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METHODOLOGICAL STUDY ON SELECTION OF CONDITIONS FOR IMPROVING *Taraxacum kok-saghyz* Rodin SEED GERMINATION

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Abstract

Currently, Kok Sagyz is considered to be the most promising source of natural rubber. In many countries significant investments in the development of rubber production technologies based on the use of this plant are being made. Ensuring a high percentage of rapid and uniform germination is important for Kok Sagyz cultivation. In this research we have obtained results significantly extending the idea of temperature as a factor affecting the characteristics of Kok Sagyz seeds germination, which is important for this rubber plant reproduction practice. The aim of our study was to determine a practical and simple method to improve the germination of *Taraxacum kok-saghyz* seed. An orthogonal array design L_9 (3^4)] was used to optimize three factors: concentration of $KMnO_4$ solution (0.07 %, 0.1 %, and 0.2 %), soaking time (1, 2, and 4 h), and germination temperature (4/17, 17, and 23 °C). Germination parameters, including germination percentage, T_{50} (time taken for 50 % of seeds to germinate), germination index, and vigor index, were evaluated. Using analysis of variance, the optimum conditions for germination were determined, i.e. 2 h of soaking time in 0.07 % $KMnO_4$ solution and a germination temperature of 23 °C. The optimum conditions were subsequently validated. Under the optimized conditions, we achieved a germination percentage of 71 %, a T_{50} value of 11 days, and germination and vigor indices of 7.22 and 14.40, respectively. Moreover, we found that in addition to moisture, temperature was the main factor influencing the germination of *T. kok-saghyz* seeds. Solution composition and concentration and soaking time had little or no effect on germination.

Keywords: germination percentage, germination uniformity, $KMnO_4$, potassium permanganate, *Taraxacum kok-saghyz* Rodin, Russian dandelion

Taraxacum kok-saghyz Rodin (Russian dandelion) is a perennial herbaceous plant. The root contains about 2.89 to 27.89% (mass fraction) of natural rubber, molecular weight of which is 2180 [1, 2] and 25-40% (mass fraction) of inulin [3]. It can also be studied as a model plant for revealing mechanism of rubber synthesis [4]. Now it is developing as one of the most promising natural rubber plants, which attracts huge investments in many countries [1, 2, 5].

Securing a high percentage of rapid, uniform and otherwise germination was important for *T. kok-saghyz* production [6]. It is well known that *T. kok-

saghyz seed enters dormancy during the after-harvest ripening period and so how to break dormancy effectively is the key point to achieve high percentage of rapid and uniform germination. Stratification, prechilling [6], soaking and chemicals methods were found and applied. And relation of temperature, moisture, and several other factors to germination had been revealed [6]. When applying these methods, we found at least 24 hours of pretreatment were needed, which caused some inconveniences.

Some studies showed that $KMNO_4$ (Potassium Permanganate) can sterilize and break seed dormancy [7-9]. So what about effect of $KMNO_4$ solution on seed germination of *T. kok-saghyz*? Moreover, most of previous studies mainly focus on effect of methods on germination percentage of *T. kok-saghyz* seeds, except other germination indices. While germination uniformity, germination index and so on, are also important for seed germination and seedling growth afterwards.

In this study, we'll try to find a practical method to improve seed germination of *T. kok-saghyz* with $KMNO_4$ solution. And answer one question, what effects of temperature, concentration of solution, soaking time are on seed germination of *T. kok-saghyz*? These findings will contribute to facilitating its use as a natural rubber plant. At the same time, this work aims to demonstrate the potential of orthogonal array designs for establishing efficient seed germination protocols.

Our subjective was to optimize the conditions for the germination of Russian dandelion seeds using KMnO4 solution, and also to investigate the effect of temperature, solution concentration, and time for soaking on germination.

Techniques. Seeds used in all germination tests were harvested from k-445 accession of *T. kok-saghyz* which provided by Vavilov All-Russian Institute of Plant Industry. Seeds were extracted from physiologically ripe fruits of plants cultivated in an open trial field of Heilongjiang Academy of Sciences (45°34'59.9"N, 126°34'18.8"E) on June, 2014, when plants were one year of age. The region is characterized by a typical continental climate with black soil. Harvested seeds were air-dried for about two weeks and then hand threshed to minimize seed damage. Round hole sieves (0.45 mm diameter) and a seed blower were used to clean seeds. Seeds were stored for 5 months under room temperature before being treated. Seeds were pre-tested for viability with 1% concentration of TTC solution for 30 min [10].

Depending on the experiment, seeds were germinated in Petri dishes (9.0×2.5 cm) on two layers of filter paper which remained moist. One hundred seeds after being soaked in solutions were placed evenly on filter paper. Seeds for each treatment were sown under different temperatures depending on designs. The Petri dishes were watered regularly to keep the seeds moistened.

The main effects of such three factors studied at three levels were evaluated using an orthogonal array design L9 (3^4) method consisting of nine treatments. For each treatment, three replicates (three Petri dishes, with 50 seeds per Petri dish) were performed. Four factors, concentration, soaking, temperature, blank (for estimating data errors) with three possible levels (level 0, L0; level 1, L1; level 2, L2) for each factor were evaluated for their effect on the germination of *T. kok-saghyz* seeds. The levels for each factor were as follows:

(a) Concentration: L0 = 0.07% (mass fraction) concentration of $KMNO_4$ solution; L1 = 0.1% $KMNO_4$; L2 = 0.2% $KMNO_4$.

(b) Soaking time: L0 = soaking seeds in $KMNO_4$solution for 1 hour; L1= soaking seeds in $KMNO_4$ solution for 2 hours; L2 = soaking seeds in $KMNO_4$ solution for 4 hours.

(c) Temperature: L0 = seeds germinated at 23 °C after being soaked in $KMNO_4$ solution; L1 = seeds germinated at 17 °C; L2 = seeds germinated at 17 °C for 16 hours, and then switch to 4 °C for 8 hours.

Seed germination was evaluated for 20 days after the seeds were placed in Petri dishes for the first experiment, aiming to determine the levels of different factors for improving germination of *T. kok-saghyz*. For the experiments aimed at validating the improved treatment, the seed germination (Petri dishes) was evaluated for 14 days since the seeds were placed in the germination cabinet. Seeds were considered germinated when the radicle was 1 mm or greater.

The following 4 parameters, which evaluate germination process, including time, rate, homogeneity and synchrony [11], were considered for an analysis of variance (ANOVA) statistical evaluation: (a) Germination percentage (%) = (total number of germinated seeds/number of total seeds) × 100%; (b) Germination uniformity, expressed as T_{50}, namely the time for 50% seeds getting germinated. (c) Germination index: ∑(sum of germinated seeds per day/days of germination counted from the first day of testing). (d) Vigour index: germination index × radicle length (mm), reflects growth velocity and biomass comprehensively.

The effect of each factor on the characteristic properties were determined by analysis of variance (ANOVA) and Duncan's multiple range test.Data analyses was made by SPSS (version 19.0, IBM, USA) in which multivariate method of general linear model was applied. The range analysis was employed to discriminate the comparative significance of each factor in OAD. To make results more clearly and intuitive, the average scores of the summations of level1-3 of three factors were shown in line plus symbol shape in graph. To further identify the reliability of the OAD results, additional experiments under the optimum condition of the corresponding parameters plus some control experiments were performed, with three replications of every treatment.

Results. Seed germination indices we estimated are sufficient for a comprehensive assessment of the ongoing processes of growth and biomass accumulation. For three values of each of the factors, a three-way variance analysis L_9 (3^4) was finally designed (Table 1).

1. Orthogonal array matrix L_9 (3^4) to optimize seed germination of *Taraxacum kok-saghyz* Rodin

Treatment	Factors			
	solution $KMnO_4$ (%)	time, h	temperature, °C	Blank
1	1 (0,07 %)	3 (4)	3 (23)	1
2	2 (0,1 %)	2 (2)	3 (23)	2
3	3 (0,2 %)	1 (1)	3 (23)	3
4	3 (0,2 %)	2 (2)	1 (4-17)	3
5	1 (0,07 %)	1 (1)	1 (4-17)	1
6	1 (0,07 %)	2 (2)	2 (17)	2
7	2 (0,1 %)	1 (1)	2 (17)	2
8	2 (0,1 %)	3 (4)	1 (4-17)	3
9	3 (0,2 %)	3 (4)	2 (17)	1

Seeds viability test indicated that 81.57% of seeds were viable.

Impact of three factors on seed germination of *T. kok-saghyz*. Concentration of KMNO4 solution had significant effect on vigour index of seed germination of *T. kok-saghyz* ($p < 0.05$), while no significant effect on germination percentage, germination uniformity, and germination index ($p > 0.05$). Soaking time had no significant effect on all indices of seed germination of *T. kok-saghyz* ($p > 0.05$); Temperature produced significant effect on all indices of seed germination of *T. kok-saghyz* ($p < 0.05$), and highly significant effect on vigour index ($p < 0.01$) (Table 2).

2. Degrees of freedom, the *F*-ratio and its probability obtained from the ANOVA analyses for the effects of treatments and for the orthogonal comparisons between three levels for each factor tested on the *Taraxacum kok-saghyz* Rodin seed germination parameters

Source	Dependent variables	df	Square value	*F*	Sig.
Concentration	Germination percentage (%)	2	34.111	1.415	0.414
	Germination uniformity (days)	2	4.370	1.489	0.402
	Germinability	2	3.356	5.446	0.155
	Germination index	2	10.414	36.596	0.027
Time	Germination percentage (%)	2	15.444	0.641	0.610
	Germination uniformity (days)	2	0.961	0.328	0.753
	Germinability	2	0.200	0.325	0.755
	Germination index	2	2.487	8.738	0.103
Temperature	Germination percentage (%)	2	707.111	29.327	0.033
	Germination uniformity (days)	2	230.633	78.583	0.013
	Germinability	2	14.339	23.266	0.041
	Germination index	2	38.644	135.793	0.007
Error	Germination percentage (%)	2	24.111		
	Germination uniformity (days)	2	2.935		
	Germinability	2	0.616		
	Germination index	2	0.285		

Establishing an improved seed germination treatment. As far as germination percentage was concerned, the bigger is the better. When concentration of $KMNO_4$ increased, germination percentage decreased; more or less soaking time in $KMNO_4$ solution was not adequate for germination percentage; 4 °C-17 °C treatment was better than 4 °C treatment, but not as good as 23 °C treatment. So the best conditions for germination percentage of *T. kok-saghyz* were 1.0% $KMNO_4$ concentration, 2 h soaking time and 23 °C treatment (Fig. 1).

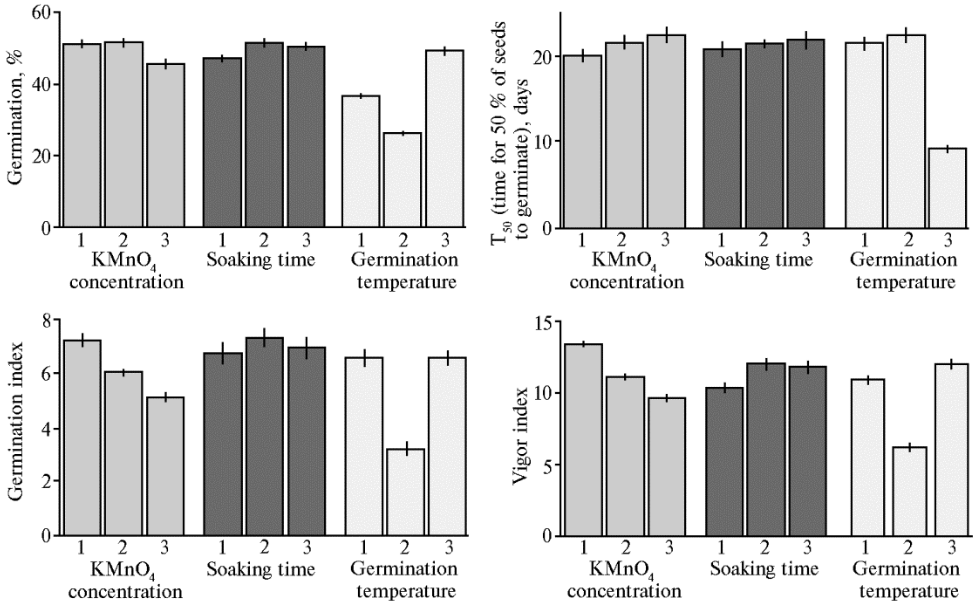

Fig. 1. Effects of three levels of factors on the *Taraxacum kok-saghyz* Rodin seed germination (description of the experimental design see in the section *Techniques*).

T_{50} (time for 50% seeds germinated) stands for germination uniformity. So the smaller one is the better. When concentration of $KMNO_4$ increased, germination uniformity increased; soaking time increased, germination uniformity increased; 23 °C treatment was better than 4-17 °C and 17 °C treatments. So the best conditions for germination uniformity were 0.07% of $KMNO_4$ concentration, 1 h of soaking time and 23 °C of germination temperature (see Fig. 1).

As far as germination index was concerned, the bigger is the better. When concentration of $KMNO_4$ increased, germination index decreased; 2 h of soaking time was better than 4 h, which was better than 1 h; 23 °C of germination temperature was better than 17-4 °C treatment, which was better than 4 °C treatment. So the best conditions for germination index were 0.07% concentration of $KMNO_4$ solution, 2 h of soaking time, 23 °C of germination temperature (see Fig. 1).

As far as vigour index was concerned, the bigger is the better. When concentration of $KMNO_4$ increased, vigour index decreased; 2 h of soaking time was better than 4 h, which was better than 1 h; 23 °C of germination temperature was better than 17-4 °C treatment, which was better than 4 °C treatment. So the best conditions for vigour index were 0.07% concentration of $KMNO_4$ solution, 2 h of soaking time, 23 °C of germination temperature (see Fig. 1).

As above showed, when concentration was 0.07%, germination temperature was 23 °C, all of the germination indices of *T. kok-saghyz* were the best; While when soaking time was 2 h, all of the germination indices were the best too, except germination uniformity, but considering soaking time in $KMNO_4$ solution had no significant effect on all indices of seed germination of *T. kok-saghyz* (see Table 2), 2 h of soaking time could be recognized as the best. We finally concluded that all germination indices of *T. kok-saghyz* were the best when seeds of *T. kok-saghyz* were soaked into 0.07% (mass fraction) concentration of $KMNO_4$ solution for 2 h, and then made germinated under 23 °C.

Validation of the improved germination treatment. The improved germination protocol was not among the treatments tested in the orthogonal array design. To validate the proposed treatment for its germination in Petri dishes, wedesigned and implemented the validated experiment. Some works [13, 14] suggested that low temperature (usually 0-5 °C) may increase germination uniformity of seeds, so we set two low-temperature treatments to determine effect of low temperature on germination of *T. kok-saghyz* seeds and compare results of two different time-disposal treatments. Levitt and Hamm [12] suggested that a certain concentration of KNO_3 solution can improve germination percentage and other indices through promoting physiological maturation of seeds, so we also set a 3% KNO_3 treatment to compare the results between proposed treatments. Finally, we took distilled water treatment as the control (Table 3).

3. Validating experiment design to test suggested protocol for the *Taraxacum kok-saghyz* Rodin seed germination

Treatment	Solution	Concentration, %	Soaking time, h	Temperature
1	$KMnO_4$	0.07	2	23 °C
2	$KMnO_4$	0.07	2	One week under 4 °C, then under 23 °C
3	$KMnO_4$	0.07	2	Two weeks under 4 °C, then under 23°C
4	KNO_3	3.00	24	23 °C
5	Distilled water		2	23 °C

The proposed treatment 1 had the highest germination percentage (71%), compare to other treatments; KNO_3 solution increased germination percentage of *T. kok-saghyz* seeds, but not as high as the proposed treatment, with significant difference.

One-week of 4 °C-disposal increased germination percentage, with no significant difference from control, namely distilled water treatment. Two-week of 4°C-disposal didn't increase germination percentage, with significant difference from one week of 4 °C -disposal (Fig. 2).

One-week 4 °C-disposal decreased germination uniformity (increased time of T_{50}) significantly compared to other treatments, up to 17 days, while there were no significant differences among other treatments.

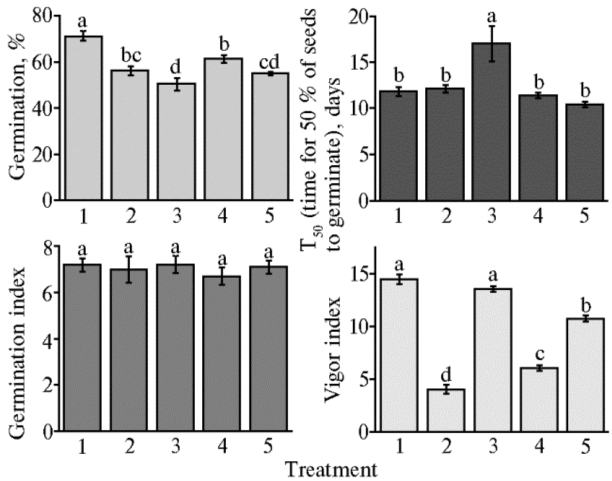

Fig. 2. Validation experiment — effect of treatments on germination of the *Taraxacum kok-saghyz* Rodin seeds: 1, 2, 3, 4, 5 — treatments (see Table 3).

Compared to control, the four treatments didn't affect germination index significantly (see Fig. 2). Considering the five treatments (all germinated under the same temperature), we may make a conclusion: germination temperature maybe the only factor which affects germination index of *T. kok-saghyz* seeds, while no matter what kinds of pretreatments, including sorts of solution pre-soaked orthe concentration of the solution, or the seeds of pre-stored under low temperature or not.

Vigour indexes of the proposed treatment were the highest, up to 14.40; One-week 4 °C disposal treatment got the lowest vigour index, while two-week of 4 °C disposal treatment increased vigour index apparently, up to the same level as the proposed treatment ($\alpha = 0.05$); KNO_3 treatment also decreased vigour index significantly, compare to the control.

In this study, we have shown that temperature had significant effect on all seed germination indices of *T. kok-saghyz*, thus we can state that temperature is the main impact factor on seed germination of *T. kok-sazhyz* besides moisture. The other factors, nature of solution, concentration of solution and soaking time had slight or none effect on seed germination of *T. kok-saghyz*. The conclusion is consistent with Levitt and Hamm's studies [12] in some ways. So if we kept *T. kok-saghyz* seeds certain moisture and made them germinated at suitable temperature (23 °C), we can get desired results in most cases.

The best seed germination conditions for *T. kok-saghyz* is 0.7% concentration of $KMNO_4$ with 2 h of soaking time, 23°C of germinated temperature, which was validated later. Under the condition, germination percentage can be up to 71%, germination uniformity (period for 50% seeds germinated) is about 11 days, germinability is about 20, germination index is about 7.22, vigour index is about 14.40. Germination percentage in our investigation were not as high as previous studies [6, 12], as well as germination uniformity. The most possible reason is seed quality. Because we found almost all the left seeds without germination were empty. And in validating experiment, treatment 4 applied KNO_3 solution to break seed dormancy as Levitt and Hamm [12] suggested, the results were not those their expected. So the only possible reason is seed quality, the maturity, which made some empty seeds and less physiological ripen seeds. And so the method needs more seeds with high quality to validate. Seeds used in the present study were one-year old, so how about two-year old seeds or more. Is the proposed method still applicable? We need more experiments to answer these questions.

According to Roberts [15], there are three separate physiological processed in seeds affected by temperature: first, temperature, together with moisture content, determines the rate of deterioration in all seeds; secondly, temperature affects the rate of dormancy loss in dry seeds and the pattern of dormancy change in moist seeds; and, thirdly, in non-dormant seeds temperature determines the rate of germination. So before discussing effect of temperature on seed germination, we have to answer one question: are *T. kok-saghyz* seeds dormant? *T. kok-saghyz* seed enters dormancy during the after-harvest ripening period. Seed dormancy refers to a state in which the viable seeds fail to germinate when provided with conditions normally favorable to germination such as adequate moisture, appropriate temperature regime and light [16]. Our validating experiment showed that treatment 1 got about 71% of germination percentage, significantly higher than the control (54% of germination percentage) under the same other conditions. This means about 17% seeds were dormant and necessary to be stimulated by $KMNO_4$ to germinate. So we can state that most of one-year old *T. kok-saghyz* seeds are not dormant, only a small amount of seeds (about 17%) are dormant. Therefore, temperature determines the rate of germination of *T. kok-saghyz* seeds.

It has been known for a long time that pretreatment of seeds with Oxidants such as $KMNO_4$, H_2O_2 leads to breaking seed dormancy [17]. In the present study, we found seeds under $KMNO_4$ treatments didn't go moldy, compare to those under no $KMNO_4$ treatments with about 15% of moldy rate. So we stated that $KMNO_4$ has two effects on *T. kok-saghyz* seeds, one is to improve seed germination by breaking seed dormancy. $KMNO_4$ can remove seed coat obstacle, and provide oxygen and Manganese elements, which can strengthen respiration and trophism for germination. Anothereffect is to avoid seeds going moldy by sterilization. Because we found most of the moldy seeds were blank, so $KMNO_4$ increased seed germination of *T. kok-saghyz* mainly by breaking seed dormancy. The concentration should be controlled no more than 0.1% and 0.07% is better, otherwise vitality of germinated seeds maybe decreased.

We suggest 23 °C is the optimal temperature for seeds germination of *T. kok-saghyz*. *T. kok-saghyz* seeds don't germinate at all at 4 °C. The conclusion is not subject to Levitt and Hamm [12], whose investigation showed seeds germination percentage can be up to 71% after pretreatment. As seeds were soaked in $KMNO_4$ solution for 4 hours, they lasted not long as that of Levitt and Hamm's investigation. Because the longest soaking time of seeds were 4 h, far less than that in the soaking time of seeds in Levitt and Hamm [12] studies. So the sole reason maybe is that seeds at 4 °C need more moisture to germinate than that under other temperatures to lessen the effect of low temperature.

In the present study, 4-17 °C treatment showed better than single 17 °C treatment on all germination indices, which is consistent with Hegarty's study [18], which showed that seeds may germinate faster than would be predicted on the basis of thermal time alone in fluctuating temperature environments [19]. So maybe 4-23 °C alternative treatment improve seeds germination better more than single 23 °C treatment. Related experiments can be carried out in the future.

Thus, our findings demonstrate that temperature is the main impact factor on seed germination of *T. kok-saghyz*, besides moisture. Nature, concentration of solutions and soaking time had none or slight effect. The best germination conditions for one-year old seeds of *T. kok-saghyz* are soaking in 0.7% $KMNO_4$ solution for 2 h before germinating at 23 °C. The method need to be validated in field plot. The 23 °C is the optimal temperature for seed germination of *T. kok-saghyz*, 4 °C is not. Most of one-year old seeds of *T. kok-saghyz* are not dormant. These conclusions shall be truly verified by high-maturity seeds.

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