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ENHANCING THE VIABILITY AND VIGOR OF SELECTED SUNFLOWER SEEDLINGS THROUGH HORMONAL PRIMING TECHNIQUES

A. R. Mohammed [1] **N. N. Mutlak** [2] **D. K. Duraideh** [1*]
S. E. Seadh [3]

[1] **Department of General Sciences, College of Basic Education/Haditha, University of Anbar.**
[2] **Ministry of Higher Education and Scientific Research.**
[3] **Agronomy Department, Faculty of Agriculture, Mansoura University, Egypt.**

***Correspondence to:** Dhaif K. Duraideh, Department of General Sciences, College of Basic Education/Haditha, University of Anbar, Iraq.
Email: dhaif.k.d@uoanbar.edu.iq

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Abstract

This study was conducted in May 2022 and 2023 to assess the impact of different concentrations of the brassinolide hormone (0, 0.5, 1.0, and 1.5 mg L^{-1}) on the viability and vigor of seedlings derived from specific sunflower varieties (Ishaqi-1, Aqmar, and Sakha). The factorial experiments consisted of four replications using a completely randomized design (CRD) for each. The results revealed that sunflower variety significantly influenced both the viability and vigor of their seedlings in the two years. The Sakha variety outperformed the others in producing high-quality seeds and robust seedlings during germination and vigor tests. Specifically, sunflower seeds primed with 1.0 mg L^{-1} of brassinolide hormone exhibited superior viability and seedling vigor compared to other test doses in both conventional germination tests and seedling characteristics.

Keywords: Brassinolide, Hormone, Sunflower, Seed priming, Germination, Seedling vigor.

تعزيز حيوية وقوة بادرات اصناف مختارة لزهرة الشمس من خلال تقنية التنشيط الهرموني

احمد رجب محمد [1] نجاح نعيم مطلك [2] ظيف كطاع دريدح [1]*
صالح السيد سعده [3]

[1] قسم العلوم العامة، كلية التربية الأساسية/ حديثة، جامعة الأنبار.
[2] وزارة التعليم العالي والبحث العلمي، العراق.
[3] قسم الزراعة، كلية الزراعة، جامعة المنصورة، مصر.

***المراسلة الى:** ظيف كطاع دريدح، قسم العلوم العامة، كلية التربية الأساسية – حديثة، جامعة الانبار، العراق.
البريد الالكتروني: dhaif.k.d@uoanbar.edu.iq

الخلاصة

نفذ البحث في مختبر الأحياء، قسم العلوم العامة، كلية التربية الأساسية في حديثة، جامعة الأنبار، العراق. هدفت الدراسة إلى تقييم تأثير معاملة نقع البذور بتركيزات مختلفة من هرمون البراسينولايد (0، 0.5، 1.0، و1.5 ملغ/لتر) على حيوية وقوة بادرات أصناف محددة من زهرة الشمس (إسحاقي-1، أقمار، وساخا). تضمنت التجارب العاملية أربع مكررات باستخدام تصميم عشوائي كامل (CRD) لكل منها. بينت النتائج أن اصناف زهرة الشمس قد استجابت بشكل كبير لمعاملة النقع من حيث التأثير في الحيوية وقوة البادرات في كلا العامين. اذ تفوق صنف ساخا على إسحاقي-1 وأقمار في إنتاج بذور عالية الجودة وبادرات قوية خلال اختبارات الإنبات وقوة البذور. على وجه التحديد، أظهرت بذور زهرة الشمس المعالجة بتركيز 1.0 ملغ/لتر من هرمون البراسينولايد حيوية وقوة بادرات أفضل مقارنة بالمعاملات الأخرى المختبرة (0.0، 0.5، و1.5 ملغ/لتر) في كل من اختبارات الإنبات التقليدية وصفات البادرات.

كلمات مفتاحية: هرمون، زهرة الشمس، تحضير البذور، الإنبات، قوة البادرات.

Introduction

The sunflower (*Helianthus annuus* L.), a member of the Asteraceae family, is a major oilseed used in edible oil production. Ranking third among major oilseed crops, following soybean and peanut, it shares this position with others like canola and cotton, making substantial contributions to global edible oil production (8). Renowned for its cholesterol-free qualities and rich content of unsaturated fatty acids, sunflower has become a key constituent of the edible vegetable oil industry worldwide (20). As such, implementing and developing up-to-date methods for processing oilseeds, including sunflower seeds, is important (6).

The nutritional significance of sunflower oil extends to its excellent properties, being virtually free of major toxic compounds and characterized by a relatively high concentration of linoleic acid (26). Sunflower demonstrates significant agricultural importance, not only due to its high quality and quantity relative to other oilseed crops but also because of its limited adaptability to high temperatures and drought conditions worldwide (2). The primary objective of cultivating sunflower is to obtain seeds noted for their oil (36-52%) and protein (28-32%) content, as reported by (23). Selecting varieties with high production capabilities is crucial to enhance sunflower productivity per unit area.

This study assessed both old and newly developed sunflower varieties to identify the most promising cultivars with optimal germination performance. The evaluation of sunflower cultivars revealed considerable variation in both germination rates and seedling vigor. In this context, (19) observed that the highest germination percentage was achieved by the hybrid USDA 894, whereas hybrid EX 47 exhibited slower germination rates at only 50%. Additionally, (3) highlighted the superior performance of the Aqmar over other studied cultivars (Ishaqi-1 and Ishaq-2) across various laboratory characteristics, including germination percentage at the first count, standard germination percentage, radicle length, plumule length, seedling vigor index, and plumule fresh and dry weights. Furthermore, (25) reported that cv Catissol-01. demonstrated higher germination percentage and seedling vigor. Seed priming, a pre-germination technique, involves allowing seeds to absorb water until the initiation of germination processes, without radicle emergence. The seeds are subsequently naturally dried until they return to their initial moisture content (4 and 22). This enhances the viability of low-vigor seeds (12) and improves germination rates and percentage, uniformity, and speed of germination (11).

Brassinosteroids (BRs) have growth-promoting phytohormones that work actively in controlling cell elongation and distinctive plant growth responses across multiple test systems (21), and have emerged as a crucial group of plant hormones. Brenolide plays diverse physiological roles in promoting growth and influencing developmental processes, including seed germination properties and photosynthesis (9, 10 and 13). Brassinolide enhances the antioxidant capacity of plants, ascorbic acid, and glutathione biosynthesis pathways under various conditions. Enhanced levels of nucleic acids, soluble proteins, and carbohydrates have been associated with improved stress tolerance (14).

According to (29), alfalfa seeds treated with brassinolide exhibited significantly higher germination percentages, and germination and vigor indices compared to untreated seeds in each cultivar. Additionally, seed priming with brassinolide led to a significant increase in shoot fresh and dry weights, and root dry weight in two cultivars, along with a notable increase in root length and root vigor. In addition, the antioxidation of enzymatic activity such as peroxidase, superoxidase dismutase and catalyse increased in Victoria and Victor seedlings after treatment. (27) recorded significant values of germination and seedling vigor when bell pepper seeds were treated with brassinosteroids. Seed priming is a method for enhancing the germination and seedling emergence capabilities of aged sunflower seeds. Understanding the molecular mechanisms involved in the germination and seedling emergence processes of older seeds provides additional insight into the scientific study of sunflowers. It confirms that the role of seed priming in enhancing the germination of aged sunflower seeds and seedling appearance may be intimately linked to the fatty acid, glycometabolism, and ABA (16).

Priming sunflower seeds with plant hormones is an effective treatment for improving the quality parameters of the seeds (28). Some maize seed germination, germination speed length of seedling root and shot, dry matter accumulation and seedling vigor index recorded significant means when the seeds were treated with salicylic acid, brassinolides, and sodium nitroperoxide. This research might provide future reference as to the potential applications of seed priming in increasing aged crop seed vigor. Hence, the aim of this study was to employ varying concentrations of the hormone brassinolide as seed priming to enhance the physiological performance and homogeneity in seedling growth of selected sunflower varieties. This intervention aimed to potentially impact germination and seedling vigor.

Materials and Methods

Experiments were conducted under ambient conditions at the Biology Laboratory, Department of General Sciences, College of Basic Education, Haditha, University of Anbar, Iraq in May 2022 and repeated a year later to confirm the findings on seeds produced in 2021-22. Both experiments followed the same arrangements, utilizing a factorial design in a completely randomized setup, involving two factors. The first factor comprised three sunflower varieties (Ishaqi-1, Aqmar, and Sakha), while the second involved four concentrations of the brassinolide hormone (0, 0.5, 1.0, and 1.5 mg L^{-1}), each with four replications. The objective was to assess the response of these varieties to the hormone in regard to germination and seedling characteristics.

Prior to the experimentations, seed samples from each sunflower variety underwent through mixing for homogeneity. Subsequently, they were soaked for 6 hours in the specified concentrations of brassinolide hormone solution at room temperature (25±3 °C). After soaking, the treated seeds were dried (after washing with tap water) to their original moisture content of 12.5% at room temperature (25 °C).

Laboratory tools and workplaces were sterilized using 99% pure medical alcohol. Samples comprising 400 seeds per treatment were sown in sterile sand culture (0.8 mm diameter) within sterile Petri dishes (14 cm diameter). Four Petri dishes comprising 25 seeds each were arranged closely together, and treated as a replicate of 100 seeds. The seeds were placed in a germination cabinet at 25±0.5 °C and 80%±1 relative humidity for ten days, following the guidelines of the International Seed Testing Rules Association (17).

Studied characteristics:

1. Germination percentage (%): Germination was monitored daily, and the count of normal seedlings in each replicate was conducted at the final assessment (after 10 days from planting). The germination percentage was calculated using the equation (15):

$$\text{Germination \%} = \frac{\text{No. of normal seedlings at the final count}}{\text{No. of total seeds}} \times 100 \qquad (1)$$

2. Root length (cm): The average root length of ten seedlings was randomly selected for each replicate, measured from the seed to the tip of the root, and recorded in centimeters (cm) as the root length at the end of the standard germination test (5).
3. Shoot length (cm): The average shoot length of the ten seedlings was randomly chosen for each replicate and measured from the seed to the tip of the leaf blade at the end of the standard germination test (5).
4. Seedlings dry weight (g): Ten seedlings were randomly selected per replicate, placed in perforated paper bags, and subjected to an electric oven at of 80 °C until reaching constant weight. They were then weighed using a sensitive balance to four decimal places and expressed in grams (5).
5. Seedling vigor index (SVI): Calculated according to the formula suggested by (1):

$$\text{SVI} = \frac{(\text{Radical } + \text{Plumel lengths}) \times \text{Germination } \%}{100} \qquad (2)$$

Statistical analysis: All data underwent statistical analysis as a factorial experiment in a completely randomized design (CRD using the Genstat Computer software program. The least significant of difference (LSD) test was employed to assess differences between treatment means at a 5% probability level.

Results and Discussion

Germination percentage: The results in Table 1 indicate significant differences in the germination percentage among the three sunflower varieties studied in both the 2022 and 2023 experiments. The highest germination percentages were observed in the Sakha variety in 2022 and the Aqmar variety in 2023, reaching 86.25% and 88.75%, respectively. These values contrasted sharply with the Ishaqi-1 variety, which had the lowest germination percentage at 82.75% and 82.83% in the first and second years, respectively. The considerable variation is attributed to genetic differences, aligning with the findings of (3, 7 and 25).

The seed priming technique, employing various concentrations of the brassinolide hormone (0, 0.5, 1.0, and 1.5 mg L^{-1}), significantly influenced germination rates over the two years (Table 1). Seeds treated with the 1.0 mg L^{-1} concentration exhibited the highest rates at 85.67% and 87.56% for the first and second years, respectively. While not significantly different from the seeds treated with 0.5 mg L^{-1}, both concentrations were markedly superior to the control treatment (without seed priming with brassinolide), which recorded the lowest germination rates at 83.33% and 81.67% for the two years, respectively. These results underscore the positive impact of brassinolide on in vitro germination rates. Statistically significant differences were also reported by (18) in the percentage of normal seedlings in the laboratory germination test for seeds treated with brassinolide.

The interaction between sunflower varieties and seed priming techniques, involving different concentrations of the brassinolide hormone, significantly influenced germination rates in both years (Table 1). Notably, the interaction of the Aqmar variety with a brassinolide concentration of 1.0 mg L^{-1} in 2022 and 1.5 mg L^{-1} in 2023 resulted in the highest germination rates of 90.00% and 91.67% in the first and second seasons, respectively. On the other hand, Ishaqi-1 and the control (with no priming) interaction showed the lowest germination rates at 81.67% and 78.33% during first and second seasons, respectively.

Table 1: Germination rates (%) of the sunflower varieties at different concentrations of the brassinolide hormone, 2022 and 2023.

Sunflower varieties	Brassinolide concentrations (mg L^{-1})				Means of varieties
	0	0.5	1.0	1.5	
2022					
Ishaqi-1	81.67	83.33	82.00	84.00	**82.75**
Aqmar	81.67	85.00	90.00	85.00	**85.42**
Sakha	86.67	86.67	85.00	86.67	**86.25**
LSD at 5 %	2.79				**1.39**
Means for brassinolide	**83.33**	**85.00**	**85.67**	**85.22**	
LSD at 5 %	1.61				
2023					
Ishaqi-1	78.33	84.67	84.33	84.00	**82.83**
Aqmar	86.67	86.67	90.00	91.67	**88.75**
Sakha	80.00	86.67	88.33	85.67	**85.17**
LSD at 5 %	3.19				**1.59**
Means for brassinolide	**81.67**	**86.00**	**87.56**	**87.11**	
LSD at 5 %	**1.84**				

Root length: Table 2 shows the mean differences in root length for the 3 sunflower varieties over the two experiment seasons, with Sakha recording the highest at 9.84 and 8.28 cm in 2022 and 2023, respectively. The lowest means were for the Ishaqi-1 at 8.31 cm (2022) and 6.68 cm (2023). Similar findings were reported by (3 and 25). The same table also shows the significant influence of brassinolide concentrations on root length. The 1 mg L^{-1} concentration produced the highest means at 9.68 cm for 2022 and 7.82 cm in 2023, while the control had the lowest means for both seasons at 8.59 cm (2022) and 6.69 cm (2023). The interaction between variety and brassinolide hormone had a significant impact on root length for both seasons, with the highest mean recorded for the Sakha and 1 mg L^{-1} interaction at 10.23 and 8.57, respectively while the lowest was that between Ishaqi-1 and the control.

Table 2: Root length (cm) of the sunflower varieties at different concentrations of the brassinolide hormone, 2022 and 2023.

Sunflower varieties	Brassinolide concentrations (mg L^{-1})				Means of varieties
	0	0.5	1.0	1.5	
2022					
Ishaqi-1	7.50	8.23	8.67	8.83	**8.31**
Aqmar	8.63	9.77	10.13	9.70	**9.56**
Sakha	9.63	9.77	10.23	9.73	**9.84**
LSD at 5 %	0.46				**0.23**
Means for brassinolide	**8.59**	**9.26**	**9.68**	**9.42**	
LSD at 5 %	0.27				
2023					
Ishaqi-1	5.53	7.07	7.13	6.97	**6.68**
Aqmar	6.47	8.10	8.03	7.60	**7.55**
Sakha	8.07	8.30	8.17	8.57	**8.28**
LSD at 5 %	0.62				**0.30**
Means for brassinolide	**6.69**	**7.82**	**7.78**	**7.71**	
LSD at 5 %	**0.36**				

Shoot length: The results in Table 3 demonstrate significant differences between mean shoot lengths of the sunflower varieties in this study, with Sakha registering the highest at 10.17 and 7.81 cm, and Ishaqi-1 the lowest at 7.99 and 7.15 cm for 2022 and 2023, respectively. This is probably related to the variations in embryo activity and genetic factors that affect the response of embryo growth under different conditions (3 and 25). Priming the sunflower seeds with the different concentrations of the brassinolide hormone significantly impacted the characteristic in the first season, with the 1.0 mg L^{-1} concentration showing the highest mean at 9.411 cm, though this was not significantly different from the other concentrations. The differential effect of brassinolide concentrations on different parts of the developing embryo is attributed to its varying impact on embryo vitality. This finding is supported by previous research indicating the significant influence of brassinolides on root and shoot length (9, 10 and 13), and corroborated by (24, 27 and 29).

The interaction between sunflower varieties and seed priming treatment at different brassinolide hormone concentrations significantly affected shoot length in the first year, and vice versa in the second year, as outlined in Table 3. Additionally, the interaction of the Sakha variety and the 0.5 mg L^{-1} brassinolide concentration resulted in the highest average shoot length of 10.63 cm compared to the Ishaqi-1 variety and the control treatment (without seed priming with brassinolide), which gave the lowest average shoot length (7.60 cm) in 2022.

Table 3: Shoot length (cm) of the sunflower varieties at different concentrations of the brassinolide hormone, 2022 and 2023.

Sunflower varieties	Brassinolide concentrations (mg L^{-1})				Means of varieties
	0	0.5	1.0	1.5	
2022					
Ishaqi-1	7.60	7.63	9.03	7.70	**7.99**
Aqmar	7.63	9.40	8.80	8.63	**8.62**
Sakha	9.77	10.63	10.40	9.87	**10.17**
LSD at 5 %		0.45			**0.23**
Means for brassinolide	**8.33**	**9.22**	**9.41**	**8.73**	
LSD at 5 %		0.26			
2023					
Ishaqi-1	6.57	6.97	7.53	7.53	**7.15**
Aqmar	7.37	7.70	7.53	7.53	**7.53**
Sakha	7.50	7.73	8.00	8.00	**7.81**
LSD at 5 %		NS			**0.40**
Means for brassinolide	**7.14**	**7.47**	**7.69**	**7.69**	
LSD at 5 %		**NS**			

Dry weight of seedlings: The dry weight of seedlings is a reliable indicator of their vigor, reflecting their rapid growth in the early stages of plant formation. Seeds that exhibit swift growth produce larger, robust seedlings, directly impacting their dry weight.

The results in Table 4 reveal significant differences in dry weight among the three sunflower varieties for both years. The Sakha variety exhibited the highest average dry weight recording 0.251 g in 2022 and 0.322 g in 2023, while the Ishaqi-1 variety displayed the lowest at 0.227 g, respectively. The superior dry weight of the Sakha seedling variety can be attributed to its excellent germination traits, and root and shoot lengths (Tables 1, 2, and 3), underscoring its exceptional seedling development properties. These findings align with prior research by (3, 7 and 24).

Table 4 illustrates the significant impact of seed priming with the different concentrations of the brassinolide hormone on the dry weight of the sunflower seedlings, particularly in the second year. In the first year, differences did not reach the level of significance. The seeds treated with 1.0 mg L^{-1} brassinolide concentration produced the highest average seedling dry weight of 0.317 g, while those from the control (without brassinolide) yielded the lowest weight at 0.297 g. The enhanced dry weight of the former is attributed to the superior growth of its embryonic parts (Tables 2 and 3), as also noted by (10, 13 and 29).

The interaction between sunflower varieties and seed priming with different brassinolide hormone concentrations significantly affected the dry weight of seedlings in both years. Treating the Sakha variety seeds with 0.5 mg L^{-1} brassinolide concentration in the first year, and the Aqmar variety seeds with 1.5 mg L^{-1} concentration in the second year resulted in their highest dry weight averages of 0.263 g and 0.333 g, respectively. In comparison, the Ishaqi-1 variety seeds in the control treatment (without seed priming with brassinolide) exhibited the lowest weight averages, recording 0.216 g in 2022 and 0.280 g in 2023.

Table 4: Dry weights (g) of the sunflower seedlings at different concentrations of the brassinolide hormone, 2022 and 2023.

Sunflower varieties	Brassinolide concentrations (mg L^{-1})				Means of varieties
	0	0.5	1.0	1.5	
2022					
Ishaqi-1	0.216	0.226	0.246	0.220	**0.227**
Aqmar	0.246	0.246	0.236	0.240	**0.242**
Sakha	0.250	0.263	0.240	0.253	**0.251**
LSD at 5 %	0.017				**0.008**
Means for brassinolide	**0.237**	**0.245**	**0.241**	**0.237**	
LSD at 5 %	NS				
2023					
Ishaqi-1	0.280	0.290	0.306	0.300	**0.294**
Aqmar	0.296	0.316	0.323	0.333	**0.310**
Sakha	0.316	0.316	0.323	0.306	**0.322**
LSD at 5 %	0.012				**0.006**
Means for brassinolide	**0.297**	**0.307**	**0.317**	**0.313**	
LSD at 5 %	**0.007**				

Seedling Vigor Index: The results in Table 5 show significant differences in the means for seedling vigor index (SVI) among the sunflower seedings. The Sakha variety recorded the highest mean at 17.25 and 13.62 while the Ishaqi-1 had the lowest at 13.44 and 11.40 in 2022 and 2023, respectively. The low SVI means for the Ishaqi-1 variety was expected as the other indicators for seedling vigor such as germination percentage, root length, and shoot length (Tables 1, 2, 3) were also low for this variety. These findings are consistent with prior research by (3, 7 and 25).

Table 5 also illustrates the significant effect of seed priming on the SVI for both seasons. Seeds treated with the 1.0 mg L^{-1} concentration showed the highest mean for SVI at 16.34 in 2022 and 13.59 in 2023, outperforming the others, including the control which had the lowest mean index of 14.15 and 11.15, respectively. The superiority of the 1.0 mg L^{-1} brassinolide concentration may be attributed to its positive effects on germination percentage, root length, and shoot length (Tables 1, 2, 3), resulting in an enhanced SVI. This relationship is directly proportional, with the SVI correlating with germination percentage and the lengths of the root and shoot. Similar findings were reported by (7, 24 and 29).

The interaction between the sunflower varieties and seed priming treatments with different brassinolide hormone concentrations significantly affected the SVI in both years. Seeds from the Sakha variety, at 0.5 mg L^{-1} brassinolide concentration in 2022 and 1.0 mg L^{-1} in 2023, achieved the highest averages for the index at 17.67 and 14.29, respectively. In contrast, seeds from the Ishaqi-1 variety without seed priming exhibited the lowest SVI averages at 12.33 in 2022 and 9.48 in 2023.

Table 5: Seedling vigor index averages of the sunflower varieties at different concentrations of the brassinolide hormone, 2022 and 2023.

Sunflower varieties	Brassinolide concentrations (mg L^{-1})				Means of varieties
	0	0.5	1.0	1.5	
2022					
Ishaqi-1	12.33	13.20	14.47	13.76	**13.44**
Aqmar	13.29	16.27	17.03	15.59	**15.54**
Sakha	16.82	17.67	17.54	16.98	**17.25**
LSD at 5 %	0.49				**0.25**
Means for brassinolide	**14.15**	**15.71**	**16.34**	**15.44**	
LSD at 5 %	0.28				
2023					
Ishaqi-1	9.48	11.59	12.47	12.08	**11.40**
Aqmar	11.99	13.69	14.01	13.87	**13.39**
Sakha	12.41	13.90	14.29	13.86	**13.62**
LSD at 5 %	0.45				**0.22**
Means for brassinolide	**11.15**	**13.06**	**13.59**	**13.27**	
LSD at 5 %	**0.26**				

Conclusions

This study showed significant variations in germination percentage and seedling vigor among the three sunflower varieties tested, with the Sakha outperforming the Ishaqi-1 and Aqmar varieties in most germination attributes. Also, priming of the sunflower seeds with 1.0 mg L^{-1} concentration of the brassinolide hormone significantly improved viability and seedling vigor over the 0.0, 0.5, and 1.5 mg L^{-1} concentrations as shown by the standard germination tests and seedling characteristics. Finally, further experiments should be conducted using seed priming technology at different soaking treatments, especially with environmentally friendly plant extracts, to enhance seed performance in terms of homogeneity and germination rates.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

ARM: conceptualization, methodologies, experimental setup, data collection and analysis, writing-original draft preparation; NNM: experimental setup, data collection; DKD: data collection, statistical analysis; SES: conceptualization, writing-review and editing.

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The authors declare no conflict of interest.

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