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Effects of Exogenous Hormones Like ACC on Germination of Lettuce Seeds

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In order to investigate the effects of different exogenous hormones on the dormancy and germination of lettuce seeds, this paper soaks the lettuce seeds in ACC, GA, $CaCl_2$ and SNP solutions with different concentrations, respectively, observes the quantity of lettuce seeds germinated, calculates the germination rate, and analyzes the optimum concentrations to break the period of dormancy and promote the germination of seeds to increase the germination rate of seeds. Through the observation of lettuce seeds soaked with ACC, GA, calcium chloride and SNP in different time periods, it is found that ACC, GA, calcium chloride and SNP solutions can all promote the germination of lettuce seeds. Lettuce falls within the Lactuca genus, the Asteraceae family. It likes cold climate and the suitable temperature for growth is 15-20°C. If the temperature exceeds 25°C, the high temperature in the leafy head will cause the necrosis and rot and poor growth of interior leaves (Song et al., 2013). The temperature for germination of seeds ranges between 15-20°C. If the temperature is higher than 25°C, the seed coat will not be able to absorb water, affecting the germination (Li et al., 2012). When lettuce is sowed in summer, it needs to be treated at a low temperature. After being soaked, it should be placed in the refrigerator freezer to accelerate germination and should not be sowed until the sprout turns white. Lettuce is rich in protein, sugar, vitamins, minerals and other nutrients. It is popular among consumers due to its crisp texture, fresh taste and fragrance and rich nutrition (Hu and Zhan, 2014). Few research has been conducted on the effects of exogenous hormones on the germination rate of lettuce seeds. This study takes lettuce seeds from the same source as the test material and conducts tests on the germination rate of lettuce seeds, trying to explore the effects of ACC, GA, $CaCl_2$ and SNP solutions with different concentrations on the germination rate of lettuce seeds so as to improve the germination rate.

1. Materials and methods

1.1 Test materials

The vegetable seeds for testing are lettuce seeds. The reagents selected are ACC (1-aminocyclopropane-1-carboxylate), GA (gibberellic acid), $CaCl_2$ (calcium chloride) and SNP (sodium nitroprusside), which are all analytically pure.

1.2 Treatment design

Seed treatment: soak the seeds in cold water for about 6 hours, sterilize them with 75% ethanol for 3 minutes and then wash them clean. Place 50 lettuce seeds into each culture dish and keep them at a constant temperature of 15-18°C. Replace the reagent solution every 2 days.
Control group treatment: use water as control. Obtain two groups of data for each kind. Calculate the mean value of the germination rates.
Treatment group treatment: select four different exogenous hormones ACC, GA, $CaCl_2$ and SNP four different exogenous hormones, and also obtain two groups of data for each kind. The concentration gradient of the four reagents is 5umol/L, 10umol/L and 20umol/L for ACC; 50umol/L, 100umol/L and 200umol/L for GA; 2.5mmol/L, 5mmol/L and 10mmol/L for $CaCl_2$; and 100umol/L and 200umol/L for SNP.

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After four different reagents are added, observe them for 14 consecutive days.

1.3 Measurement and calculation of indices

When half of the germ comes out of the seed, it is regarded as germinated. Record the germination of lettuce seeds from the first day of germination (Huang et al., 2010; Zhang et al. ,2010). Calculate the germination rate of lettuce seeds by recording the number of buds. The germination index can be used to measure the germination capacity and vitality of the plant (Wang et al., 2016).

Germination rate = (quantity of germinated seeds / quantity of seeds for test) ×100% (Liu et al., 2013)

Germination potential refers to the percentage of seeds normally germinated in the total quantity of seeds for test during the initial germination period. High germination potential of the seeds means strong seed vitality, high uniformity of germination, consistent emergence and great yield-increasing potential.

The germination index can be used to measure the germinating ability and vitality of the plant.

Germination potential=(number of seeds germinated on Day 5/number of seeds for test)×100%.

Germination index (GI)=$\sum$[Gt/Dt)].

where, Dt stands for the number of seeds germinated on Day t; and Gt stands for the number of germination days.

Record the germination status of the seeds every day on an Excel file, sort the data and calculate the germination rate, germination potential and germination index, and draw a line chart based on the germination rat.

Use the software SPSS22.0 to do statistics and carry out significance test on the mean values through one-way analysis of variance (Wang et al. ,2014).

2. Results and analysis

2.1 Effects of different solutions at different concentrations on the germination of lettuce seeds

Table 1: Effects of different treatments on germination of lettuce seeds

	Concentration	Germination rate (%)	5% difference significance	Germination potential (%)	5% difference significance	Germination index	5% difference significance
CK	0	40±0.56	ab	39±0.08	ab	42±0.35	ab
	5	46±1.12	ab	43±0.14	a	49±0.15	a
ACC	10	50±0.56	a	45±0.08	a	41±0.01	a
	20	64±3.36	a	41±0.14	a	53±0.07	a
	50	48±1.68	ab	37±0.20	ab	49±0.30	a
GA	100	50±1.12	a	22±0.06	b	37±0.01	ab
	200	49±1.96	ab	34±0.22	ab	49±0.21	a
	2.5	52±1.68	a	18±0.11	b	30±0.19	ab
$CaCl_2$	5	59±0.28	a	32±0.11	a	46±0.19	a
	10	65±0.28	a	22±0.06	ab	34±0.16	ab
	100	72±0.22	a	30±0	a	40±0.08	a
SNP	200	60±0.06	a	25±0.03	b	32±0.03	ab

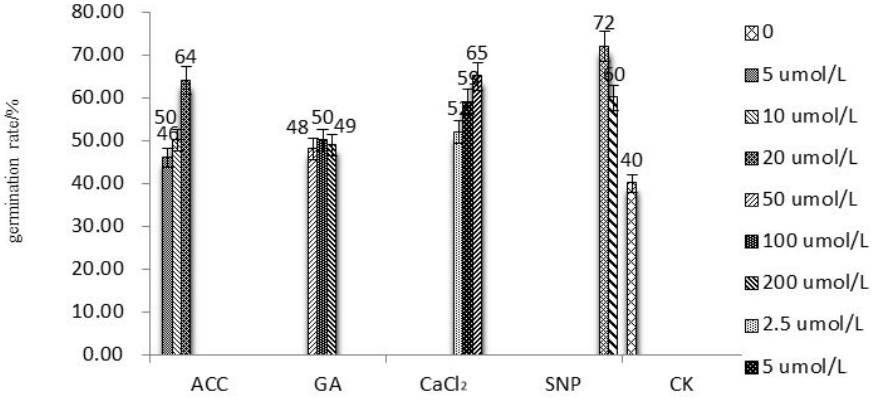

Figure 1: Germination rates of lettuce seeds soaked with different solutions at different concentrations

As can be seen in Figure 1, with the addition of ACC and $CaCl_2$ solutions, the germination rate of lettuce seeds increases, and the greater the concentration is, the higher the germination rate of the seeds will be, indicating that greater concentrations of ACC and $CaCl_2$ solutions can promote the germination of lettuce seeds. After GA and SNP solutions are added, the germination rate of lettuce seeds first increases and then declines, and the germination rate in both cases in the test range is higher than that of the control group, indicating that GA and SNP solutions at a certain concentration can promote the germination of lettuce seeds, but that if the concentration exceeds the value, the germination rate will show a downward trend.

2.2 Effects of different solutions at different concentrations on the germination potentials of lettuce seeds

As can be seen from Table 1 and Figure 2, the germination potential of the seeds in the control group is 42%, while the seed germination potential in ACC5, ACC10 and ACC20 solutions was 49%,41%,53%, respectively. Indicating that in the solution at such concentration, the lettuce seeds have great vitality, high germination speed and yield-increasing potential. The lettuce seeds in ACC20 have the greatest germination potential and the highest germination speed.

According to Table 1, in this test range, the germination indices of the seeds in ACC and GA solutions decline first and then increase, but finally, the germination index of the seeds in highly concentrated solution is all higher than that of the seeds in the control group. In $CaCl_2$ and SNP solutions, the germination index of lettuce seeds also declines first and then increases, but the germination index of the seeds in $CaCl_2$ solution at any concentration is lower than that of the seeds in the control group, indicating that the seeds in $CaCl_2$ and SNP solutions have relatively weak germination abilities.

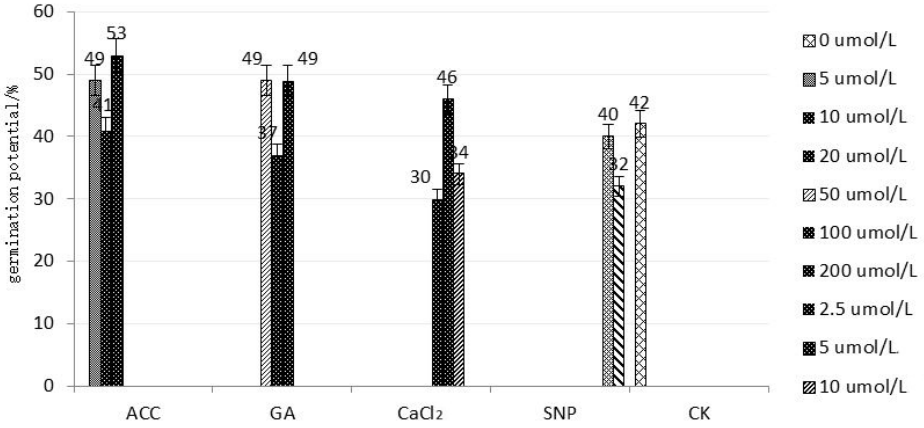

Figure 2: Germination potentials of lettuce seeds at different concentrations

2.3 Effects of ACC solution on the germination of lettuce seeds

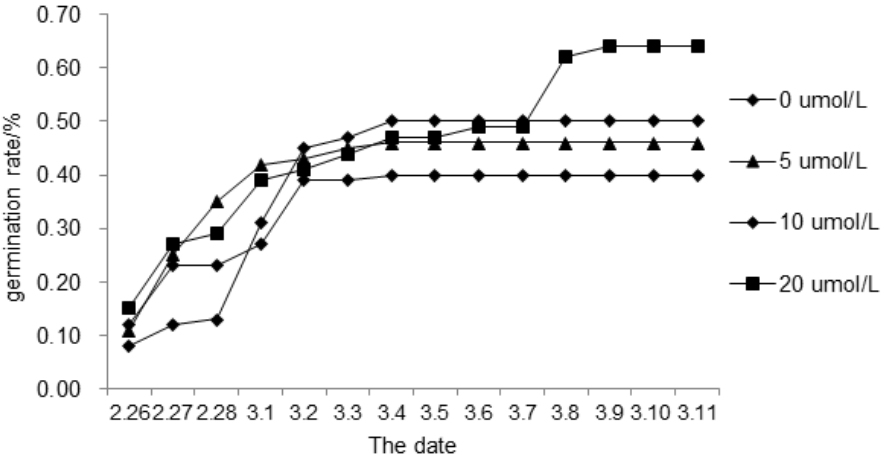

Figure 3: Effects of the ACC treatment concentration on the germination of lettuce seeds

As can be seen in Figure 3, the overall germination of lettuce seeds increases over time. The germination rate of the control group increases from 12% to 40%. After the ACC solution is added, the germination rate increases from 12% to 46% for 5umol/L ACC solution, from 8% to 50% for 10umol/L ACC solution and from 15% to 64% for 20umol/L solution. The germination rates of the lettuce seeds in the test group and control group increase significantly during Day 2-5, and become basically stable after Day 7. After 20umol/L ACC solution is added on Day 11, the germination rate rises significantly again. Generally speaking, when ACC solution of any concentration is added, the germination rate of lettuce seeds is higher than that of the control group. Therefore, the ACC solution can accelerate the germination of seeds, and the higher the concentration is, the more obvious the acceleration effect will be.

2.4 Effects of GA solution on the germination of lettuce seeds

As can be seen in Figure 4, the overall germination of lettuce seeds increases over time. The germination rate of the control group increases from 12% to 40%. After the GA solution is added, the germination rate increases from 21% to 48% for 50umol/L GA solution, from 12% to 50% for 100umol/L GA solution and from 15% to 49% for 200umol/L GA solution. The germination rate of the lettuce seeds in the control group increases significantly during Day 2-5, and then becomes basically stable. The germination rate of the test group all increases with different concentrations of GA solution and tends to be flat on Day 12. Generally speaking, from Day 8 on, the germination rate of the lettuce seeds added with GA solution is higher than that of the control group, but when the GA concentration is 100umol/L, the germination rate reaches the maximum. Therefore, high-concentration GA solution can inhibit the germination of seeds while low-concentration GA can accelerate the germination.

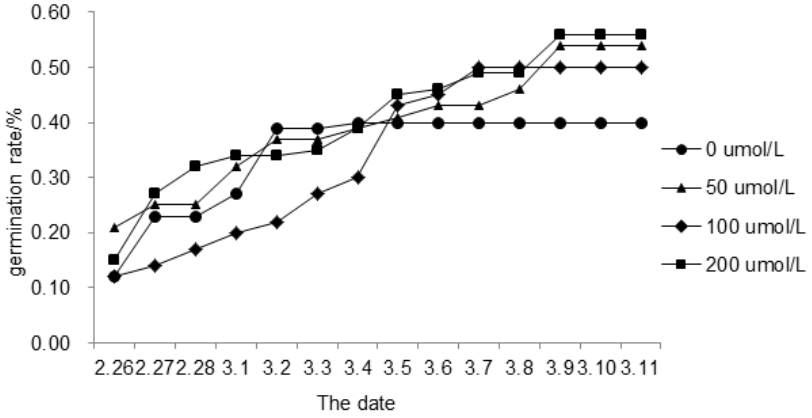

Figure 4: Effects of the GA treatment concentration on the germination of lettuce seeds

2.5 Effects of $CaCl_2$ solution on the germination of lettuce seeds

As can be seen in Figure 5, the overall germination of lettuce seeds increases over time. The germination rate of the control group increases from 12% to 52%. After the $CaCl_2$ solution is added, the germination rate increases from 6% to 52% for 2.5umol/L $CaCl_2$ solution, from 11% to 59% for 5umol/L $CaCl_2$ solution and from 2% to 65% for 10umol/L $CaCl_2$ solution. On Day 10, the germination rates of the control group and the lettuce seeds soaked with 5umol/L $CaCl_2$ become flat, and that of the seeds soaked with 2.5umol/L $CaCl_2$ solution keeps increasing during Day 2-12. From Day 13 on, the germination rate is lowered and then becomes flat. The germination rate of the seeds soaked with 10umol/L $CaCl_2$ solution keeps increasing until it reaches the maximum value 65%. Therefore, $CaCl_2$ solution can promote the germination of lettuce seeds, but the effect is not obvious. Low-concentration $CaCl_2$ solution has little effect in accelerating the germination of lettuce seeds, but the greater the concentration gets, the more significant effect it will have.

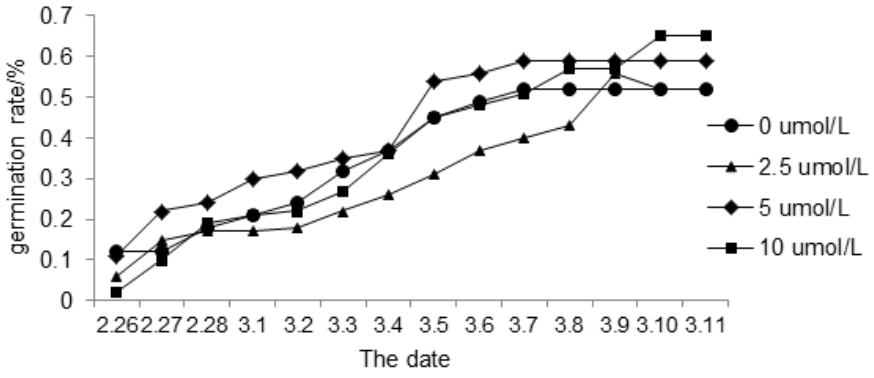

Figure 5: Effects of the $CaCl_2$ treatment concentration on the germination of lettuce seeds

2.6 Effects of SNP solution on the germination of lettuce seeds

As can be seen in Figure 6, the overall germination of lettuce seeds increases over time. The germination rate of the lettuce seeds in fresh water increases from 12% to 52%. After the SNP solution is added, the germination rate increases significantly. The germination rate increases from 2% to 72% for 100umol/L SNP solution and from 11% to 59% for 200umol/L SNP solution. From Day 2-10, the germination rates of lettuce seeds in fresh water keeps increasing and then it becomes stable on Day 10. The germination rate of the seeds soaked with the 100umol/L SNP solution starts to stabilize and reaches the maximum value 72% from Day 11 on, and the germination rate of the seeds soaked with the 200umol/L SNP solution starts to stabilize from Day 12. Therefore, the SNP solution can promote the germination of lettuce seeds, and low-concentration SNP has very significant effect.

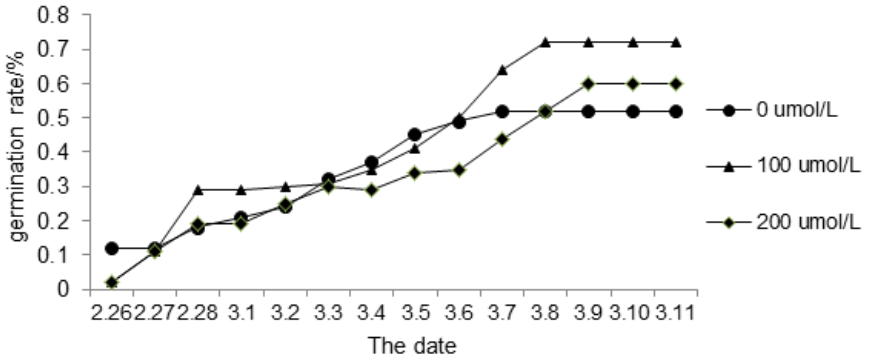

Figure 6: Effects of the SNP treatment concentration on the germination of lettuce seeds

3. Conclusions

The ACC deaminase growth-promoting bacteria refers to a class of bacteria with ACC deaminase activity which promotes plant growth (Hua, 2014). According to the definition and categorization of the bacteria promoting plant growth, Click has explained the promoting mechanism of the ACC deaminase growth-promoting bacteria, and classified it as part of the plant growth-promoting bacteria (Zhao et al., 2009). Through the recording and calculation of the germination status of lettuce seeds, it is found that ACC solution lettuce can promote the germination of lettuce seed, and the greater the concentration is, the higher the germination rate will be; the germination potential is the greatest and the germination speed is the fastest at ACC10, the germination index is the highest and the germination capacity is the greatest at ACC20.

Gibberellic acid (GA) is a plant hormone that promotes plant growth. It enhances seed germination by promoting cell division and controlling the balance of promoters and inhibitors (Liu et al., 2006). This experiment shows that GA solution can promote the germination of lettuce seeds, and has more significant effect at GA100. The greater the concentration is, the higher the germination rate will be.

Calcium is one of the essential nutrients for normal plant growth and development and plays an important role in the metabolism of plant elements. A large number of studies have shown that calcium helps maintain the integrity of cell cornea and reduce the membrane permeability under adverse conditions (Liu and Li, 2011).

Through the recording and calculation of the germination status of lettuce seeds, it is found that certain concentration of $CaCl_2$ solution can promote the germination of lettuce seeds; however, after the GA solution is added, the germination potential and index of the lettuce seeds are both lower than those of the control group, indicating that the germination speed of the lettuce seeds is slower and the germination capacity is weaker in GA solution.

Nitric oxide (NO) sodium nitroprusside (SNP) has different effects on the germination of vegetable seeds at different concentrations. NO has effects on the respiration of plants, photo morphogenesis, germination of seeds, growth and development of roots and leaves, stomatal movement, and responses to various stresses(She et al.,2011). Through the recording and calculation of the germination status of lettuce seeds, it is found that the germination rate of lettuce seeds is the highest at SNP100, indicating that seed soaking with SNP solution can improve the vitality of lettuce seeds, and effectively promote the germination of lettuce seeds at certain concentrations. However, the germination potential of the lettuce seeds is lower than the control group, indicating that lettuce seeds soaked with SNP solution sprout slowly; at SNP5, the germination index is high and the germination capacity is great.

Experiments show that, ACC and GA solutions can shorten the dormancy period of seed, so that the seeds germinate earlier than the control group. At the same time, they can also bring forward the concentrated germination of the seeds. $CaCl_2$ and SNP solutions can promote the germination of lettuce seeds, but the speed is slower and the germination capacity is weaker. And between ACC and GA solutions, the ACC solution has better promoting effect on the germination of lettuce seeds.

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