]. It is a normal practice reduce the time of germination, harmonize germination, increase the rate of germination, nutrient uptake and increase enzyme activity [7]. Seed priming is the simple, inexpensive and low hazard technique and can assist farmers to decrease the extreme use of fertilizers [8]. It involved fractional germination of seed by soaking in either water or in a suitable solution for a given period of time to activate biochemical processes, then re-drying them just before the radicle emerges [9]. Different priming methods are advanced to provide best seed quality such as hydro-priming, halo-priming, osmo-priming, matrix priming, hormonal priming, nutria-priming, thermo priming and bio-priming [10].

The very active method of all for maize seed priming is hormonal priming especially the use of gibberellic acid (GA3) which is one of the plant growth regulators that have stimulating effects on most plants and plays important role in activating the cell elongation and breaking its dormancy [11]. Siadat et al indicated that the soaking of maize seeds with gibberellic acid (400 ppm for 12 hours) gave the best germination rate compared with control treatment [12]. Maize seed soaking in GA3(300 mg/L for 24 hours) increase the ratio of germination at the first and final count, radical and plumule lengths and fresh and dry weight of seedling [13] Salicylic Acid (SA) is a phenolic compound found in most plant species. It is a group of endogenous plant hormones that acts as one of the specific growth regulators for plant growth and development and stimulate the flowering of plants [14]. Salicylic Acid promotes or inhibits physiological processes, controlling the absorption of ions, hormonal balance and stomata movement [15].

Some plant extracts were intervened with the plant growth due to their chemical products, which affected seed germination and seedling of seed [16]. *Glycyrrhiza glabra* L. is known as liquorice and sweet wood, belong to Fabeacea family which is used as a medicinal and therapeutic plant [17]. The root of liquorice contains some compounds such as flavonoids, triterpenoids, saponins (4-20%), vitamins, minerals, phenolic compounds and mevalonic acid which are used in gibberellins synthesis [18]. The action of liquorice root extract is similar to gibberellic acid because it contains the biosynthesis of gibberellin starter which stimulates the increase in the germination of seeds and accelerates cell division and elongation [19] The objective of this study was to evaluate the effects of various seed priming techniques on seed germination of hybrid maize with different durations.

Materials and Methods

A laboratory experiment was carried out at the College of Agriculture / University of Tikrit in the season of 2021 according to Complete Randomized Design (CRD) with three replicates and two factors. The first factor was the seeds priming treatments as shown in table [1]and the second factor was the two priming duration (12, 18 hours)

Table 1: the symbol of different seeds priming

Symbol	Treatments
T0	Dried seeds (Non-soaked seeds)
T1	Seeds soaking with water (12hours)
T2	Seeds soaking with gibberellic acid (25mg/L) (12 hours)
T3	Seeds soaking with salicylic acid (20mg/L) (12 hours)
T4	Seed soaking with gibberellic acid + salicylic acid (12 hours)
T5	Seeds soaking with extract of liquorice (5mg/L) (12 hours)
T6	Seeds soaking with extract of liquorice + salicylic acid (12 hours)
T7	Seeds soaking with water (18 hours)
T8	Seeds soaking with gibberellic acid (25mg/L) (18 hours)
T9	Seeds soaking with salicylic acid (20mg/L) (18 hours)
T10	Seeds soaking with gibberellic acid + salicylic acid (18 hours)
T11	Seeds soaking with extract of liquorice (5mg/L) (18 hours)
T12	Seeds soaking with extract of liquorice + salicylic acid (18 hours)

Seeds of *Zea mays* L. (Hybrid Tikrit variety) were obtained from the state of commission for the testing and certifying the seeds of the Salahuddin branch. The seeds were sterilized with 5% sodium hypochlorite (Na₂ HCl) solution for 1 minute to avoid contamination and were thoroughly rinsed four times with sterile distilled water, then seeds were dried back near the original weight, all Petri dishes were sterilized and labelled for various treatments to avoid mixing up. The seeds were soaked with various solutions of treatments for 12 and 18 hours at room temperature without light. After the completion of priming durations, seeds were washed carefully with distilled water three times and dried up to their original weight.

Three replicates of 25 seeds were germinated in covered disposal Petri dishes on two layers of whatman No. 1 filter paper which were irrigated with distilled water daily during the experiment to keep the filter paper moist for seedling development for a period of 12 days. All Petri dishes were placed at room temperature, in addition to the control treatments (Dry seeds) also were placed on filter paper planted Petri dishes. A seed was scored as germinated when the radicle emerges by about 2 mm in length [20]. The germination of seeds was recorded daily up to day 12th after the start of the experiment and germination parameters were determined as the following:

Y1) Seed germination percentage at the first count after four days from planting

Y2) Seed germination percentage at the final count when the experiment was ended

$G\% = (\text{Number of normal germinated seeds} / \text{Number of total germinated seeds}) \times 100$ [21]

Y3) Plumule length

Y4) Radical length

The plumule and radical length of ten randomly selected seedling were separated carefully from their radical and measured using a ruler in cm [22]

Y5) Seedling vigor index at the first count

Y6) Seedling vigor index at the final count

$SVI = G\% (\text{length of plumule} + \text{length of radical}) / 100$ [23]

Y7) Speed of germination = Number of normal germinated seed/ Number of days since the start of planting

Y8) Response index = (Number of germinated seeds in the treatments/ Number of seeds in the control) –[24]

Y9) Mean of seedling fresh weight

Y10) Mean of seedling dry weight

Y11) Radical: plumule ratio = (Mean of radical dry weight/ Mean of plumule dry weight)

Y12= Daily seed growth rate (gm/day) = Seedling dry weight/ Number of planting days

Y13) Seed vigor = (G% × length of seedling) / 100[25]

The collected data were submitted to analysis of variance (ANOVA), and differences obtained in the mean value were categorized through the Least Significant Difference (LSD) test at 0.05 probability [26].

Results and Discussion

The present study focused on the effect of different soaking treatments under two duration periods to activate the seeds of maize. Data presented in table [2] and [3] clearly showed significant differences for the most parameters studied, the first germination count (%), final germination (%), radical and plumule length (cm), seedling vigor index in the first and final count, speed of germination (seed/day), mean of seedling fresh and dry weight (mg), radical: plumule ratio, response index, and seed vigor except the daily seed growth rate parameter which did not show a significant effect among the treatment.

Results in the table [2] indicated that there was a significant difference in the average germination percentage in the first and final count. The highest main value (86.67) of germination percentage during the first count Y1 was recorded to both treatments of distilled water T1 and liquorice extract T5 for 12 hours, which was statistically followed by the treatments T11 and T12 which recorded the same mean value (82.67). On the other hand, the lowest value of the first germination percentage (50.67) was recorded for the treatment of seeds which soaked in distilled water for 18 hours T7. Tekrony & Egli, (1977) were reported that the first count was considered as a good predictor of field emergence [27]. As for the parameter of final germination percentage Y2 the treatment T1 was recorded the highest mean value (84.00), this treatment was significantly different from the rest treatments, while the least value (42.67) was recorded to T7 treatments. In general, rapid germination was attributed to the high synthesis of DNA, RNA and protein during priming [28].

Plumule length parameter Y3 was recorded a maximum value of (8.750) with the T1 treatment and followed by the treatment of soaking with liquorice extract T5 which was recorded (7.333), while the minimum value (3.283) was observed in seeds soaking in distilled water for 18 hours T7 treatment. Other parameters radical length Y4, seedling vigor index at first and final count Y5, Y6 and speed of germination Y7 showed the highest value for the T1 treatment (6.633, 136.6, 130.8 and 1.889) While lowest value of these parameters was recorded to T7 treatment (3.150, 33.6, 25.7 and 1.222) respectively. The reason for the increase of the plumule and radical lengths is also attributed to their increase in the percentage of germination, or they took a long time to grow than those which did not germinate. In contrast the reason for the decrease may be due to the presence of a toxic substance that inhibits the increase of radical length or presence of some active compounds

such as alkaloids, tannins and glycosides which inhibit cell division and elongation and then reduce the length of plumule [29]. An increase in radicle length might be the result of higher embryo-cell wall extensibility. The trait of germination speed gives evidence for the quality of the seeds and their tolerance to field conditions and gives homogeneity during germination [30, 31]. Agrawal (1986) (31) indicated that the first count of natural seedlings was a guide to the speed of germination and a measure of seed vigor.

Table (2): The effect of different soaking treatments on some parameters: Y1. Y2 (G% at first and final count), Y3 (plumule length), Y4 (radical length), Y5, Y6 (seedling vigor index at first and final count), Y7 (speed of germination)

Parameters	Y1	Y2	Y3	Y4	Y5	Y6	Y7
Treatments							
Dry seed T0	90.67	84.00	8.750	7.100	143.9	133.5	1.972
D.W (12h)T1	86.67	84.00	8.750	6.633	136.6	130.8	1.889
GA3 (12) T2	77.33	60.00	3.950	4.400	64.8	50.5	1.527
SA (12h) T3	74.67	60.00	4.133	4.967	67.3	54.1	1.500
GA3+SA (12h) T4	78.67	57.33	3.717	3.833	59.2	44.6	1.555
Liquorice extract (12 h) T5	86.67	77.33	7.333	5.333	109.8	97.9	1.805
Liquorice extract+ SA (12) T6	74.67	64.00	3.667	3.967	58.0	45.8	1.639
D.W (18) T7	50.67	42.67	3.283	3.150	33.6	25.7	1.222
GA3 (18h) T8	64.00	49.33	3.967	3.667	48.5	41.3	1.555
SA (18) T9	69.33	58.67	4.900	4.617	66.2	55.9	1.527
GA3+SA (18h) T10	70.67	61.33	5.183	4.567	68.0	59.9	1.555
Liquorice extract (18h) T11	82.67	62.67	5.000	5.500	86.5	65.9	1.611
Liquorice extract+ SA (18h) T12	82.67	65.33	5.283	4.467	81.6	63.4	1.722
L S D	5.97430	8.56947	1.09208	0.954141	16.4226	18.2325	0.142565

The results in table [3] showed significant difference among treatments. The treatment of soaking seeds in distilled water for 12 hours T1 was showed a maximum value (-0.0412, 0.7120) for parameters of response index Y8 and mean of seedling fresh weight Y9 while the lowest value (-0.3798, 0.4967) was observed from T7 and T4 treatments. Mean of seedling dry weight Y10 was showed the highest value (0.15300) in seeds soaked with distilled water for 18 hours T7 and T12 treatment which seeds soaked with salicylic acid and liquorice extract for 18 hours, while the treatment T1 was showed the lowest value (0.10867).

Maximum radical: plumule ratio Y11 (0.5593) was observed in seeds that were soaked with GA3 for 18 hours T8, whereas the minimum value (0.2367) was observed in seeds soaked with liquorice extract (T11). The parameter of daily seed growth ratio Y12 was showed non-significant differences among treatments. The seed vigor Y13 was showed the highest value [16.21] with the T1 treatment, whereas the lowest value (5.36) was recorded for T7 treatment.

Table (3): The effect of different treatment on some parameters: Y8 (response index), Y9, Y10 (Mean of seedling fresh and dry weight), Y11 (R/P ratio), Y12 (daily seed growth ratio), Y13 (seed vigor).

Parameters	Y8	Y9	Y10	Y11	Y12	Y13
Treatments						
Dry seed T0	-0.1110	0.6467	0.11333	0.26833	0.013567	18.08
D.W(12h) T1	-0.0412	0.7120	0.10867	0.3793	0.014900	16.21
GA3(12h) T2	-0.2246	0.6083	0.14233	0.5303	0.014867	8.91
SA (12h) T3	-0.2373	0.5497	0.13567	0.5037	0.014300	9.12
GA3+SA (12h) T4	-0.2107	0.4967	0.14333	0.4257	0.014567	8.42
Liquorice extract (12h) T5	-0.0839	0.6173	0.11367	0.2437	0.013067	13.97
Liquorice extract+ SA (12h) T6	-0.1703	0.5037	0.15167	0.4510	0.014800	8.67
D.W(18h) T7	-0.3798	0.5213	0.15300	0.4133	0.014900	5.36
GA3(18h) T8	-0.2101	0.5630	0.14500	0.5593	0.014800	6.79
SA(18h) T9	-0.2258	0.6143	0.13400	0.4580	0.013767	8.96
GA3+SA (18h) T10	-0.2107	0.5533	0.14467	0.4260	0.014933	9.65
Liquorice extract (18h) T11	-0.1817	0.5663	0.13967	0.2367	0.014600	10.87
Liquorice extract+SA (18) T12	-0.1268	0.5957	0.15300	0.3160	0.016000	10.94
L S D	0.0831630	0.0478304	0.00929019	0.0905109	Ns	1.75020

This study concluded that different priming technique may have various effects on the germination of maize seeds. Results showed that, for most evaluated germination parameters, hydro priming was more effective and the duration for 12 hours was more effective than the duration for 18 hours possibly, this indicated that applied hydro-priming treatment did not damage seed structure or metabolic activity and there has no toxic effect of ion accumulation on the embryo. These results were in agreement with Pegah et al (2008) who reported that germination was significantly enhanced by water soaking [32]. The increasing of some parameters in the treatment of soaking in liquorice extract may be due to its contents of several compounds like saccharides, proteins, and mineral nutrition (P, K, Cu, Mg, Mn, Zn, Cu) [33].

Other treatments showed little effects on germination parameters probably due to the low concentration of plant regulators used and the short soaking periods, or may be due to the maize seeds were not exposed to any kind of environmental stresses. Several studies reported the major role of SA and GA in modulating the plant response to several abiotic stresses including salt and water stress [34, 35].

Conclusion

It may be concluded from the present study that priming with water for 12 hours was better than other priming media tested for high vigor and rapid seed germination. However, the priming ability of proposed chemicals depend on the concentrations of these chemicals as well as, the priming duration. Then, these priming techniques do not need to be adopted by the farmer when sowing the crop carried out in drought and salinity- free conditions.

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تقييم تأثير معاملات التحفيز المختلفة في أنبات بذور الذرة الصفراء (*Zea mays L.*) تحت الظروف المختبرية

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أنبات البذور واحدة من المراحل الأساسية والحيوية في دورة حياة النبات والتي تتأثر بعوامل عديدة. وهناك تقنيات مختلفة بالأمكان استخدامها لتحسين الأنبات والمحاصيل، مثل تحفيز البذور والتي تعتبر من المعاملات الفسيولوجية التي تعمل على تحسين أداء البذور وتقدم أنبات سريع ومتزامن. تهدف الدراسة الى تقدير فعالية طرق التحفيز المختلفة في أنبات بذور الذرة الصفراء الهجينة بفترتين مختلفتين (18,12 ساعة) وتحت الظروف المختبرية. تم تعريض بذور الذرة الصفراء الى معاملات التحفيز المائي، التحفيز الهرموني بالجبرلين وحامض الساليسليك والتحفيز بمستخلص عرق السوس بالإضافة الى التداخل بين معاملات التحفيز مقارنة مع معاملة السيطرة (بذور جافة). تم إجراء التجربة المختبرية في كلية الزراعة/ جامعة تكريت للموسم 2021 بأستعمال التصميم العشوائي الكامل وبثلاث مكررات. بينت النتائج بأن التحفيز المائي لمدة 12 ساعة أعطى زيادة معنوية في معدل أغلب عوامل الأنبات (نسبة الأنبات الأولي والنهائي 86.67 %، 84.00 %، (طول الرويشة والجذير 8.750 سم، 6.633 سم)، (دليل قوة البادرة في العد الأولي والنهائي 136,6، 130,8)، سرعة الأنبات (1,889 بذرة/يوم)، دليل الاستجابة (-2140.0)، معدل الوزن الرطب للبادرة (0.7120 غم) وقوة البادرة (16.21). وكان أعلى معدل وزن جاف للبادرة قد سجل لمعاملة التداخل بين حامض الساليسليك ومستخلص عرق السوس (15.300) لمدة 18 ساعة. بينما لم يسجل معدل النمو اليومي أي اختلاف معنوي بين المعاملات. هذه النتائج أثبتت فعالية تحفيز البذور المائي لمدة 12 ساعة عن بقية المعاملات.