

EFFECTS OF GA₃ AND ABA ON THE GERMINATION OF DORMANT OAT (*AVENA SATIVA* L.) SEEDS

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Abstract. The dormancy characteristics of oat seed that can germinate after one season or one year are used to build and maintain vegetation to protect soils from desertification in Northern China. The aim of this study was to estimate the effects of endogenous and exogenous GA₃ and ABA on oat seed (*Avena sativa* L. BaiYan 7) germination. The results showed that seed without peel hull had lower endogenous ABA content and the ratio of ABA/GA₃ than seeds with peel hull. The best GA₃ treatment duration for milky ripe, wax ripe, full ripe seeds were 60 min (m) or 120 m, 60 m and 30 m, respectively. Seed germination rate, germination potential and germination index all increased then decreased with the increasing of GA₃ concentrations. The best GA₃ concentration treatment was 100 mg L⁻¹, while the turning point was 200 mg L⁻¹. The dormancy rate of low temperature storage seeds was higher than those stored in room temperature at each storage time, and both decreased with the increase of the storage time. New seeds or stored for 1-2 months, had significantly enhanced germination rate by exogenous GA₃. GA₃ treatment had no effect on germination rate for the seeds that had been stored for over 3 months. Germination rate decreased with the increase of ABA concentrations. The most inhibitive effect, which lead to reduction of seed germination by 37.7% and 4.0%, when the concentration of ABA was 500 mg L⁻¹ and 1000 mg L⁻¹, respectively. GA₃ could decrease the inhibition effect of ABA on seed germination.

Keywords: oat, germination rate, gibberellic acid (GA₃), abscisic acid (ABA) endogenous, exogenous

Introduction

China's desertification area is about 1.74 million km², accounting for 18% of the total land area, and the area is going to increase in the future (Islam et al., 2011; Cheng et al., 2018). To solve this problem, it is important to build and maintain plant biology to increase surface coverage and restore native vegetation, which is the main reason why Chinese government has set up China Agriculture Research System to support the production and study of oat (Lin et al., 2012; Qian et al., 2018). Oat (*Avena sativa* L.) is a annual herbaceous plant belonging in gramineous precocious subfamily. It has strong ability to resist wind erosion and easy to sow, and easy to build plant (Lin et al., 2012; Zang et al., 2018). Oat can be cultivated in the marginal land with less water and fertilizer requirement so well adapted in desert area (Ren et al., 2007; Rabiei et al., 2012; Zang et al., 2015; Khan et al., 2019). Li et al. (2009) proved the strong anti-erosion ability of oat than corn, sunflower and mung bean, which will help to prevent farmland from desertification. "Baiyan7", breed by Baicheng Academy of Agricultural Sciences, has dormancy characteristics. Some other researches showed the dormancy strength of "Baiyan 7" can affect the vegetation establishment. Now the studies of oat

planting have focused on breeding, cultivation, physiological and biochemical aspects, oat seed science research focuses on the germination optimum temperature, germination substrate, salinity stress and seed storage, but the researches about the effect of phytohormones on germination of dormant oat are quite few. It is an innate property of seed that defines the environmental conditions in which the seed is able to germinate. It is determined by genetics with a substantial environmental influence which is mediated, at least in part, by the plant hormones abscisic acid and gibberellins (Finch-Savage and Leubne, 2006). Many previous studies show that GA₃ is the main regulator on seed germination and it could lift the bud and seed dormancy and promote bud instead of light or low temperature (Mukhtar and Singh, 2006; Ozkaya et al., 2006; Liu et al., 2016). Other study also found that for the germination of brown rice seed, in addition to water and a certain temperature, GA₃ was a promoting substance of seed germination (Damaris et al., 2019). ABA is a phytohormone which has been shown to be involved in a wide range of plant physiology (Wilkinson and Davies, 2010; Zhang et al., 2017). ABA could inhibit seed germination requirement concentration varies.

Therefore, we aims to 1) estimate the best GA₃ concentration and treat time for germination of oat seed with different maturity; 2) evaluate the oat seed germination as affected by ABA input; 3) test the combination effect of GA₃ and ABA for oat seed germination.

Materials and methods

Study site and materials

The experiment was conducted at Baicheng Academy of Agricultural Sciences, Baicheng, Jilin province, China (45° 37'N, 122° 48'E, 152 m elevation). The detail information of experiment sites could be found in Zang et al., 2018 and Qian et al., 2018. Bai Yan 7 oat (*Avena sativa* L.) seed used in this experiment supplied by the Baicheng Academy of Agricultural Sciences. Part of the seeds collected immediately for germination test, and other stored at room temperature under dry conditions or at 4 °C refrigerator for later use.

Experimental design

a) The effects of GA₃ treat time on germination of oat seed with different maturity

Each different matured oat seed were soaked in 100 mg L⁻¹ GA₃ for different times in order to test the germination. The treatments time were T1 = 0 m, T2 = 30 m, T3 = 60 m, T4 = 120 m, and T5 = 240 m, different maturity were S1 = milky ripe, S2 = wax ripe, and S3 = full ripe. So the experiment had 15 treatment combinations, with three biological replications, and each replicates have 200 seeds. First, the seeds soaked and disinfected in 4.0% sodium hypochlorite solution for 30 min, then washed 5-6 times with distilled water. Second, tweezers were used to put the seeds in Petri dishes lined with filter paper, each plate contained 200 seeds and were kept at sufficient distance. Third, the Petri dishes were placed in an incubator maintaining temperature 20 °C, distilled water was used to keep the filter paper moist, sprout numbers in each treatment were recorded every morning, for 10 days, and the whole process were repeated 3 times. (These materials and methods are applicable to the following experiments.)

b) The effects of GA₃ concentrations on wax ripe seed germination

The wax ripe seeds were soaked in different GA₃ concentrations for 120 min, and then test the germination. Six treatments were established with increasing GA₃ concentrations as 0, 12.5, 25, 50, 100, and 200 mg L⁻¹ (i.e. A1, A2, A3, A4, A5, and A6, respectively).

c) The effects of GA₃ on germination of oat seed in different maturity with or without peel

Different maturities were cream ripe, wax ripe and full ripe. Each maturity seeds had four treatments: B1 = seeds with peel, B2 = seeds without peel, B3 = unpeeled seeds soaked in GA₃ for 2 h, B4 = peeled seeds soaked in GA₃ for 2 h.

d) The effects of GA₃ on germination of different matured oat seeds under low or room temperature

Oat seeds with different maturity were stored under low or room temperature. Different maturities were cream ripe, wax ripe and full ripe. Each kind of seeds had five storage durations: 0, 1, 2, 3, and 4 months (i.e. C1, C2, C3, C4, and C5).

e) The interaction effect of GA₃ and ABA on germination of oat seed

GA₃ and ABA were dissolved in a small amount of ethanol, constant volume with distilled water, GA₃ preparation of 100 mg L⁻¹, the ABA 1000 mg L⁻¹ as stock solution, respectively, using the GA₃ liquid and different concentrations of ABA dilution to deal with the sterilized seed, soak 30 min, remove seeds, and dry with filter paper, then do germination test. For GA₃ and ABA application, first the seed soak with different concentrations of ABA solution 30 min, after that soak with GA₃ solution for 30 min, and then start germination test. This experiment has nine treatments: D1 = 1000 mg L⁻¹ ABA; D2 = 500 mg L⁻¹ ABA; D3 = 250 mg L⁻¹ ABA; D4 = 100 mg L⁻¹ ABA; D5 = 100 mg L⁻¹ GA₃ + 100 mg L⁻¹ ABA; D6 = 100 mg L⁻¹ GA₃ + 250 mg L⁻¹ ABA; D7 = 100 mg L⁻¹ GA₃ + 500 mg L⁻¹ ABA; D8 = 100 mg L⁻¹ GA₃ + 1000 mg L⁻¹ ABA; CK use distilled water as control.

The detail experimental design of all experiment in present study also have been summarised as *Table A1* in the *Appendix*.

Determination indexes and methods

a) Seed collection standard

Oat seed development process was divided into three maturity phase. Full ripe: glumes white and open, the appearance of seed is yellowish-white, hard; wax ripe: glumes the shallow semi-open, the appearance of seed is yellow-green, slightly harder, volume reach mature state; milk ripe: glumes green and closed, the appearance of seed is green, tender, the volume does not reach mature state.

b) Determination of germination rate, germination potential, germinating, germination index and T50

Test method reference to the international seed testing and GB/T2930.4-2001.

Germinating refers to the ratio of the sum of the maximum number of germination within three days and total number of germination.

T₅₀ refers to seed germination rate of the time required in half of the final germination.

$$\text{Germination rate (\%)} = (n / N) \times 100 \quad (\text{Eq.1})$$

In this equation n refers to the seed within the specified time normal germination accumulated grains, N refers to the total number of tested seeds.

$$\text{Germination potential (\%)} = (A / N) \times 100 \quad (\text{Eq.2})$$

In *Equation 2*, “A” refers to the cumulative germination rate of 3 d before the test.

$$\text{Germination index} = \Sigma Gt / Dt \quad (\text{Eq.3})$$

In *Equation 3*, “Gt” refers to the number of germination of the time t, “Dt” refers to the germination days.

Statistical analyses

Experimental raw data use the Excel (2007 version) statistical software to collate, then use both SAS (8.0 version) and Mstate-C statistical software to analyse. A factorial layout within randomized complete block design with 3 replications was used to analysis the variation of GA₃ processing time effect on germination characteristics of seeds in different maturity. Seed maturity were milky ripe seed, wax ripe seed and full ripe seed. GA₃ processing times were included 0, 30, 60, 120 and 240 min. In order to determine the influence of GA₃ concentration on different experimental characteristics, a randomized complete block design with three replications were used. GA₃ concentrations were 0, 12.5, 25, 50, 100 and 200 mg L⁻¹.

Results and discussion

As shown in *Table 1*, fully ripe seeds with or without peel, had significant differences ($p < 0.05$) in endogenous GA₃, ABA and GA₃/ABA at different storage periods. Endogenous GA₃ in fully ripe seeds without peel in each storage period were significantly higher than those with peel ($p < 0.05$). With the extension of storage time, endogenous GA₃ in full ripe seeds without peel were increased by 68.7%, 121.4%, 59.2%, 18.2%, and 29.0%, respectively when compared with seeds with peel. GA₃ contents showed a tremendous increasing trend with the storage time. Endogenous ABA in full ripe seeds with peel in each storage period was significantly higher than that in the seeds without peel ($p < 0.05$), with the extension of storage time, endogenous ABA in full ripe seed with peel than seed without peel were increased by 8.4%, 49.0%, 37.7%, 74.5%, and 36.7%, respectively, which means that it contains a certain amount of ABA within the peel. The ratio of GA₃/ABA in full ripe seeds without peel in each storage period was significantly higher than that in the seeds with peel ($p < 0.05$), with the extension of storage time, the ratio of GA₃/ABA in full ripe seeds without peel than seed with peel were increased by 82.8%, 229.9%, 119.2%, 106.2%, and 76.3%, respectively (*Table 1*).

Table 1. Full ripe seed phytohormones content at different storage periods with or without peel

Treatment	Determination index	Storage time (month)				
		0	1	2	3	4
With peel	GA ₃ (ng/g.FW)	9.25d	5.24e	11.50c	19.18b	39.95a
Without peel		15.61d	11.60e	18.31c	22.67b	52.52a
With peel	ABA (ng/g.FW)	146.18c	137.41e	148.42b	169.33a	138.01d
Without peel		134.91a	92.23e	107.81b	97.03d	100.94c
With peel	GA ₃ /ABA	0.06d	0.04e	0.08c	0.11b	0.29a
Without peel		0.12d	0.13d	0.17c	0.23b	0.51a

Different letters within a row indicate significant differences between the mean ($p < 0.05$)

Table 2 showed that, wax ripe seed with or without peel, have significant differences ($p < 0.05$) in endogenous GA₃, ABA and GA₃/ABA at different storage periods. GA₃ content in wax ripe seed which was stored for three months was significantly lower in the seed without peel than seed with peel ($p < 0.05$), the rest are significantly higher than wax ripe seed with peel ($p < 0.05$), with the storage time of 0 m (month), 1 m, 2 m, 4 m, and endogenous GA₃ in wax ripe seed without peel than seed with peel, the increase by 67.7%, 15.5%, 80.1%, and 59.9% was seen. Endogenous ABA in wax ripe seed with peel in each storage period are significantly higher than that without peel ($p < 0.05$), with the extension of storage time, endogenous ABA in wax ripe seed with peel than seed without peel were increased by 55.6%, 152.8%, 165.9%, 270.9%, and 72.9%, respectively; this means that it contains a certain amount of ABA within the peel. The ratio of GA₃/ABA in wax ripe seed without peel in each storage period are significantly higher than seed with peel ($p < 0.05$). Moreover, with the extension of storage time, the ratio of GA₃/ABA in full ripe seed without peel than seed with peel were increased by 161.0%, 191.9%, 378.8%, 209.9%, 176.3% (Table 2).

Table 2. Wax ripe seed phytohormones content of the different storage period with or without peel

Treatment	Determination index	Storage time (month)				
		0	1	2	3	4
With peel	GA ₃ (ng/g.FW)	19.79c	20.10c	16.45d	23.12b	37.09a
Without peel		33.20b	23.20d	29.63c	19.32e	59.29a
With peel	ABA (ng/g.FW)	223.03a	158.33d	169.16c	202.80b	131.84e
Without peel		143.37a	62.63d	63.62c	54.67e	76.27b
With peel	GA ₃ /ABA	0.09d	0.13b	0.10cd	0.11bc	0.28a
Without peel		0.23d	0.37c	0.47b	0.35c	0.78a

Different letters within a row indicate significant differences between the mean ($p < 0.05$)

Table 3 showed that, milky ripe seed with peel or without peel, the endogenous GA₃, ABA and GA₃/ABA at different storage periods have significant differences ($p < 0.05$). Milky ripe seed without peel GA₃ content in addition to storage for four month had no significant with seed with peel ($p < 0.05$), the rest were significantly higher than seed

with peel ($p < 0.05$), with the storage time of 0 m (month), 1 m, 2 m, 3 m, endogenous GA₃ in milky ripe seed without peel than seed with peel were increased by 44.0%, 37.7%, 76.9%, and 25.7%, respectively; the endogenous ABA in milky ripe seed with peel in each storage periods are significantly higher than seed without peel ($p < 0.05$), with the extension of storage time, endogenous ABA in milky ripe seed with peel than seed without peel were increased by 38.9%, 18.8, 162.2, 107.9, 71.3%, this means it contain a certain amount of ABA within the peel. Milky ripe seed without peel the ratio of GA₃/ABA in addition to storage for 0 month had no significant difference with the seed with peel ($p < 0.05$), the rest were significant ($p < 0.05$); with the storage time of 2 m (month), 3 m, 4 m, and the ratio of GA₃/ABA in milky ripe seed without peel than seed with peel were increased by 48.2%, 65.4%, and 73.6%, respectively (Table 3).

Table 3. Milky ripe seed phytohormones content of the different storage period with or without peel

Treatment	Determination index	Storage time (month)				
		0	1	2	3	4
With peel	GA ₃ (ng/g.FW)	8.62e	10.53d	16.15c	25.35b	55.07a
Without peel		5.99e	7.65d	9.13c	20.17b	55.82a
With peel	ABA (ng/g.FW)	179.97c	154.57e	211.17a	174.54d	189.59b
Without peel		129.59a	130.10a	80.53d	83.94c	110.70b
With peel	GA ₃ /ABA	0.05d	0.07c	0.08c	0.15b	0.29a
Without peel		0.05d	0.06d	0.11c	0.24b	0.50a

Different letters within a row indicate significant differences between the mean ($p < 0.05$)

Seed maturity has significant influence on germination potential, germination rate, germination index and T₅₀(d). Uniformity was not significantly influenced by seed maturity. Germination potential, germination rate and germination index were significantly affected by GA₃ processing time; however, this effect on uniformity and T₅₀(d). Like, seed maturity, the interaction between seed maturity and GA₃ processing time has significant effect on all experimental characteristics expect uniformity (Table 4).

Table 4. Analysis variance the effect of GA₃ processing time on germination characteristics of seeds in different maturity

S.O.V	d.f.	Germination potential (%)	Germination rate (%)	Germination index	Uniformity	T ₅₀ (d)
Seed maturity (S)	2	0.573**	0.569**	3296.65**	0.002	1.622**
GA ₃ processing time (T)	4	0.041**	0.039**	166.62**	0.008 ^{ns}	0.222 ^{ns}
S×T	8	0.31**	0.028**	94.07*	0.004	0.122 ^{ns}
Error	28	0.008	0.007	29.173	0.005	0.094

ns: non significant; *significant at 0.05 significance in F-tests; **significant at 0.001 significance in F-tests

The highest germination potential was related to full ripe seed and the lowest one was obtained by milky ripe seed, there was no significant difference between wax and

full ripe seed, but both of them have significant differences with milky ripe seed. The maximum germination rate and germination index also achieved in full ripe seeds. No significant differences were found in these two experimental traits between milky and ripe seed, but both of them had significant differences with full ripe seed. There were no significant differences among milky ripe seed, wax ripe seed and full ripe seed. Wax ripe seed has obtained the highest T₅₀, but its difference with full ripe seed was not significant. However, not only wax ripe seed, but also full ripe seed had significant difference with milky seed maturity. The maximum germination potential and germination rate was occurred in 120 and 60 min GA₃ processing time, respectively. 120 min GA₃ processing time had obtained the maximum germination index which just had significant differences with control treatment (0 min). There were no significant differences among treatments in uniformity index, furthermore, the maximum one was obtained by control treatment (0 min). On the one hand, the highest T₅₀ was related to control treatment; on the other hand, the lowest one was obtained by 60, 120 and 240 min. Moreover, there were no significant differences among treatments. The results show that seeds immersed for 60 m by GA₃ had best effect to promote germination to ripe seeds, and they inhibited germination when immersed for 240 m. The maximum germination potential and germination rate was related to interaction between full ripe seed and 30 min of GA₃ processing time, and the highest germination index was achieved in full ripe seed and 120 min GA₃ processing time interaction. There were no significant differences among interaction traits in uniformity. Both, interaction between milky ripe seed and control treatment in processing time, interaction between milky ripe seed and 30 min of GA₃ processing time had obtained the highest T₅₀, which had significant differences with all other interaction (*Table 5*).

Table 5. Mean comparison for germination characteristics

Treatment	Germination potential (%)	Germination rate (%)	Germination index	Uniformity	T ₅₀ (d)
Seed maturity (S)					
Milky ripe seed (S1)	38.22b	35.47b	21.37b	0.9647a	3.60a
Wax ripe seed (S2)	49.78a	34.67b	21.72b	0.9520a	3.06b
Full ripe seed (S3)	53.33a	68.80a	47.22a	0.9767a	3.00b
GA ₃ processing time (m) (T)					
0 (T1)	38c	45c	23.62b	1.00a	3.33ab
30 (T2)	49ab	52c	29.65a	0.95a	3.44a
60 (T3)	53a	53a	33.58a	0.99a	3.11b
120 (T4)	54a	43d	34.45a	0.94a	3.11b
240 (T5)	44bc	28b	29.20a	0.93a	3.11b
Seed maturity × GA ₃ processing time (S × T)					
S1T1	24h	22h	12.53e	1.00a	4.00a
S1T2	29h	24gh	15.27de	1.00a	4.00a
S1T3	49defg	46def	28.31c	0.97a	3.33b
S1T4	52def	50cde	29.25c	0.96a	3.33b
S1T5	37fgh	33fgh	21.49cde	0.89a	3.33b
S2T1	21h	21h	13.33e	1.00a	3.00b

S2T2	36fgh	34fgh	21.17cde	0.93a	3.33b
S2T3	46efg	45def	28.44c	1.00a	3.00b
S2T4	33gh	33fgh	20.64cde	0.91a	3.00b
S2T5	37fgh	38efg	25.00cd	0.90a	3.00b
S3T1	69abc	68ab	45.00ab	1.00a	3.00b
S3T2	84a	78a	52.52a	0.92a	3.00b
S3T3	64bcd	64abc	44.00ab	1.00a	3.00b
S3T4	77ab	76a	53.46a	0.96a	3.00b
S3T5	57cde	57bcd	41.11b	1.00a	3.00b

Means with common letters within each column do not differ significantly. The effect of seed maturity, GA₃ processing time, and their interaction were evaluated. The treatments for seed maturity were milky ripe seed (S1), wax ripe seed (S2), and full ripe seed (S3). The treatments for GA₃ processing time were 0 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 m (T5)

Germination potential has positive and significant correlation with germination rate and germination index, which means that with increase of germination potential, germination rate increase significantly. However, germination potential has negative and significant correlation with T₅₀ and non-significant positive correlation with uniformity. The positive significant correlation was found between germination rate and germination index. T₅₀ also had negative and significant correlation with both germination rate and germination index. Furthermore, the correlation between uniformity and T₅₀ was positive, but it was not significant (*Table 6*). GA₃ concentration had significant influence on germination potential, germination rate and germination index, but uniformity and T₅₀ were not affected by it (*Table 7*).

Table 6. Simple correlation among experimental characteristics in different seed maturity and GA₃ processing time

Traits	Germination potential	Germination rate	Germination index	Uniformity	T ₅₀
Germination potential	1				
Germination rate	0.988**	1			
Germination index	0.978**	0.987**	1		
Uniformity	0.009 ^{ns}	0.060 ^{ns}	0.063 ^{ns}	1	
T ₅₀	-0.439**	-0.498**	-0.510**	0.004 ^{ns}	1

ns: non significant; *significant at 0.05 significance in F-tests; **significant at 0.001 significance in F-tests

Table 7. Analysis of variance for the influence of different GA₃ concentrations on wax ripe seed germination

S.O.V	d.f.	Germination potential	Germination rate	Germination index	Uniformity	T ₅₀
Replication	2	0.006	0.006	16.98	0.012 ^{ns}	0.056
GA ₃ concentrations	5	0.055**	0.088**	195.77**	0.022 ^{ns}	0.489 ^{ns}
Error	10	0.008	0.007	24.89	0.008	0.182

ns, non significant; *significant at 0.05 significance in F-tests; **significant at 0.001 significance in F-tests

The highest germination potential and germination rate was related to 100 mg L⁻¹ GA₃ concentration, which had significant differences with all treatments, except 200 mg L⁻¹ in both experimental traits. The highest and the lowest germination rate were achieved in 100 mg L⁻¹ and control treatment (0 mg L⁻¹) GA₃ concentration, which had significant difference with each other. 100 GA₃ concentration had obtained the maximum germination index, which had significant differences with 0 mg L⁻¹ and 12.5 mg L⁻¹. There were no significant differences among treatments in uniformity. The highest T₅₀ was related to 50 mg L⁻¹ and 100 mg L⁻¹, respectively. Like uniformity, no significant difference was found among treatments (*Table 8*).

Table 8. Mean comparison for experimental characteristics of wax ripe seed germination in different GA₃ concentration

Treatment	Germination potential (%)	Germination rate (%)	Germination index	Uniformity	T ₅₀ (d)
GA ₃ concentration (mg L ⁻¹)					
0	21.3d	21.3c	13.33c	1.00a	3.00a
12.5	26.7cd	26.7c	15.99bc	1.00a	3.33ab
25	37.3bcd	49.3b	25.75a	0.82a	3.33ab
50	41.3abc	49.3b	25.01ab	0.85a	4.00a
100	57.3a	66.7a	33.98a	0.84a	4.00a
200	49.3ab	53.3ab	30.09a	0.82a	3.66ab

Means with common letters within each column do not differ significantly

Influence of different maturity seed germination by dealing with GA₃

The results showed that, after manual peel out seed, the immersed with GA₃, seeds in different maturity had significantly improved germination rate, germination energy and germination index, which had significant difference with other treatments. Manual removal treatment (B2) and GA₃ treatment (B3) had significant difference with B1 in germination rate. Milky ripe seeds had significant differences in germination potential by dealing with GA₃; seeds treated by B4 were significantly higher than other treatments in germination rate, there was no significant difference between B2 and B3, but also it was significantly higher than B1; seeds treated by B4 was significantly higher than other treatments in germination index, when others have no significant difference; T₅₀ of B4 and B3 is shortened one day than B2 and B1. B4 had no significant difference with B3 in the germination potential of wax ripe seed, but it was significantly higher than B2 and B1; furthermore, there was no significant difference between B3 and B2, but B3 was significantly higher than B1. There was no significant difference between B2 and B1; seed treated by B4 was significantly higher than other treatments in germination rate, there was no significant difference between B2 and B3, but they were significantly higher than B1. Seed treated by B4 was significantly higher than other treatments in germination rate, furthermore, B2 was significantly higher than B3 and B1 in germination index, and there was no significant difference between B3 and B1; T₅₀ of B4 and B3 was shortened one day than B2 and B1. Full ripe seed had significant difference in germination potential. Full ripe seed had significant difference in germination rate, there was no significant difference between B4 and B1, but all of them were significantly higher than other treatments; T₅₀ of B4 and B3 is shortened one day than B2 and B1 (*Table 9*).

Table 9. Influence of different maturity seed germination by dealing with GA₃

Provenances	treatments			
	B1	B2	B3	B4
Milky ripe				
Germination rate (%)	0.0d	2.5c	3.2b	3.7a
Germination potential (%)	40.7c	56.0b	58.7b	65.3a
Germination index	13.3b	13.4b	12.9b	22.1a
T ₅₀ (d)	8	8	7	7
Geminating	0.9	1.0	1.0	0.8
Wax ripe				
Germination rate (%)	2.7c	3.0bc	3.5ab	4.0a
Germination potential (%)	42.7c	65.3b	67.3b	80.0a
Germination index	11.5c	16.8b	13.2c	22.8a
T ₅₀ (d)	8	8	7	7
Geminating	0.8	0.9	0.9	0.8
Full ripe				
Germination rate (%)	6.7d	8.3c	15.3b	22.7a
Germination potential (%)	75.7d	77.3c	79.3b	92.0a
Germination index	28.1a	20.6b	16.1c	29.6a
T ₅₀ (d)	7	7	6	6

Different letters within a row indicate significant differences between the mean ($p < 0.05$)

Influence of different maturity seed germination under low temperature and room temperature storage conditions by dealing with GA₃

The results showed that using GA₃ treatment to harvest and storage one month cream ripe seed at room temperature, the germination rate were higher than comparison results, the difference reached significant level ($p < 0.05$), increasing 8 and 5.1 percentage points; using GA₃ treatment at room temperature to storage for two months, three months and four months, seed germination rate compared to the comparison was not significant. Storage at room temperature for three and four months, seed germination rate was lower than the comparison, reduced by 2 and 3 percentage points. Germination rate of the new harvest, cold storage for one and two months milk ripe seed treated by GA₃, was higher than the comparison, the difference reached significant level ($p < 0.05$), increasing 8, 6.9 and 5.5 percentage points, respectively; germination rate of cold storage two and three months milky ripe seed treated by GA₃ was lower than the comparison for 4%, the difference was not significant (Table 10).

Table 10. Influence of milky ripe seed germination under room temperature and 0 °C storage conditions by dealing with GA₃

Treatment	Germination rate (%)				
	0 month	1 month	2 month	3 month	4 month
Room temperature and no GA ₃	11e	35.1d	46.7c	55b	73a
Room temperature and GA ₃	19d	40.2c	50.3b	53b	70a
0 °C and no GA ₃	11e	30.4d	40.2c	50b	69a
0 °C and GA ₃	19e	37.3d	45.7c	51b	65a

Different letters within a row indicate significant differences between the mean ($p < 0.05$)

Using GA₃ treatment to newly harvested and stored one month wax ripe seeds at room temperature, the germination rate was higher than comparison results, the difference reached significant level ($p < 0.05$), increasing by 10 and 5.5 percent; Using GA₃ treatment at room temperature and stored for two, three and four months, seed germination rate compared to the comparison was not significant, storage at room temperature for four months, seed germination rate was lower than the comparison, reducing it by 3.5%. Germination rate of the new harvest, cold storage for one and two months wax ripe seeds treated with GA₃ was higher than the comparison, the difference reached significant level, increasing by 10, 7.1 and 9.6%. Germination rate of cold storage two and three months wax ripe seeds treated by GA₃ was lower than the comparison by 2%, the difference was not significant (*Table 11*).

Table 11. Influence of full ripe seed germination under room temperature and 0 °C storage conditions by dealing with GA₃

Treatment	Germination rate (%)				
	0 month	1 month	2 month	3 month	4 month
Room temperature and no GA ₃	26.3d	50.8c	82.2b	92.1a	90.3a
Room temperature and GA ₃	43.2c	78.5b	90.5a	90.2a	91.6a
0 °C and no GA ₃	26.3e	45.6d	72c	82b	88a
0 °C and GA ₃	43.2d	65.6c	83b	80ab	85a

Different letters within a row indicate significant differences between the mean ($p < 0.05$)

Using GA₃ treatment to harvest and storage one and two month fully mature seeds at room temperature, the germination rate was both higher than comparison results, the difference reached extremely significant level ($p < 0.01$), increasing 16.9, 27.7 and 8.3%; using GA₃ treatment at room temperature to storage for three and four months, seed germination rate compared to the comparison was not significant ($p > 0.05$), storage at room temperature for three months, seed germination rate was lower than the comparison reducing it by 1.9 percentage points; germination rate of the new harvest, cold storage a month and two months fully mature seeds treated by GA₃ had higher than the comparison, the difference reached significant level ($p < 0.05$), increasing by 16.9, 20 and 9%, respectively; germination rate of cold storage three and four months fully mature seeds treated by GA₃ was lower than the comparison by 2% and 3%, according to storage period for the sequence, the difference was not significant (*Table 12*).

Table 12. Influence of wax ripe seed germination under room temperature and 0 °C storage conditions by dealing with GA₃

Treatment	Germination rate (%)				
	0 month	1 month	2 month	3 month	4 month
Room temperature and no GA ₃	14.4e	50.3d	69.3c	80b	85.5a
Room temperature and GA ₃	24.4d	55.8c	73.3b	81a	82a
0 °C and no GA ₃	14.4e	45d	60.7c	73b	82a
0 °C and GA ₃	24.4e	52.1d	70.3c	74b	80a

Different letters within a row indicate significant differences between the mean ($p < 0.05$)

The results of this experiment showed that the GA₃ treatment promote seed storage at room temperature or low temperature seed germination, especially for two months, but not for three or more months or even inhibition.

GA₃ and ABA interaction effects on seed germination

The test results showed the significant differences, in the germination rate of each treatment compared with CK, D5, D6, D7 ($p < 0.01$); the D8 germination rate was significantly higher than that of D4, D3, D2, D1, ($p < 0.05$). D1 and D2 were the most important treatment which inhibited seed germination compare to other treatments, seed germination in D3 and D4 were 36.0% and 24.0%, respectively; under the same concentration of ABA, the seed germination rate of ABA and GA₃ interaction treatment was higher than ABA treatment, but it was still lower than CK levels. These results suggest that ABA inhibits seed germination, and inhibition increased with the increase trend of ABA concentration; furthermore GA₃ can alleviate the inhibitory effect of ABA on seed germination and alleviating margin of D1, D2 with GA₃ is larger than the D3, D4 (*Fig. 1*).

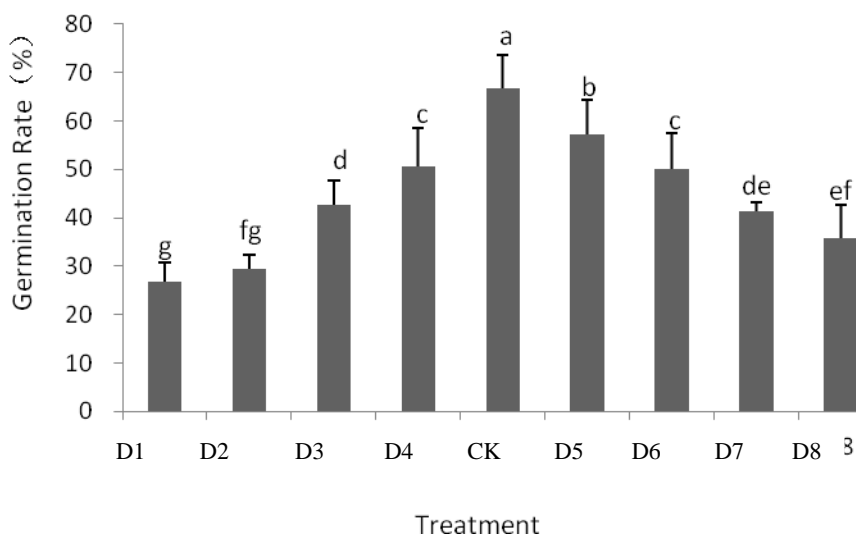


Figure 1. GA₃ and ABA interaction effects on seed germination

The results of this experiment showed that the endogenous GA₃ content of each maturity degree oat seed increased with the storage period, while endogenous ABA content decreased with storage time to extend. Different maturity seed GA₃/ABA ratio increase with the storage time, and this result is the same for maturity germination rate. Furthermore, GA₃ content of seed without peel were higher than those with peel at each storage time, while ABA content showed the opposite. Thus, the peel may contain more ABA and conducive to seed dormancy. This was in agree with the observed germination of wild oat with positive relationship between ABA/GA₃ ratio and seed germination, it further strongly affected the dormancy rate (Yu et al., 2016; Esashi, 2017). Zeng and Zhao (2001) indicated that the red string seed showed during seed development, the content of endogenous GA₃ showed a decreasing trend, that GA₃ content in dry seeds in room temperature and low temperature during storage have

shown pre gradually reduce, the latter has an upturn, and the content of endogenous GA₃ and no significant correlation with the seed germination rate. Seed germination rate significantly increased with storage time extended, the role of GA₃ to enhance the seed germination rate weakened. This is similar to the results of Zeng and Zhao (2001) that related to GA₃, a role on a red string seed germination. This experiment showed that when seeds are treated with different concentrations of exogenous ABA, seed germination rate decreased with the increase of ABA concentration, even when at the same time there is application of GA₃, the germination rate of seed was certain upward, but still lower than in CK. So ABA inhibited seed germination, but its inhibitory effect in a certain extent can be remission by GA₃. Wang et al. (2004) also reported the influence of ABA on the inhibition of rice seed germination results.

Conclusions

Seeds without peel had lower endogenous ABA content and the ratio of ABA/GA₃ than seeds with peel. The best GA₃ treatment time for milky ripe, wax ripe, full ripe seed were 60 min (m) or 120 m, 60 m, and 30 m, respectively. Seed germination rate, germination potential and germination index were all first increased and then decreased with increasing GA₃ concentrations. The best treatment concentration of GA₃ was 100 mg L⁻¹, the turning point was 200 mg L⁻¹. The dormancy rate of low temperature storage seeds were higher than those stored in room temperature at each storage time, and both were decreased with the storage time. New seeds or stored for 1-2 months, showed significantly enhanced germination rate by exogenous GA₃. GA₃ treatment had no effect on germination rate for seeds that had been stored for over 3 months. ABA inhibits the germination rate, which decreases with the increasing concentration of ABA. The most inhibitive effect, which led to a seed germination reduction by 37.7% and 4.0%, appeared when the concentration of ABA was 500 and 1000 mg L⁻¹. GA₃ could abate the effect which ABA inhibited seed germination.

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REFERENCES

- [1] Beck, E., Ziegler, P. (1989): Biosynthesis and degradation of starch in higher plants. – *Ann Rev Plant Physiol.* 40: 95-117.
- [2] Cheng, L., Lu, Q., Wu, B., Yin, C., Bao, Y., Gong, L. (2018): Estimation of the costs of desertification in China: a critical review. – *Land Degradation & Development* 29(4): 975-983.
- [3] Damaris, R. N., Lin, Z., Yang, P., He, D. (2019): The Rice Alpha-Amylase, Conserved Regulator of Seed Maturation and Germination. – *International Journal of Molecular Sciences* 20(2): 450.
- [4] Esashi, Y. (2017): Ethylene and Seed Germination. – In: Mattoo, A. K. (ed.) *The Plant Hormone Ethylene*. CRC Press, Boca Raton, FL, pp. 133-157.
- [5] Finch-Savage, W. E., Leubner-Metzger, G. (2006): Seed dormancy and the control of germination. – *New Phytologist* 171(3): 501-523.

- [6] Islam, M. R., Shahidul, A. M., Eneji, A. E., Ren, C. Z., Song, W. J., Hu, Y. G. (2011): Evaluation of water-saving superabsorbent polymer for forage oat (*Avena sativa* L.) production in arid regions of northern China. – *Journal of Food, Agriculture and Environment* 9(2): 514-518.
- [7] Khan, T. A., Nadeem, F., Gao, Y., Yang, Y., Wang, X., Zeng, Z., Hu, Y. (2019): A larger root system in oat (*Avena nuda* L.) is coupled with enhanced biomass accumulation and hormonal alterations under low nitrogen. – *Applied Ecology and Environmental Research* 17(2): 4631-4653.
- [8] Li, M., Hu, Y. G., Zeng, Z. H., Ren, C. Z., Mao, N., Song, W. J., Jia, P. F. (2009): Wind tunnel experiment on anti-wind erosion capacity of four crop stubbles in Horqin sandy land. – *Chinese Agricultural Science Bulletin* 11: 254-258 (in Chinese).
- [9] Lin, Y. C., Zeng, Z. H., Ren, C. Z., Guo, L. C., Wang, C. L., Hlatshwayo, P., Hu, Y. G. (2012): Effects of different soil moisture regimes on gas exchange in response to light, growth and biomass in naked oat (*Avena nuda* L.) – *Journal of Food, Agriculture and Environment* 10(3-4): 1473-1478.
- [10] Liu, X., Hu, P., Huang, M., Tang, Y., Li, Y., Li, L., Hou, X. (2016): The NF-YC-RGL2 module integrates GA and ABA signalling to regulate seed germination in *Arabidopsis*. – *Nature Communications* 7: 12768.
- [11] Lou, Y. L., Feng, Z. H. (1995): Lixia River region of Jiangsu Province of wheat following rice field oats the summering law research. – *Jiangsu Journal of Agricultural Sciences* 11: 25-29 (in Chinese).
- [12] Mukhtar, F. B., Singh, B. B. (2006): Influence of photoperiod and gibberellic acid (GA₃) on the growth and flowering of cowpea (*Vigna unguiculata* (L.) Walp). – *Journal of Food, Agriculture and Environment* 4(2): 201-203.
- [13] Ozkaya, O., Dundar, O., Kuden, A. (2006): Effect of preharvest gibberellic acid treatments on postharvest quality of sweet cherry. – *Journal of Food, Agriculture and Environment* 4(1): 189-191.
- [14] Qian, X., Zang, H., Xu, H., Hu, Y., Ren, C., Guo, L., ... Zeng, Z. (2018): Relay strip intercropping of oat with maize, sunflower and mung bean in semi-arid regions of Northeast China: Yield advantages and economic benefits. – *Field Crops Research* 223: 33-40.
- [15] Rabiei, E., Khodambashi, M., Pirbalouti, G. (2012): The study of the drought tolerance indices of oat (*Avena sativa* L.). – *Journal of Food, Agriculture and Environment* 10(2): 646-648.
- [16] Ren, C. Z., Ma, B. L., Burrows, V., Zhou, J., Hu, Y. G., Guo, L., Wei, L., Sha, L., Deng, L. (2007): Evaluation of early mature naked oat varieties as a summer-seeded crop in dryland northern climate regions. – *Field Crops Research* 103: 248-254.
- [17] Wang, X., Tao, L. X., Huang, X. L. (2004): Physiological mechanisms of exogenous ABA inhibition of germination of rice seeds. – *Acta Agronomica Sinica* 30: 1250-1253 (in Chinese).
- [18] Wilkinson, S., Davies, W. J. (2010): Ozone ABA and ethylene: new insights from cell to plant to community. – *Plant Cell Environ* 33: 510-525.
- [19] Yu, Y., Zhen, S., Wang, S., Wang, Y., Cao, H., Zhang, Y., ... Yan, Y. (2016): Comparative transcriptome analysis of wheat embryo and endosperm responses to ABA and H₂O₂ stresses during seed germination. – *BMC Genomics* 17(1): 97.
- [20] Zang, H., Qian, X., Wen, Y., Hu, Y., Ren, C., Zeng, Z., ... Wang, C. (2018): Contrasting carbon and nitrogen rhizodeposition patterns of soya bean (*Glycine max* L.) and oat (*Avena nuda* L.). – *European Journal of Soil Science* 69(4): 625-633.
- [21] Zeng, L., Zhao, L. J. (2001): Effects of GA₃ and ABA on seed development and germination of *Salvia splendens*. – *Journal of Shanghai Jiaotong University (Agricultural Science)* 4: 276-279 (in Chinese).
- [22] Zhang, Q., Van Wijk, R., Shahbaz, M., Roels, W., Schooten, B. V., Vermeer, J. E., Laha, D. (2017): *Arabidopsis* phospholipase C3 is involved in lateral root initiation and ABA

responses in seed germination and stomatal closure. – *Plant and Cell Physiology* 59(3): 469-486.

APPENDIX

Table A1. The summary of all experiments and treatments evaluated in present study

Experiment	Treatment
a	Factor 1 (Seed maturity): S1 = milky ripe, S2 = wax ripe, and S3 = full ripe Factor 2 (GA ₃ treat time): T1 = 0, T2 = 30, T3 = 60, T4 = 120, and T5 = 240 min
b	Factor 1 (GA ₃ concentration): A1 = 0, A2 = 12.5, A3 = 25, A4 = 50, A5 = 100, and A6 = 200 mg L ⁻¹
c	B1 = seeds with peel, B2 = seeds without peel, B3 = seeds with peel, soaked in GA ₃ for 2 h, and B4 = seeds without peel, soaked in GA ₃ for 2 h
d	Factor 1 (Seed maturity): S1 = milky ripe, S2 = wax ripe, and S3 = full ripe Factor 2 (Seed storage time): C1 = 0, C2 = 1, C3 = 2, C4 = 3, and C5 = 4 month
e	D1 = 1000 mg L ⁻¹ ABA; D2 = 500 mg L ⁻¹ ABA; D3 = 250 mg L ⁻¹ ABA; D4 = 100 mg L ⁻¹ ABA; D5 = 100 mg L ⁻¹ GA ₃ + 100 mg L ⁻¹ ABA; D6 = 100 mg L ⁻¹ GA ₃ + 250 mg L ⁻¹ ABA; D7 = 100 mg L ⁻¹ GA ₃ + 500 mg L ⁻¹ ABA; D8 = 100 mg L ⁻¹ GA ₃ + 1000 mg L ⁻¹ ABA; CK use distilled water as control