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Seed germination of Vicia hirsuta (Linn.) S.F.Gray

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In order to study the germination characteristics of Vicia hirsuta (Linn.)S.F.Gray seeds, a series experiments had been done: after the seeds which coats were cut by scalpel were cultivated in 5˚C, 10˚C, 15˚C, 20˚C, 25˚C, 30˚C separately, the results showed that 25℃ was the optimal germination temperature for the seeds; the imbibition process of vetch seeds were studied under the optimum temperature; the seeds were germinated with different concentrations (1, 10, 100, 1000 μmol/l) of GA3 and 6-BA solution; the seeds were germinated with the different temperature (30˚C /10˚C; 25˚C /15˚C); the seeds were laminated with the wet river sand in the incubator. The experimental results showed: the germination of the seeds which coats were cut by scalpel were 100% in 25℃ for 24h; the seed germination next to 3% when the seeds were nursed with the 30-day's different temperature, the 30-day's treatment GA3 and 6-BA under 25℃ , and the 60-day's lamination with river sand. The dormancy type of the Vicia hirsute (Linn.)S.F.Gray seeds which coat obstacle as water barrier were physical dormancy.

Keywords: Vicia hirsuta (Linn.)S.F.Gray; Optimum temperature; Seed coat damage; Seed dormancy

INTRODUCTION

The seed germination and dormancy, the two very important stages in the seed life, trended to the totally opposite condition through the seed life activities. They were also important things of the higher plant individual development, which concerned to the survival, reproduction and distribution of the plant community. The seed germination and dormancy had been studied for a long time, but till now a little knowledge had been get about in the seed biological field. Physical dormancy (or hard seed) was wildly found in the legume seed because the seed coat or the skin was impermeable (Hu Xiaowen 2009). In addition to the inherent factors of seed itself, the seed germination and dormancy also affected by the following factors: the temperature or temperature fluctuation in the process of system development, humidity or soil moisture, light and plant growth regulators, soil fertility, gas exchange, animal feed and etc. [1~2].

In order to improve the seed low germination rate, many scholars in and aboard had done a lot of research on the seed germination of various plants.

We found that different seed germination were related to the fluctuating temperature according to the following researches: Study on 22 Species of Perennial Grasses Seed by Yan song, Li-hua（2012）[3]; Research on Wild Rose Rhodiola Seeds by Liu Cai-fu, Zhao Wenhua.（2012）[4]; Study on the Tibet Inula Root Seeds by Bao Fugui (2011) [5~6]; and Study on the Seeds of the Castor Bean by Jiang Xiaojun and Wen Xiangduo;

The seed germination rate was improved obviously after the seed coat was cut with the mechanical method based on the related researches as the following: Treated the Seed Coat of Aohan alfalfa Seed with Frosted Quartz by Wang Li and etc. (2009) [7] Dealt the Sophora flavescens Seeds with Sandpaper Grinding by Wang Lei and etc.. (2010) [8]

The seed germinating potential, percentage, and vigor index were improved after the seed treated with different concentrations of GA3 solution. The results got from the following studies: Research on the Seeds of Cercis gigantean by SHAO Bei-bei (2010) [9], and Physical dormancy in seeds of Dodonaea viscosa (Sapindales, Sapindaceae) from Hawaii , Baskin J M, Davis B H, Baskin C C, Gleason S M, CordelS. (2004) [10]

All of the above studies, the Vicia hirsuta (Linn.)S.F.Gray Seeds were selected as the research object according to some relevant information about other several kinds of the legume Vicia genus seeds and the low germinating seed under the natural conditions[11~12]. Till now, the Vicia hirsuta Seeds germinating characteristics had not yet been found. The ornamental value and garden use of the Vicia hirsuta had not been excavated. It was only regarded as weeds in many places.

Seed germination of the Vicia hirsuta (Linn.)S.F.Gray had been studied with the following methods: fluctuating temperature treatment; seed imbibition process; different concentrations of plant hormone treatment; treatment of lamination with river sand. The researchers attempted to discover the tiny vetch germination characteristics in-depth and to provide

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reference for exploitation, protection and cultivation of tiny vetch.

Vicia hirsuta (Linn.)S.F.Gray is annual herbaceous of vicia L. species. The stems are thin and soft, angled, nearly glabrous, trailing or climbing; paripinnate leaves, tendril branched; stipules linear, base with 2-3 cracked tooth; 4-8 pairs leaflets, linear or narrowly oblong, 0.5-1.5 cm long, apex truncate, with short pointed, base ministry gradually narrow, glabrous; Racemes is shorter than leaves obviously, 2-4 (-7) flowers; Calyx campanulate, calyx teeth lanceolate, about 2 mm; corolla length of 3-5 mm, white, light blue, green or purple white, thin pink, upper petal elliptic, about 5 mm, apex truncate or slightly concave, different petals nearly spoon shaped, the length equal to the upper petal, keel shorter; Ovary sessile, densely brown hirsute, with 2 ovules, the surrounding of the upper part is glabrous; The pod shaped oblong, 0.5-1 cm long, densely brown hirsute; 2 Seeds, oblate-spheroid, two sides protruding; Flower and fruit of 2-7 months [13]; it widely introduced and naturalized in wheat fields or hill slopes in Jiangsu, Zhejiang, Sichuan, Shanxi, Taiwan and etc. The Vicia hirsuta has strong adaptability and barren resistance, but it's good in the sandy loam with loose and fecund, good drainage as well.

The Vicia hirsuta (Linn.)S.F.Gray is applied to garden greening as the new plant for the groundcover and vertical greening. It has good ornamental value with its foliage green and beautiful shape. In addition, it is also used as the green manure and feed because the animal likes to eat it. The whole plant is used as the medicine for it is good for promoting blood circulation, stomach-calming, eyesight and anti-inflammatory and etc. [14].

MATERIAL AND METHOD

Experimental material, reagent, instrument

Experimental material

The Vicia hirsuta (Linn.)S.F.Gray seed(collected at the square of Qiliping Campus of Shaoyang University from 2014.10 to 2014.11

River sand(buy at market), filter paper(buy at market)

Experimental reagent

Potassium Permanganate KMnO4 (AR): Tianjin bodi chemicals co.;

GA3 (analysis of pure): National Pharmaceutical Group Chemical Reagent Co., ltd.;

6-BA (analysis of pure): the Chemical Reagent Co., Ltd.

Distilled water (made)

Experimental instrument

CXZ intelligent seed germination box (Ningbo Jiangnan instrument factory);

AYU220 type electronic balance (SHIMADZU): a sense of 0.0001g;

A 100 ml volumetric flask, 1000ml volumetric flask, beaker, glass rod, cylinder, test tubes, filter paper and washed ear ball, thermometer, plastic head dropper, tweezers, a Petri dish.

EXPERIMENTAL METHOD

Treatment of the experimental material

The Vicia hirsuta (Linn.)S.F.Gray seeds: The Vicia hirsuta (Linn.)S.F.Gray fruits were treated by rubbing, shelling, washing, rinsing in water, natural drying and then preserved in a cool and dry place.

River sand: The sand was gone through 10 meshes screen, disinfected with 0.5% KMnO4 solution, immersed for half an hour, cleaned with water and drained.

Determination of the weight of 1000 seeds

The natural dried Vicia hirsuta (Linn.)S.F.Gray seeds were divided into 12 parts with the four –way division. One thousand grain seeds were randomly selected from each part, weighted with a sense of volume to 0.0001g electronic analytical balance. This process was repeated three times, and the average value was calculated.

Test of seed water imbibition

300 grain Vicia hirsuta seeds randomly selected were divided into two parts, each part including 150 grains, one of them including 150 grain seeds which coats were cut. The two part seeds were equal divided into 3 groups respectively (1, 2, 3; cut 1, cut 2, cut 3). The seeds were weighted first and then recorded o hour. The seeds were immersed in the clean water and imbibed water. The weight of each group were measured and recorded down at 1h, 2h, 3h, 4h, 6h, 8h, 10h, 12h, 16h, 20h, 24h, 30h, 36h, 48h, 60h, 72h respectively.

Reagent preparation 0.50 % KMnO4

To prepare 1000ml KMnO4 solution with concentration of 0.50%, 5.000g KMnO4 (which was accurately weighted by an electronic balance) was needed. The KMnO4 were purred into the beaker, we added water and stirred until the solution fully dissolved and injected into volumetric flask with the capacity of 1000 ml along the glass rod. The beaker wall was washed with distilled water, and then each wash solution was

injected into a volumetric flask [15~18]. Distilled water was injected slowly into the volumetric flask, when surface near the volumetric flask scale 1 ~ 2cm stop. The glue dropper was used to add, and eyes with the surface of the concave surface at the bottom of the flat, when the solution titrated to scale line stop dropping. The volumetric flask was capped and shaken upside down till the solution well.

Configuration the GA3 and 6BA solution of 1000μmol/L, 100μmol/L , 10μmol/L, 1μmol/L

100mL GA3 and 6BA with different concentration (1μmol/L, 10μmol/L, 100μmol/L, 1000μmol/L) were Prepared respectively. Firstly, 100mL GA3 and 6BA solution with concentration of 1000μmol/L were configured: firstly, quantitative 6-BA and GA3 were weighted and put into a clean beaker respectively, dissolved with appropriate sodium hydroxide solution. Secondly, until completely dissolved, the solution was neutralized with hydrochloric acid; a proper amount of distilled water was added into the solution and the solution was injected into a100 ml volumetric flask along the glass rod. Thirdly, the inside of the beaker was cleaned several times with a small amount of distilled water till no residue and each washing solution was purred into a volumetric flask [15~18]. The distilled water was added to a volumetric flask, when the surface near capacity graduated bottle 1cm to 2cm stop. The glue dropper was used to head dropping, and eyes with the surface of the concave surface at the bottom of the flat, when the solution titrated to scale line stop dropping. The volumetric flask was capped and shaken upside down till the solution well. Fourthly, with this method, the solution was diluted according to the concentration gradient. 100mL solution with concentration of the100 mmol / L, 10 mmol / L, 1 mmol / L were prepared respectively.

The optimum temperature of seed germination

18 Petri dishes (diameter 9cm, high 2cm) were picked out, cleaned, and labeled with A1-A4, B1-B4, C1-C4, D1-D4, E1-E4. Eighteen part of the Vicia hirsuta (Linn.)S.F.Gray seeds (each 50 grains) which had been winnowed were randomly selected. The seeds were get out after immersed in KMnO4 solution with concentration of 0.5% for 30 minutes, rinsed with distilled water for 3 - 4 times until no disinfectant residual in the seed. The seeds which coat was cut were placed in 18 culture dishes (4 culture dishes as a group). The dishes were put into the incubators with temperature at 5˚C, 10˚C, 15˚C, 20˚C, 25˚C, 30˚C and nursed for one month.(Table 1)

Table 1. The optimum temperature of seed germination

Test	The temperature（˚C）
A1-A4	5
B1-B4	10
C1-C4	15
D1-D4	20
E1-E4	25
F1-F4	30

Seed imbibition test

6 Petri dishes (diameter 9cm, high 2cm) were picked out and cleaned. 6 part of the Vicia hirsuta (Linn.)S.F.Gray seeds (each 50 grains) which had been winnowed were randomly selected. Three of them were pierced the seed coat with a blade (be careful not to row to the embryo), and divided into F1 to F3 group (parallel experiment) and cultured in the optimum temperature for 72h.(Table 2)

Table 2. The germination of seed which coat was broken.

Number	The way of deal
F1, F2, F3,	Cut or the whole

Breaking the seed dormancy by GA3 and cytokinins 30 petri dishes were picked out (diameter 9cm, high 2cm) and cleaned. 30 part of the Vicia hirsuta (Linn.)S.F.Gray seeds (each 50 grains) which had been winnowed were randomly selected, divided into five groups G, h, I, J, K(n = 3, parallel experiments) , added different concentrations (0,1, 10, 100, 1000 μmol/l) of GA3, cultured in optimum temperature for a month.(Table 3)

Table 3. To break the seed dormancy by GA3

Number	Concentration（μmol/L）
G1, G2, G3, G4	0
H1, H2, H3, H4	1
I1, I2, I3, I4	10
J1, J2, J3, J4	100
K1, K2, K3, K4	1000

Lamination treatment on the seed germination

Four beakers with the capacity of 100ml were picked out and cleaned. Four part of the Vicia hirsuta (Linn.)S.F.Gray seeds (each 50 grains) which had been winnowed were randomly selected. The seeds were wrapped with disinfect clean nylon gauze, divided into A to L group (n = 4, parallel experiments), put into the clean river sand, and nursed in optimum temperature for one to two months (Table 4)

Table 4. The seed germination of seed lamination

Number	Material of lamination
L1, L2, L3, L4	River sand

RESULTS

Basic information of the seed

The seeds were randomly selected and weighted with the four - way division. Average 1000 seeds were weighted for 5.1638g; the maximum differences of 1000 seed weight for each group were below 0.05. In this way, we ensured that the used seed quality, particle size and seed germination activity were almost the same.

In order to test the seed water content, firstly, the sample boxes were dried, cooled, weighted, and then marked. Secondly, three parts of the seeds (each part are 100 grain) were chosen, put into the boxes we had prepared, weighted (accurate to 0.0001g), and put into the oven with temperature 80℃ for 48h. The box was covered with crucible tongs (the box sealed). Thirdly, the boxes which contained the sample seeds were picked out, put into the dryer, cooled to room temperature, weighted in 30-40 minutes later. After calculating, we found that the water contents of the sample tiny vetch seeds were 9.3%.

Referred to international rules for seed testing, the seed viability was determined with TTC (2, 3, 5-Triphenyl Telrazolium Chloride, TTC)staining method. The prepared seeds which coats were cut were randomly selected, invaded into Tetrazole solution (concentration of 1%) for 4 hours under the temperature 20℃. This proceeding was repeated for 3 times, and each time 50 grain seeds were used. As a result, the percentage of the seed viability was 99% after the seeds were treated and silted. (See Table 2.1)(Table 5)(Fig. 1)

The seed imbibitions test

As shown from Fig. 1, we knew that the water absorption rate of the seed which coat was cut was 97.12%, while the whole seed water absorption rate was 14.98% during 0 to 6 hours. After 12 hours the water absorption of the whole seed no longer increased, and the water absorption of the seeds which coat were cut still increased. As a result, the seed coat of the small vetch was a barrier for the seed water absorption.

The consequence and analysis of the optimum temperature for seed germination (Table 6)

Table 5. The basic information of seed

Species	Harvest year	Collection site	Life form	1000Weight (g)	Moisture content (%)	Viability (%)
Vicia hirsuta (Linn.) S.F.Gray.	2014	Shaoyang, Hunan	Annual	5.1638	9.3	99

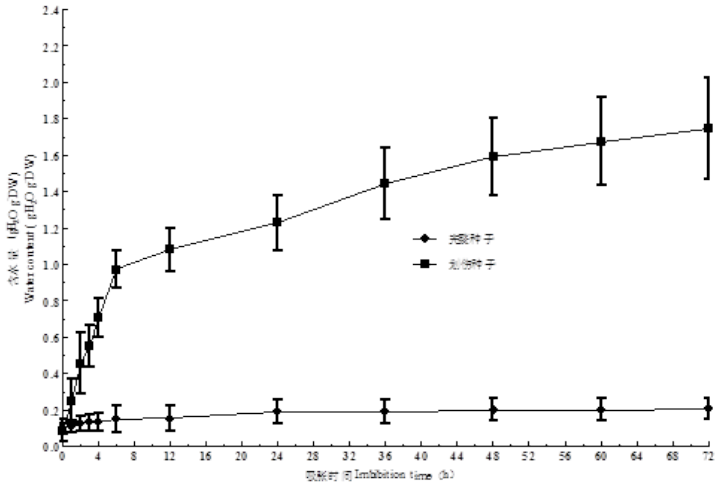

Fig. 1. The curve of whole seeds and the seeds which coats were cut

Table 6. The germination of seed under different temperature

Temperature（˚C）	Number	Germination（%）
5	0	0c
10	0	0c
15	0	0c
20	38	76b
25	47	94a
30	34	68b

Note: values in same column the different small letters indicate significantly different at P 0.05, the below is same.

As shown from Table 6, we found that the seed germination rate of the tiny vetch which coat was cut was zero after 30 hours nurturing under the temperature 5 to 15 degrees Celsius, while under the temperature 25 degrees Celsius, the seed germination rate reached to 94%. As a consequence, the optimum temperature for the seed germination of the tiny vetch was 25 degrees Celsius

The consequences and analysis of the seed germination by plant hormones or by fluctuating temperatures (Table 7)(Table 8)(Table 9)

Table 7. To break the seed dormancy by GA3

Concentration（μmol /L）	Number	Germination（%）
0	0	0
1	4	8
10	3	6
100	3	6
1000	2	4

As shown from Table 7~9, it was seen that when the seeds were soaked in different concentrations of GA3 and 6-BA solution and cultivated in variety temperature, the 3 groups of Vicia hirsuta (Linn.)S.F.Gray seed germination rate were very low (the average germination rate was 3.2%), which indicated that plant hormones and different cultivating temperature hadn't promote the tiny vetch seed germination.

Consequence and analysis of the seed germination of seed lamination(Table 10)

Table 8. To break the seed dormancy by 6-BA

Concentration（μmol /L）	Number	Germination（%）
0	0	0
1	3	6
10	2	4
100	3	6
1000	0	0

Table 9. To break the seed dormancy by changing temperature

Temperature（˚C）	Number	Germination（%）
25/15	4	8
30/10	3	6

Table 10. The seed germination of seed lamination

Experiment time (d)	Number	Germination (%)
30	1	2
40	2	4
50	2	4
60	2	4

As shown from Table 10, it was seen that the seed germination percentage was 3.5% after the seed lamination with river sand in optimum temperature. Seed lamination hadn't helped the seed germination.

DISCUSSION

During experiment, the seeds we select for the test are freshly collected in October, 2014 because the proportion of hard seeds are 42.5% at early stage and the seed coats permeability are relatively good. With the time going, although the seed coats haven't obviously dehydration, the water inside the seeds evaporates and the seed water entrance decrease gradually. The proportion of hard seeds increases from early 42.5% to 93% in experiment in June, 2015.

After the seed coat is cut, the seed imbibition rate of the Vicia hirsuta (Linn.)S.F.Gray seeds reaches to 100% in 5˚C, 10˚C, 15˚C, 20˚C, 25˚C, 30℃ after 24 hours. The seed germination rate reaches to 94% after the seeds are nurtured in 25 degrees Celsius for 30 hours. The germination rate reaches 76% and 68% in 20˚C and 30˚C while the germination rate is zero in 5˚C, 10˚C and 15˚C. All above, the optimum temperature for the Vicia hirsuta seeds is 25 ˚C.

The germination rate of the tiny vetch seeds is 3.2% with 30 day's treatment by plant hormones and fluctuating temperature. The seed germination rate is just 3.5% with the 60-day's lamination treatment with river sand. The plant hormones and lamination treatment have no help for the seed germination. The water absorption barrier of the seeds has great impact on the seed germination. The Vicia hirsuta seeds have the physical dormancy, so to break the seed physical dormancy is an effective way to improve the seed germination rate.

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