Effect of Seeding Depth and Abscisic Acid on Barley *Hordeum vulgare* L. Germination, Growth and Seed Yield

Mudhir I. Hwaidi

Lec., Department of Field Crops, College of Agriculture, Tikrit University, Tikrit, Iraq.

Abstract

The field experiment was conducted at field crops research station, college of agriculture/ Tikrit University. The factors of study were seeding depth and abscisic acid. The first factor was seeding depth at 3, 6, and 9 cm. The second was abscisic acid concentrations of 0, 150, 250 and 300 mg L⁻¹. A randomized complete block design (RCBD) with three replications was used. The results showed that a seeding depth of 3 cm recorded the highest means of germination, number of days to seed filling, plant height, number of spikes per m², number of seeds per spike, spike length, 1000 seed weight and seed yield. The means were 91%, 6.31, 85.87 cm, 385.2, 27.38, 13.25 cm, 52.99g and 5.59 ton ha⁻¹. However, the longest coleoptile of 9.21 cm was observed at 9 cm seeding depth. In addition, 300 mg L⁻¹ gave the highest number of days to seed filling, spike length, 1000 seed weight and yield, and means were 41.98 day, 13.96 cm, 50.95 g and 4.20 ton ha⁻¹. Our data revealed that the Interaction between 3 cm and 300 mg L⁻¹ showed the highest means of the number of days to seed filling, spike length, 1000 seed weight and seed yield, and means were 44.58 day, 16.31 cm, 54.17 g and 5.74 t ha⁻¹.

Keywords: Barley, abscisic acid, germination, growth, seed yield.

تأثير عمق البذار وحامض الأبسسك في إنبات ونمو وحاصل بذور الشعير . Hordeum vulgare L

مظهر اسماعيل هويدي

مدرس, قسم المحاصيل الحقلية, كلية الزراعة, جامعة تكريت, تكريت, العراق.

المستخلص

الكلمات المفتاحية: الشعير، حامض الأبسسك، الإنبات، النمو، حاصل البذور .

Introduction

Barley has several unique features among our crop plants. It was one of the earliest plants to be domesticated and continues to play an essential role in modern agriculture today. It is a versatile crop used for human nutrition and animal feed. It plays an essential role as an experimental model plant allowing advances in plant genetics, plant physiology, plant pathology, plant biochemistry, and, more recently, in plant biotechnology (Anderson and Garling, 2000). It influences germination, emergence, establishment and, subsequently, seed production. However, deep seeding leads to poor seed germination. It could redirect the produced energy during germination toward coleoptile elongation instead of supporting radical and plumule growth and development (Aikins et al., 2006; Mohan et al., 2013).

More than 3 cm seeding depth harmed germination, seedling emergence, coleoptile length, and the number of tillers (Hucl and Baker, 1990). Furthermore, Bazzaz et al. (2018) discovered that seedling emergence was greater and quicker at 2 cm when seeds were sown at various depths. Seeding depth was superior in all examined parameters except spike length at 4 cm, followed by 2 cm, and (Hadjichristodoulou et al., 1977) found a decline in the number of tillers as seed depth increased from 2 cm to 10 cm. Mahdi et al. (1998) reported that seeding depths of 3, 9, and 12 cm produced weaker plants than plants seeded at a depth of 6 cm, reducing the number of tillers. Additionally, (Photiades and Hadjichistodoulou, 1984) mentioned that a seeding depth of 5 cm significantly differed from depths of 10, 15, and 20 cm in wheat plant height under dry

circumstances. The findings of Bazzaz et al. (2018) found that sowing depth had a substantial influence on plant height. The tallest plants were grown from seeds planted at a depth of 4 cm, followed by 2 and 6 cm. In comparison, the shortest plants were grown from seeds planted at a depth of 8 cm. Results of Rebetzke et al. (2007) showed a clear relationship between seeding depth and spikes number, with deep planting resulting in fewer spikes per planted area. When seeds were planted at a depth of 4 cm, the most spikes were produced, while fewer spikes were found at a depth of 8 cm (Alam et al., 2014). In addition, a planting depth of 4 cm produced the most seeds per spike, and a depth of 2 cm had no significant difference. While, Yagmur and Kaydan (2009) observed that a 7 cm seeding depth yielded the maximum mean number of seeds in a spike. According to Alam et al, (2014), varied planting depths did not affect spike length, although the tallest spike was recorded at a 4 cm seeding depth and the shortest at 8 cm. In the same regard, the study found that seeding depth substantially influenced the number of seeds per spike, with 4 cm yielding the most significant number. Seeding depths of 2 cm and 8 cm had a direct impact and recorded the maximum 1000 seed weight. Sowing depth directly impacted seed yield, and (Aikins et al., 2006; Mo et al., 2017) found that sowing depths of 2 cm or 8 cm with low soil moisture dramatically lowered seed production. Yagmur and Kaydan (2009) found that sowing seeds deeper than 7 cm diminished seed production considerably, and 5 cm yielded more seeds than 3, 7, and 9 cm. According to Alam et al. (2014), varied seeding depths did not affect spike length,

although the tallest spike was obtained from 4 cm and the smallest from 8 cm. In addition, the same study found that seeding depth substantially influenced the number of seeds per spike, with 4 cm being the greatest and 8 cm being the lowest. From the research conducted by Sandhi (2007), the variation in 1000 seed weight among different planting depths, with increasing seeding depth, resulted in increased seed size.

Abscisic acid was known as a plant growth inhibitor. However, it could have a dual role in plants (Finkelstein et al., 2002). It is a stress hormone that accumulates faster in plants under water shortage than in wellwatered. It induces critical functions of metabolisms that allow plants to survive (Zhang et al., 2006), and its role in modulating morphological, physiological and biochemical processes was proven before in barley (Skowronand and Trojak, 2020) and maize (Ramya et al., 2022) under water stress conditions. The foliar application of abscisic acid at plant enlargement and later on at the anthesis stage with 300 mg L⁻¹ on wheat plants grown under water stress conditions for three consecutive years of experiments with total precipitations of 62, 181 and 201 mm, respectively, increased total seed yield significantly comparing to no spraying (Travaglia et al., 2010). In addition, spraying wheat with 300 mg L⁻¹ abscisic acid throughout plant jointing and anthesis under water shortage circumstances of 181 mm total rain precipitation significantly enhanced the accumulated carbohydrates levels and remobilization. Then there was а considerable rise in 1000 seed weight (Travaglia et al., 2007). In addition, Yang et al. (2013) reported a substantial increase in

wheat seed filling time when abscisic acid was sprayed on wheat leaves during the postanthesis stage under water shortage circumstances of rainfalls, 50.2 mm in 2009 and 43.3 mm in 2010. Furthermore, spraying wheat with 10 mg L⁻¹ abscisic acid improved yield considerably (Yang et al., 2014) and significantly boosted the 1000 seed weight (Nayyar and Walia, 2004; Zhiqing et al., 2011; Bano et al., 2012; Mohammadi et al., 2013). Findings of (Zhiqing et al., 2011) showed that abscisic acid at a concentration of 45 mol L⁻¹ enhanced seed production by 9.81%, and Yang et al. (2014) recorded an overall wheat seed yield of 14%.

The adverse impact of water stress on the plant is widely recognized (Kumari et al., 2018). A few studies examined the effect of exogenous application of abscisic acid in increasing plant tolerance to water deficit situations and using other agricultural elements that may aid in increasing barley seed yield in addition to abscisic acid. Therefore, this research aimed to explore the influence of seeding depth and abscisic acid foliar spray at the anthesis stage on barley germination, growth, and seed production.

Materials and methods

Location and meteorological data

This study was conducted in 2019 at the Department of Field Crops Research Station-College of Agriculture, Tikrit University. (34° 35' 59.99" N, 43° 40' 59.99" E). It was designed to investigate the impact of seeding depth (S) and abscisic acid concentrations (C) at the anthesis stage on the Aksad barley variety under water stress. Samples of soil were extracted at a depth of approximately 30 cm for physical and chemical properties, and results are illustrated in (Table 1). The agricultural practice was performed, and light watering was given until the heading stage. Zadoks decimal codes (Zadoks et al., 1974) were used to determine the growth stages of the planted crop. Urea as a nitrogen source and triple super phosphate was added as recommended. Temperature degrees and rainfalls were obtained from the meteorological organization, and averages of temperature degrees for the months from November to June were 19.5, 17.2, 11, 9.9, 16.3, 22.5, 33.2, and 39.4 C°, respectively. Collected amounts of rain from the experiment field for crop growth were 133.1 mm, and severe water shortage started before the anthesis of about five days.

Properties		Units	Values
РН			745
EC		dS m ⁻¹	25
Available N		mg kg ⁻¹ soil	1950
Available P		mg kg ⁻¹ soil	430
Available K		mg kg ⁻¹ soil	8720
Organic matter		g kg ⁻¹ soil	245
Soil Separators	Sand	g kg ⁻¹ soil	524.00
	Silt	g kg ⁻¹ soil	266.00
	Clay	g kg ⁻¹ soil	210.00
Texture	Sandy loam		

Table 1. Chemical and physical properties of experimental site samples.

Statistical analysis

Two factors were tested in this study. The first factor was seeding depths of 3, 6, and 9 cm, represented with symbols of S_1 , S_2 , and S₃, respectively, and the second factor was Abscisic acid concentrations 0, 150, 250, and 300 mg L^{-1} , represented with symbols of C_0 , C₁, C₂, and C₃, respectively. Concentrations of abscisic acid sprayed in this study were chosen according to the recommendations and used before by Travaglia et al. (2007); Travaglia et al. (2010); Travaglia et al. (2012) on fields such as wheat and maize grown under water stress. The experimental design was (RCBD) with three replications; there were 12 experimental units in each replication. The experimental unit size was 3×3 m. The planting density was about 250 plants in m². Abscisic acid purity of 90% was used in the experiment. The required solutions of desired concentrations were prepared at the laboratory. A mix of distilled water and alcohol was used to dissolve the abscisic acid granules to obtain the required concentrations. Application of the previously prepared concentrations on the plants was at dusk until full wetness and dripping of the solution from the plants.

Collected data were analysed using SAS for F-test to check the significance of factors impacts and their interactions, along with the Duncan test to illustrate the differences between means. Collected data was from the characteristics of Germination %, radical length (cm), plant height (cm), number of active tillers, number of spikes per m⁻², number of days to seed-filling, number of seeds per spike (seed spike⁻¹), spike length (cm), 1000 seed weight (g), and total seed yield (t ha⁻¹). Collected data from germination to spikes number were from all the experimental units, but the statistical analysis was done only for seeding depth's impact.

Results and discussion

Results from ANOVA Table 2 showed a significant impact of seeding depth on all the measures except the number of days to seed filling. The effect of abscisic acid was significant in the number of days to seed filling, spike length, 1000 seed weight and seed yield. The interaction was obvious between study factors, especially in the number of days to seed filling, spike length, 1000 seed weight and seed yield.

Source of variation	R	S	С	S*C	Error
d.f	2	2			4
Germination %	3.63	26.56^{*}			3.14
Coleoptile Length (cm)	0.35	14.4^{**}			0.65
Number of active tillers	0.05	0.98^*			0.111
Plant height (cm)	4.34	38.78^{**}			2.13
Number of spikes (spike m ²)	765	746.34**			85
d.f	2	2	3	6	22
Number of days to seed	35	45.66ns	90.56**	45.35**	15.22
filling(day)					
Number of seeds per pike	1.22	12.34**	2.21ns	1.95ns	1.76
Spike length (cm)	0.07	1.21**	2.33**	1.03^{*}	0.27
1000 seed weight (g)	2.42	12.31**	25.01**	2.57^{*}	0.57
Seed yield (t ha ⁻¹)	0.11	2.32**	0.63**	0.072^{*}	0.017

 Table 2. Analysis of variance means squares for studied characteristics.

Germination and coleoptile length

Results in Table 3 indicated obvious and significant differences between means in germination percentage and coleoptile length. A seeding depth of 3 cm recorded the highest mean of germination, 91%. However, the lowest percentage of germination was obtained from seeds that sown deeply. Coleoptile length significantly increased with increasing seeding depth, and the longest coleoptile was recorded at 9 cm seeding

at 3 cm. Our results were in line with Kirby, (1993) and Bazzaz et al. (2018). However, Bazzaz et al. (2018) and Hadjichristodoulou et al. (1977) indicated different seeding depths for more vigorous germination and seedling. Soil texture, temperature, variety, and a range of other elements all influence germination and emergence. The soil texture of our study was sandy loam (Table 1), whereas the other studies soil textures and structures differed.

depth. However, the shortest coleoptile was

Furthermore, climate change has resulted in rising temperature degrees worldwide and a scarcity of water for irrigation and rainfall, which regulates soil surface mechanical resistance and coleoptile length in some Moreover, advancements places. in agricultural sciences and the production of grade seed hybrids with varying germination and emergence percentages might also be sources of fluctuation in the results. Seed germination initially depends on endosperm reserves for supporting germination and coleoptile elongation. Therefore, when the emergence is delayed, coleoptile trays emerge using high energy, which may aid the seed for successful emergence. This probably was the reason for low germination percentages and long coleoptiles, as indicated by the results of this study.

Growth

The effect of sowing depth on plant height and the number of active tillers was significant, and the highest means were recorded at 3 cm, Table 3. However, the lowest means were recorded at 9 cm. Findings of this study are supported by previous research that found a reduction in the number of tillers and plant height with increasing seeding depth (Mahdi et al., 1998; Photiades and Hadjichistodoulou 1984; Hadjichristodoulou et al., 1977). The decrease in the number of tillers is most likely due to the impact of germination and seedling growth since deep seeding reduces the available energy for the seedling to grow and produce a large number of tillers. Deep planting channelled endosperm stores toward supplying energy for coleoptile elongation, resulting in weak seedlings that did not have more active tillers. In addition to tillers launched from the crown node, there is a tillering known as coleoptile tillering. It is widely known that the jointing stage of the barley plant begins after tillering and concludes at booting as a result of plant development. As demonstrated by our study, the detrimental impact of increased seeding depth on germination and seedling emergence was reflected in plant height.

Seeding	Germination	Coleoptile	Number of	Plant height	Number of
depth (S)	(%)	length (cm)	active tillers	cm	spikes per m ²
\mathbf{S}_1	91 a	3.21 c	6.31 a	85.87 a	385.2 a
S_2	85 b	7.04 b	4.83 b	65.44 b	321.7 b
S_3	66 c	9.21 a	2.51 c	59.85 c	225. 5 c

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Yield components

Results in Table (3) showed an increase in spikes with decreasing seeding depth. The highest number of spikes of 385.2 spike m² was obtained from seeding at 3 cm. However, the lowest mean of 225.5 was obtained from a seeding depth of 9 cm. the Findings of this study were comparable to those previously

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reported by Rebetzke et al. (2007) and Alam

et al. (2014). The number of spikes depends

on the number of active tillers, as is widely

known and previously reported (Bulman and

Hunt, 1988). As a result, planting depth was

the primary factor in reducing the number of

spikes in this study. When seeds are planted deeply, all germination, seedling and plant

growth are affected and causes a kind of weakness in plant growth that decreases the number of tillers and then the number of spikes per unit of area.

Table 4 showed no significant influence of varied planting depths on the number of days to seed filling. Still, there were significant changes in the concentrations of abscisic acid spraying and the interaction. Spraying plants with abscisic acid during anthesis resulted in a considerable increase in seed filling time. Plants sprayed with 300 mg L⁻¹ produced the most significant mean of 41.98 days compared to the control treatment, which had the lowest mean of 33.65 days. The interaction between the two study factors was significant, with the combination of 3 cm and 300 mg L⁻¹ producing the highest mean (44.58 days), while the interaction of 3 cm and abscisic acid control had the lowest mean of 30.15 days. According to the findings of

current and earlier research (Yang et al., 2014; Yang et al., 2013), the acid plays an essential impact in extending the number of days to seed filling and speeding seed filling rate, which may be regarded as an important feature for seed yield, especially under adverse situations such as water stress during reproductive stages. Abscisic acid promotes associated physiological mechanisms that support plants to tolerate water deficit (Ramya et al., 2022; Yue-Xia et al., 2011). Exogenous application of the acid under water stress triggers the action of the D1 protein. It increases its concentration in plant cells via abscisic acid-induced psbA gene expression, which is required to compensate complex functions, for PSII thereby increasing the rate of photosynthesis, seed filling, and seed yield, as demonstrated by the findings of this study.

Abscisic acid		Seeding depth				
	S1	S2	S3	— Means		
C1	30.15 d	36.27 bc	34.55 cd	33.65 c		
C_2	40.17abc	38.33 bc	36.51 cd	38.33 b		
C3	41.14 ab	40.35abc	38.24 bc	39.91 ab		
C_4	44.58 a	40.38 abc	40.98abc	41.98 a		
Means	39.01 a	38.83 a	38.69 a			

Table 4. Effect of seeding depth and abscisic acid on the number of days to seed filling.

As indicated in Table 5, planting depths significantly influenced seeds number per spike. Results illustrated that the highest number of seeds per spike (27.38 seed spike⁻¹) was obtained from 3 cm planting depth, and the lowest number (23.67 seed spike⁻¹) was observed at 9 cm. Our study's results agree to an extent with the result previously found by (Alam et al., 2014). The number of seeds per spike is one of the yield

components that have an enormous impact and relationship in increasing seed yield. This trait is positively correlated to the growth situation of the plant. As indicated here, this study was done under water stress conditions. Abscisic acid has a significant role in eliminating the negative impact of water stress. However, the time of foliar spray of the acid at the anthesis stage did not increase seed number significantly due to the early initiation of seed primordia. Planting depth showed significant changes in the characteristic means, probably due to the maximum benefit from seed endosperm stored energy that was immaculately consumed by the root system because of the suitability of seeding depth.

Abscisic acid		Seeding depth				
	S1	S2	S3	— Means		
C1	26.22 a	25.22 a	25.86 a	25.76 a		
C_2	26.35 a	24.51 a	22.61 a	24.49 a		
C3	27.48 a	25.16 a	23.59 a	25.41 a		
C_4	29.50 a	25.46 a	22.65 a	25.87 a		
Means	27.38 a	25.08 b	23.67 c			

Table 5. Effect of seeding depth and abscisic acid on the number of seeds per spike (seed spike⁻¹).

According to means of spike length, there were significant differences between means under the effect of seeding depth, abscisic acid and interaction. The most extended spike of 13.25 cm was obtained from seeding at 3 cm, but the shortest, 11.40 cm, was at 9 cm. in addition, a spike length of 13.96 cm was recorded at 300 mg L⁻¹. However, the shortest was 11.39 at control. A spike length of 16.31 was obtained from the interaction between

300 mg L⁻¹ and seeding barley seeds at 3 cm. Hence, the shortest was at the interaction of control and 6 cm seeding depth. One of the most critical yield components is the length of the spike. Irfan Ullah et al. (2021) indicate the number of spikelets per spike. However, it might be an early indicator of an increase in another vital feature, such as seed size, which is represented by 1000 seed weight, as demonstrated by the findings of this study.

Abscisic acid		— Means		
	S 1	S2	S3	
C_1	11.54 c	11.32 c	11.33 c	11.39 c
C_2	11.70 c	11.55 c	11.24 c	11.49 c
C_3	13.46 b	11.95 c	11.32 c	12.24 b
C_4	16.31 a	13.85 ab	11.74 c	13.96 a
Means	13.25 a	12.16 b	11.40	

Table 6. Effect of seeding depth and abscisic acid on spike length (cm).

1000 seed weight responded significantly. A seeding depth of 3 cm gave the heaviest seed weight of 52.99 g. However, seeding barley seed deeply at 9 cm gave the lightest weight of 46.95 g. foliar application of ABA at a concentration of 300 mg L^{-1} recorded the

highest mean of 50.95, but the lowest was 48.21 at control, (Table 6). Interaction between the highest concentration and the lowest seeding depth recorded the 54.17 g. Hence, the interaction between 9 cm seeding depth and control gave the lowest mean of

45.64 g. The 1000 seeds weight is an excellent predictor of seed size since the heaviest weight of the same number of seeds signifies larger seeds when compared to the lightest seeds. The findings of this study demonstrated the effect of plant growth phases on this trait to produce seeds. Fast germination, optimal main root and systems (Hucl and Baker, 1990), and plant development contributed considerably to seed filling at 3cm seeding depth. Increasing seed weight at varied planting depths has also been documented in research before (Sandhi 2007; Bazzaz et al., 2018). The findings of our investigation verified a rise in seed weight with increasing ABA content, which was previously reported by Travagila et al. (2007), Travaglia et al. (2010). Navyar and Walia (2004). Zhiqing et al. (2011); Bano et al. (2012), Yang et al. (2014), and Mohammadi et al. (2018) As demonstrated by this study, spraying ABA during the anthesis stage boosted the seed filling rate, which is regulated by endogenous hormones

under water stress. As a result, a significant increase in sink capacity and seed weight was previously discovered by Yang et al. (2003) reported here. Exogenous hormones, like may ABA, raise indigenous ABA concentrations, supporting its regulatory responsibilities in the seed-filling process. Exogenous ABA caused a considerable rise in seed weight in our study. The increase is most likely due to physiological changes during seed filling duration, such as an increase in indigenous levels of zeatin, which is responsible for promoting cell division in the endosperm and then increasing sink capacity for accumulated assimilations, as previously explained by (Yang et al., 2014). Furthermore, changes in tissue structure, such as increasing in the number of vascular bundles and phloem (Travagila et al., 2012) and the development of the spike node located at the neck of it resulted in an increase in the translocated synthesized products from leaves to the under developing seed (Yang et al., 2014).

Abscisic acid		Seeding depth			
	S 1	S2	S3	— Means	
C1	50.79 c	48.21 e	45.64 g	48.21 d	
C_2	52.97 b	49.19 d	46.60 f	49.58 c	
C_3	54.03 a	49.20 d	47.38 ef	50.20 b	
C_4	54.17 a	50.50 c	48.17 e	50.95 a	
Means	52.99 a	49.27 b	46.95 c		

Table 7. Effect of seeding depth and abscisic acid on 1000 seed weight (g).

Table 8 showed that seeding depth, ABA spraying, and interaction had a substantial effect. The most significant seed yield was 5.59 ton ha^{-1,} achieved at 3 cm, whereas the lowest was 2.51ton ha^{-1,} obtained at 9 cm. Foliar spraying with various doses of abscisic

acid also had a significant influence. The maximum seed yield was 4.20 ton ha⁻¹ at 300 mg L⁻¹ and did not differ significantly from 250 mg L⁻¹. The lowest seed yield, however, was 3.74-ton ha⁻¹ at control. The interaction between seeding depth and abscisic acid

concentrations had a considerable influence, where 3 cm and 300 mg L^{-1} produced the highest seed yield of 5.74 ton ha⁻¹whereas 9 cm and control produced the lowest. The yield of seeds is the result of the plant life cycle and reflects the state of plant development from sowing to harvest. Our findings showed substantial variations in the influence of sowing depth, which agreed with the results of (Aikins et al., 2006; Mo et al., 2017; Yagmur and Kaydan, 2009). When seeds are planted at an optimal seeding depth, ideal primary root growth in length and number of branches will be the foundation of a strong crown root. Strong adequate roots allow the plant to absorb moisture and nutrients from different soil profile depths, and excellent plant growth is reflected in components. Under water-stress vield circumstances, seed production improved considerably with increasing the concentration of sprayed abscisic acid in this

study, and comparable results were reported by Travaglia et al. (2007), Travaglia et al. (2010), and Yang et al. (2014). Seed yield components have a significant influence on seed yield. Our study found that raising the concentration of abscisic acid under water stress increased 1000 seed weight, which was attributable to the role of abscisic acid in translocating photosynthetic products from leaves to seeds enhancing seed yield. Abscisic significantly increases acid morphological, physiological, and biochemical processes in plants under water stress (Ramya et al., 2022). Thus, transferring photosynthesis products from leaves and stems to developing seeds is significantly aided by increased seed sink capacity (Travaglia et al. 2010). The current study findings revealed a clear relationship between seed production and 1000 seed weight.

Abscisic acid		— Means		
	S1	S2	S3	
C1	5.35 cd	3.57 f	2.30 f	3.74 c
C_2	5.56 b	3.92 de	2.36 f	3.94 b
C_3	5.72 a	3.99 cde	2.66 e	4.12 a
C_4	5.74 a	4.15 bc	2.72 d e	4.20 a
Means	5.59 a	3.91 b	2.51 c	

Table 8. Effect of seeding depth and abscisic acid on seed yield (ton ha⁻¹).

Conclusion

The conclusion of our study can reveal that the sowing depth is one of the main parameters that might mitigate the negative impact of water stress. Selecting a suitable depth significantly improves barley plant germination, emergence, growth, and seed yield. Furthermore, abscisic acid application at the anthesis stage enhances barley plant tolerance to water stress, seed yield components, and seed yield. Moreover, the relationship between planting depth and abscisic acid indicates that using agricultural field practices in conjunction with the abscisic acid application may boost barley tolerance to water stress.

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