RESEARCH ARTICLE

Effect of compound treatments of sodium carbonate and kinetin on seed germination of *Zanthoxylum armatum* DC.

Manyi Fu[1, 2, *], Daoyuan Zhou[1]

[1]Xinyang Agriculture and Forestry University, Xinyang, Henan, China. [2]Xinyang Engineering Technology Centre for Woody Oilseed Crop Cultivation and Development, Xinyang, Henan, China

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***Zanthoxylum armatum* DC. is an important oil plant and economic crop, but the natural reproduction rate is extremely low due to the existence of dormancy in its seeds, so it is of great significance to explore methods to promote its seed germination. In this study, an orthogonal test was applied to study the effects of different factors on the seed germination indexes with four factors and four levels of different sodium carbonate degreasing time, different incubation temperature, different kinetin soaking concentration, and seed soaking time. Through this study, analysis of variance, correlation, and clustering was applied. The results demonstrated that all experimental groups showed significant improvement in different germination indexes compared with the control. Among which, group 7 treated with sodium carbonate degreasing for 12 h, kinetin soaking concentration of 50 mg/L for 48 h, and under the culture condition of constant temperature at 25°C showed the best performance in germination rate, germination vigor, vitality index, germination index, root length, and shoot length. In addition, the shoot fresh weight and dry weight, root dry weight and fresh weight of group 7 were also significantly increased compared with the control. The results of this study provided a certain theoretical basis for the efficient seed germination of *Zanthoxylum armatum* DC. in the future.**

Keywords: *Zanthoxylum armatum* DC.; orthogonal test; kinetin; seed germination.

[*]**Corresponding author:** Manyi Fu, Xinyang Agriculture and Forestry University, Xinyang 464000, Henan, China. Email: fmy556@xyafu.edu.cn.

Introduction

Zanthoxylum armatum DC. with its tiny and narrow leaves shaped like bamboo leaves is a small deciduous tree or shrub of the family *Rutaceae*, genus Pepper [1]. The various parts of it including roots, stems, leaves, peels, seeds, *etc*. all have economic and edible values [2-4]. It is a woody economic tree species that integrates edible, medicinal, and ecological value. However, at present, its standardized production degree is low, especially in seedling cultivation, the seeds have low germination rate or germination difficulties and other phenomena, which restricts the popularization and application of it. Therefore, it is of great production and practical importance to study how to deal with the seeds and promote their germination.

Temperature, moisture, and air are among the most important factors affecting the seed germination, and in addition to these factors, seed germination is also affected by the hardness of the seed coat and storage time [5, 6]. The seed coat of *Zanthoxylum armatum* DC. is thick, hard, and full of oil, which makes the germination difficult and low seedling rate. Additionally, the presence of germination inhibitors inside the

seed is one of the reasons [7]. Therefore, breaking the seed coat barrier is the key to improving the germination seeds rate. Previous study showed that the germination ability of *Zanthoxylum schinifolium* Siebold & Zucc was enhanced under constant temperature and darkness environment after cold and heat treatment and gibberellic acid (GA3) soaking [3]. Another study found that alkaline water together with sand storage under cold temperature treatment and alkaline water together with hormone treatment could improve the germination rate of pepper seeds [8]. Liu *et al*. treated pepper seeds with sodium carbonate to remove the oil skin or concentrated sulfuric acid for 5-10 mins and then soaked the seeds with 500 mg/L erythromycin to promote germination, which was favorable to seed germination [7]. Cui also found that 30 days of stratification treatment was favorable for the seed germination of *Zanthoxylum armatum* DC. [9]. The adoption of seed soaking of phytohormone is also a simple and effective way to break seed dormancy, promote seed germination, and develop strong seedlings. As a typical exogenous hormone, kinetin has an obvious promotional effect on seed germination [10, 11]. Zuo *et al*. found that the appropriate concentration of kinetin was beneficial to the seed germination of *Vallisneria natans* (Lour.) Hara [12]. Wei *et al*. showed that appropriate concentrations of kinetin could increase the germination rate and germination vigor of seeds [13].

Based on previous research, this study was to investigate suitable methods for improving the seed germination of *Zanthoxylum armatum* DC. by using sodium carbonate degreasing treatment at different time, kinetin seeds soaking treatment at different time and concentrations, and culture conditions under different temperatures, thus, providing a certain theoretical basis for the large-scale nursery of *Zanthoxylum armatum* DC. in the future.

Materials and Methods

Collection of *Zanthoxylum armatum* DC seeds

Zanthoxylum armatum DC. used in this study were collected in October 2023 in Ziba Au Village, Tiechang Town, Rong County, Zigong City, Sichuan, China. The fruits were transported to the central laboratory of Xinyang Agriculture and Forestry University (Xinyang, Henan, China) immediately after picking. After the fruits were dried in the shade and the seeds fall off naturally, the impurities were removed to obtain pure pepper seeds.

Design of orthogonal experiments

The orthogonal experimental design was carried out through an orthogonal test table. In this study, L16.4.4 orthogonal table was applied to fully utilize the four factors in seed treatment including time of sodium carbonate degreasing, kinetin concentration, time of kinetin immersion, and different incubation temperatures with a total of 16 groups and three replications of each. Meanwhile, the seeds treated without sodium carbonate degreasing, kinetin soaking, and at room temperature were used as a control group (CK) (Table 1). According to the previous report, the stored seeds were poured into the container with water and soaked for 12 h to remove the uplifted seeds, and the sunken seeds were selected for the experiment [14]. Sodium carbonate was used for the degreasing treatment at 6, 12, 24, and 48 hours with the concentration of sodium carbonate as 2.5% to make the ratio of sodium carbonate and seed weight as 1:40. After treating the seeds with sodium carbonate, seed oil was rubbed off with gauze, and then rinsed repeatedly with sterile water for several times before soaking the washed seeds in water for 12 h and rinsing repeatedly with sterile water. The remaining oil was rubbed off again with gauze. The seeds were then treated with sodium carbonate and clean water in the kinetin solution at the concentrations of 10, 25, 50, and 100 mg/L, and soaked for 12, 24, 36, 48 h, respectively. After soaking treatment, the seeds were washed several times with sterile water, and rubbed strongly and repeatedly until lost their luster. The seeds were then sterilized with alcohol for 1 min and rinsed four to five times with sterile water

Table 1. Orthogonal factors and levels combinations of 2.5% sodium carbonate and kinetin compound treatment Table L_{16} (4^4).

Number	2.5% sodium carbonate degreasing time (h)	Kinetin concentration (mg/L)	Kinetin seed soaking time (h)	Incubation temperature (°C)
1	6	10	12	25
2	6	25	24	18/25
3	6	50	36	25/35
4	6	100	48	25
5	12	10	24	25/35
6	12	25	12	18/25
7	12	50	48	25
8	12	100	36	18/25
9	24	10	36	25/35
10	24	25	48	25/35
11	24	50	12	18/25
12	24	100	24	25
13	48	10	48	18/25
14	48	25	36	25
15	48	50	24	25
16	48	100	12	25/35

Note: 18/25℃ and 25/35℃ both refers to the alternation of two temperatures under the circumstance of the darkness: light = 12 h : 12 h alternately change.

before being placed in petri dishes with sterilized sand bed. 20 seeds were placed in each petri dish and sealed with cling fil. The seeds were placed in the 18/25℃, 25℃, and 25/35℃ incubators, respectively, and the seed germination was observed every day.

Measurement of indicators

The germination vigor was calculated from the 7th day of the experiment. The germination rate was calculated from the 15th day, and every day thereafter. The germination index (GI) mainly reflected the vitality of the seeds in terms of the germination speed. The vitality index (VI) was the product of the GI and the growth potential of the seedlings. The growth potential of the seedlings was often expressed in terms of the average dry weight or the average height of the plant. At the end of the experiment, root fresh weight (mg), shoot fresh weight (mg), root dry weight (mg), shoot dry weight (mg), root length (cm), and shoot length (cm) were counted and determined.

$$Germination\ potential\ \% = \frac{\text{Total number of germination on and before the day}}{\text{Number of experimental seeds}} \times 100\%$$

$$Germination\ rate\ \% = \frac{\text{Number of seeds germinated on the day}}{\text{Number of experimental seeds}} \times 100\%$$

$$\text{Germination index (grain/d) Gi} = \sum (\text{Gt/Dt})$$

$$\text{Vitality index Vi} = \text{Gi} \times \text{S}$$

where Gt was the number of germinations on day t. Dt was the corresponding germination time. S was the average dry mass of a single shoot at the end of germination.

Statistical analysis and graphing

Data significance analysis of variance was performed using SPSS 26.0 (IBM, Armonk, NY, USA). Data statistics and graphs were prepared using Excel 2010 software (Microsoft, Redmond, WA, USA). The correlation and clustering analyses were performed using the R language (Bell Laboratories, Murray Hill, NJ, USA).

Results and discussion

Effect of compound treatments on germination rate

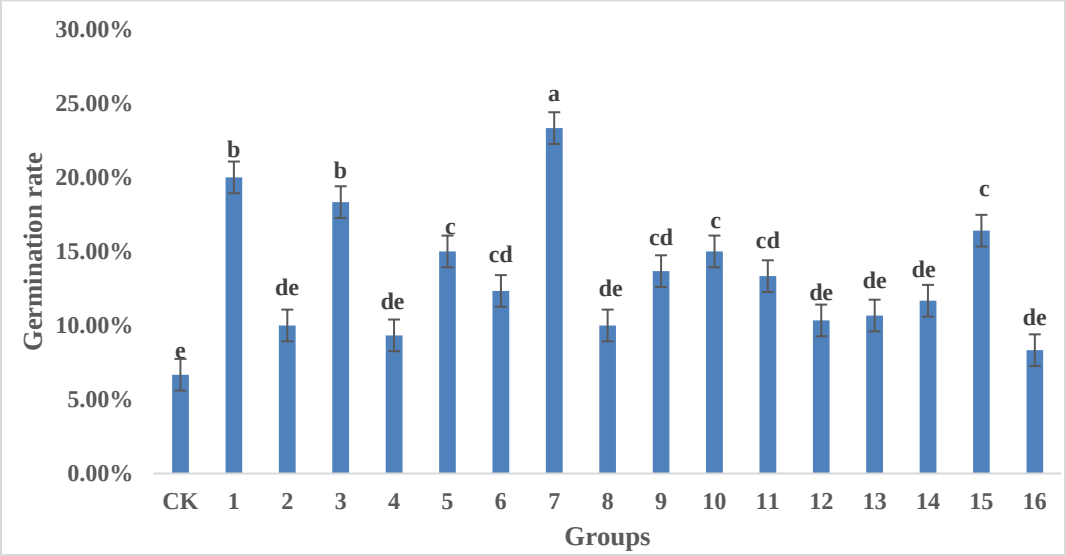

Figure 1. Effect of compound treatments on germination rate.

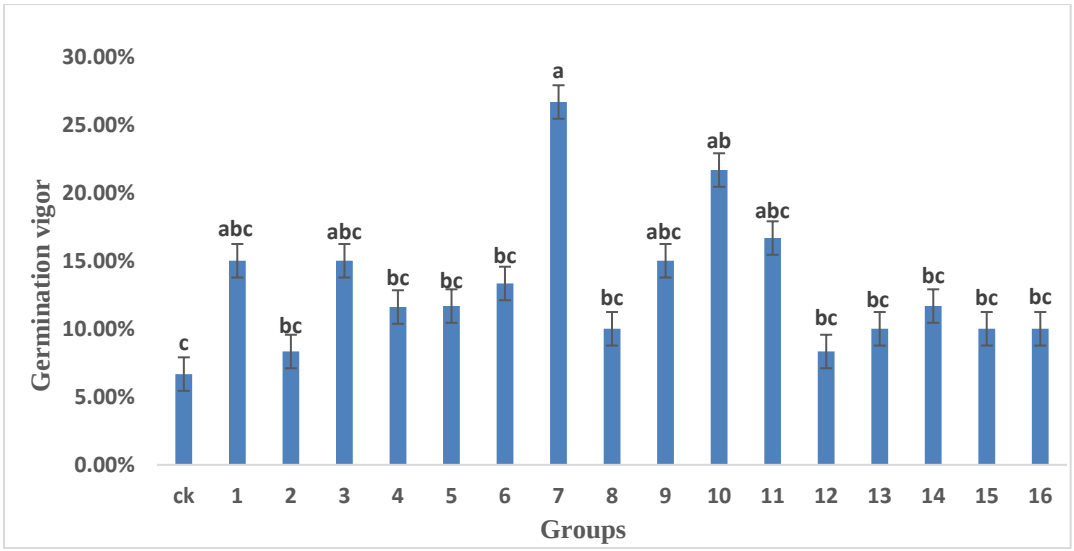

Figure 2. Effect of compound treatments on germination vigor.

The seeds were treated with 2.5% sodium carbonate for different degreasing times of 6, 12, 24, and 48 h before soaked in 10, 25, 50, and 100 mg/L of kinetin for 12, 24, 36, and 48 h. The seeds were then placed in the incubator at different temperatures of 18/25, 25, and 25/35°C (Figure 1). The results showed that the different combination of treatments had a positive effect on the germination rate. The best germination condition was found in group 7, which had a germination rate of 25%, 2.7 times higher than that of the control, and the difference was significant ($P < 0.05$). Among them, treatments 1, 3, 6, 9, 10, 11, and 15 also showed significant promotion effect compared with the control ($P < 0.05$). Group 7 treated with 2.5% sodium carbonate degreasing for 12 h, 50 mg/L kinetin for 48 h, and incubating at constant temperature of 25°C demonstrated the highest germination rate.

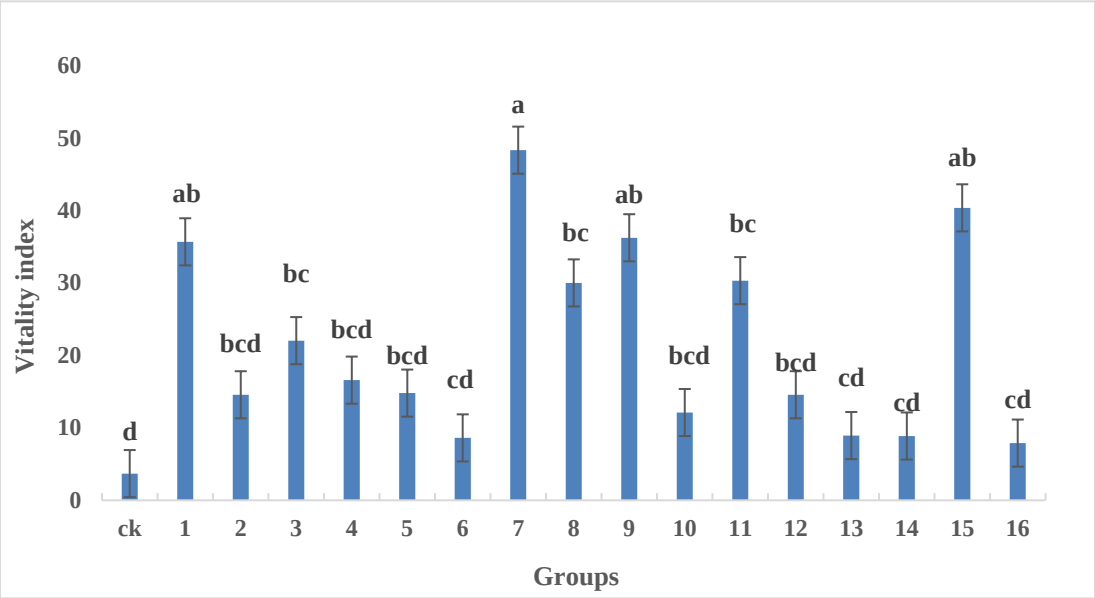

Figure 3. Effect of compound treatments on vitality index.

Effect of compound treatments on germination vigor

Among the 16 different groups, group 7 had the highest germination vigor of 26.67%, which differed significantly from the control ($P < 0.05$) and was three times higher than the control. Group 10 improved 2.25 times compared to control, while other treatments did not show significant difference from control (Figure 2). The results indicated that the highest germination vigor was achieved under group 7 culture conditions.

Effect of compound treatments on vitality index

Group 7 showed the largest vitality index of 48.32, which was significantly different from the control ($P < 0.05$) and 12 times higher than the control. Groups 1, 3, 8, 9, 11, and 15 also had significant differences in vitality index compared to the control ($P < 0.05$), while the other 9 groups showed no difference compared to the control (Figure 3). The results indicated that the highest vitality index was achieved under group 7 culture conditions.

Effect of compound treatments on germination index

The germination index under the condition of group 7 was the largest, which was significantly different from the control ($P < 0.05$) and increased 4.8 times compared with the control. There was no significant difference between control and groups 2, 6, 10, 13, 16, while the other 10 groups were also significantly different from the control ($P < 0.05$) (Figure 4). The results showed that the germination index under group 7 culture condition was the best.

Effect of compound treatments on dry and fresh weight of roots

Group 15 showed the greatest effect on the dry weight of roots with a maximum value of 15 mg, which was significantly different from the control ($P < 0.05$) and increased by 1.6 times compared with the control. Groups 1, 7, 8, and 10 also showed significant differences compared with the control ($P < 0.05$), while the remaining 11 groups showed insignificant differences compared with the control. The results indicated that root dry weight was the heaviest under group 15 conditions (Figure 5). The maximum root fresh weight was reached in group 6 with a maximum value of 94.8 mg, which was significantly different ($P < 0.05$) and 0.25 times

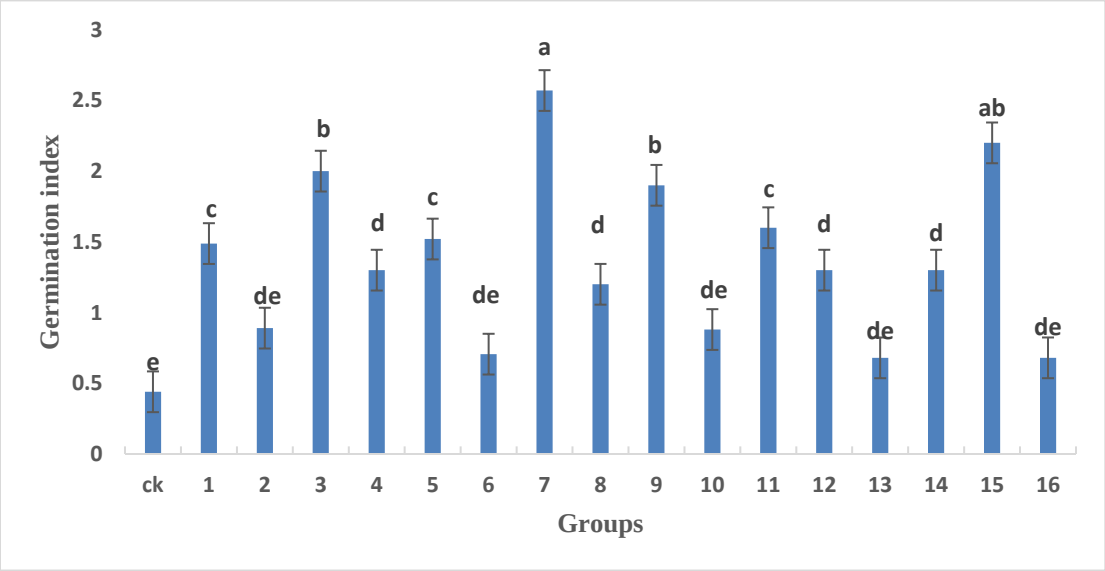

Figure 4. Effect of compound treatments on germination index.

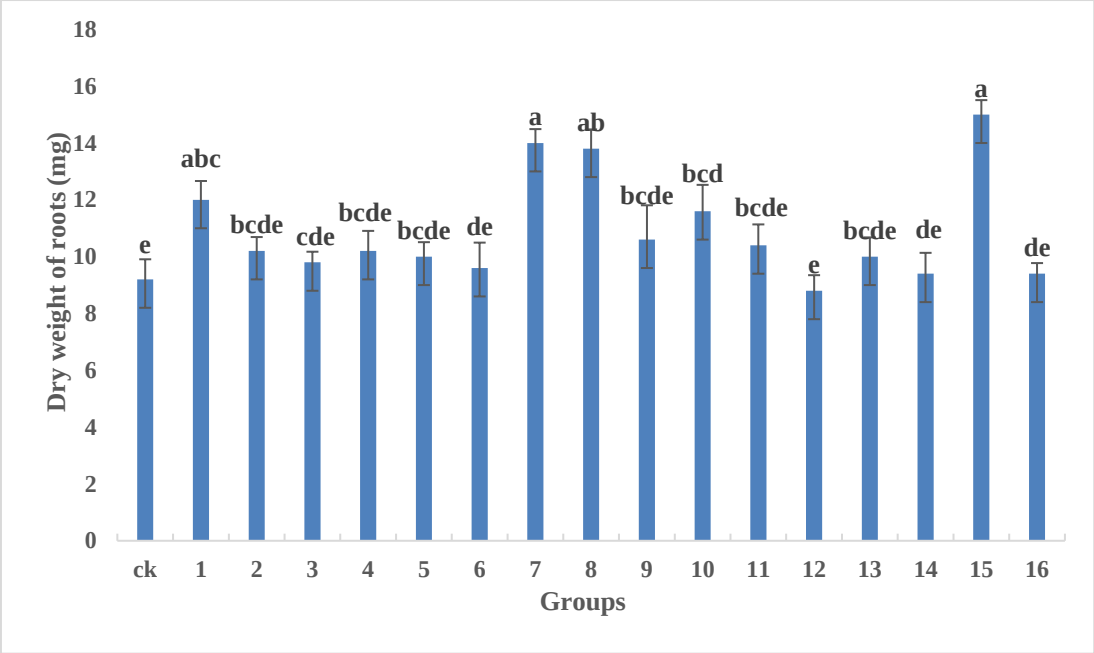

Figure 5. Effect of compound treatments on dry weight of roots.

higher than the control. Groups 1, 3, 7, 11, 12, 14, 15, and 16 were also significantly different to the control group ($P < 0.05$), while the other seven groups were not significantly different (Figure 6). The results indicated that group 6 culture conditions had the best performance in terms of fresh weight of roots.

Effect of compound treatments on dry and fresh weight of shoots

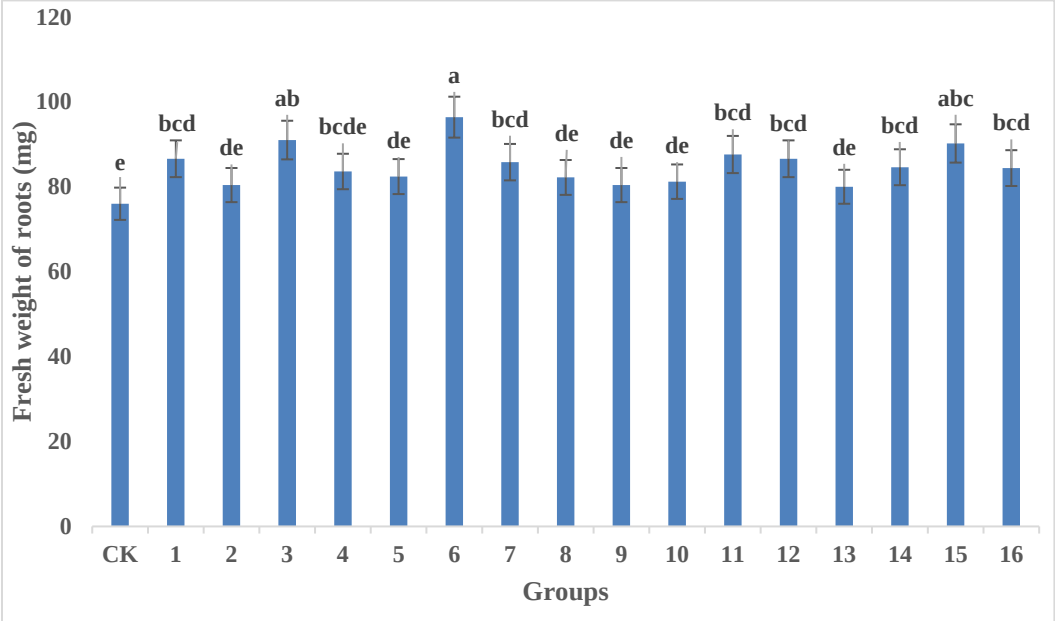

Figure 6. Effect of compound treatments on fresh weight of roots.

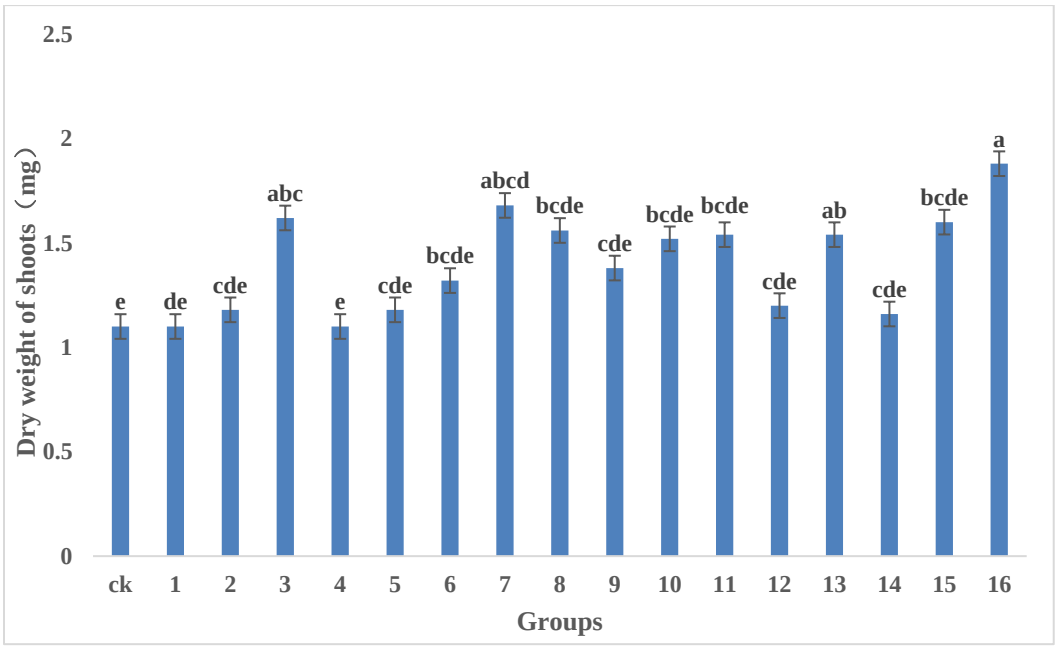

Figure 7. Effect of compound treatments on dry weight of shoots.

The dry weight of shoot can reflect the strength of the growth and development of the aboveground part of the plant. The heavier the dry weight of the shoot, the stronger the aboveground part of the plant grows. Group 16 showed the highest one with a maximum value of 1.98 mg, which was significantly different and 0.83 times higher than that of control ($P < 0.05$). Groups 3, 7, and 13 were also significantly different to the control ($P < 0.05$), while the

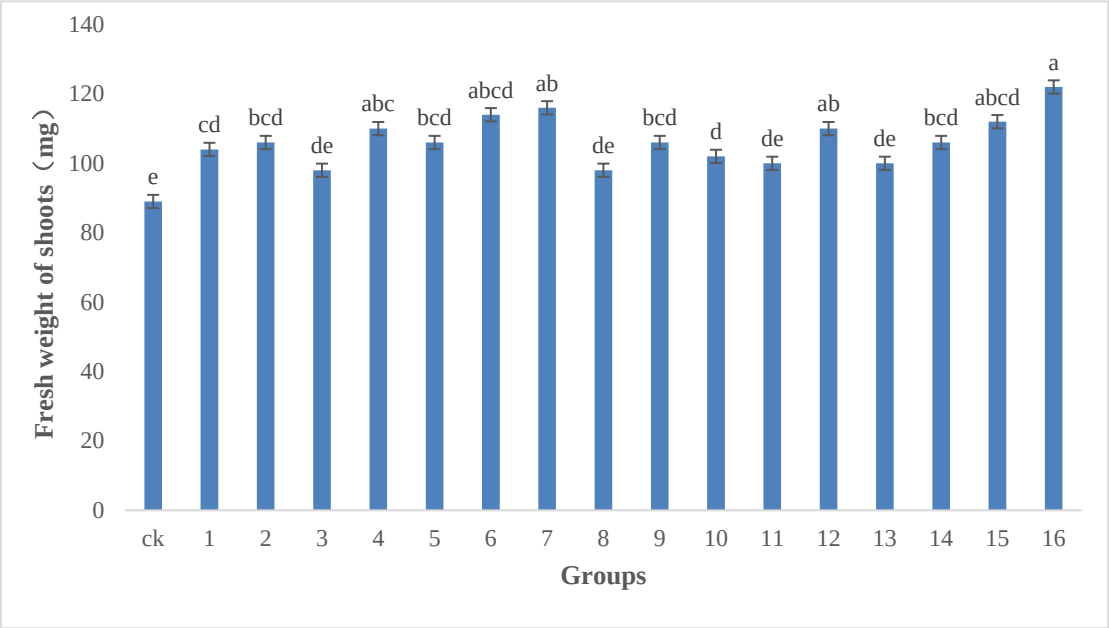

Figure 8. Effect of compound treatments on fresh weight of shoots.

remaining 12 groups were not significantly different compared to the control (Figure 7). The results indicated that the best shoot dry weight was obtained under group 16 culture conditions. The maximum fresh weight was reached in group 16 too with a maximum value of 116 mg, which was significantly different and 0.35 times higher than the control (P < 0.05). There was no difference in groups 3, 8, 11, and 13 compared to control, while the remaining 12 groups were significantly different to control (P < 0.05) (Figure 8). The results showed that group 16 treated with 2.5% sodium carbonate degreasing for 48h, kinetin soaking of 100 mg/L for 12 h, and incubating at 25/35°C had the best fresh weight of shoot.

Effect of compound treatments on root length and shoot length

The root system is the most basic source of nutrients for plants. The length of the root system refers to the total length from the root tip to the rhizome, and its length can directly reflect the quality of seedlings. The stronger the root system of a plant, the stronger its ability to absorb nutrients, which is conducive to the overall growth and development of the plant. The longest root length of 2.78 cm was observed under the condition of group 7 with a significant difference to the control (P < 0.05). The groups 1, 4, 5, 6, 10, 11, 14, and 15 were also significantly different from the control (P < 0.05), while the other seven groups showed no significant difference (Figure 9). The results indicated that group 7 had the best performance in terms of root length. Figure 10 demonstrated that the shoots under group 7 condition was the longest with a length of 1.575 cm, which was significantly different to the control (P < 0.05) and 0.47 times higher than the control. There was no difference between treatment 8 and the control, while the remaining 15 groups were significantly different from the control (P < 0.05). The results showed the longest sprouting shoot length under group 7 culture condition.

Cluster analysis of germination treatments and indicators of germination

Treating seeds with 2.5% sodium carbonate degreasing for different times of 6, 12, 24, 48 h followed by soaking seeds in different concentrations of kinetin at 10, 25, 50, 100 mg/L for 12, 24, 36, and 48 h, and germinating seeds at different temperatures of 18/25, 25, 25/35℃,

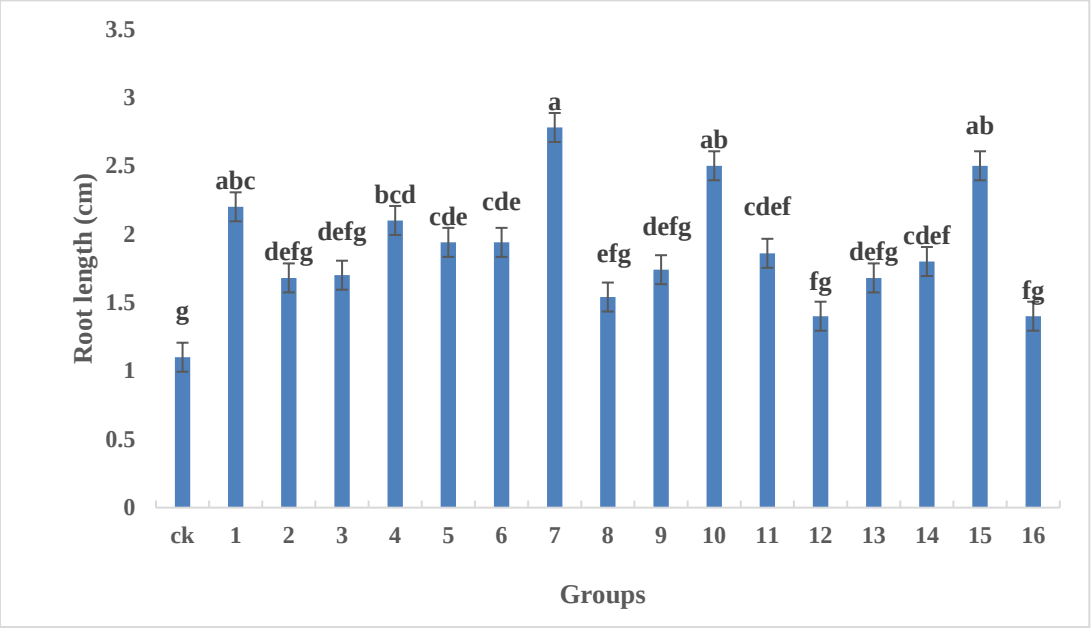

Figure 9. Effect of compound treatments on root length.

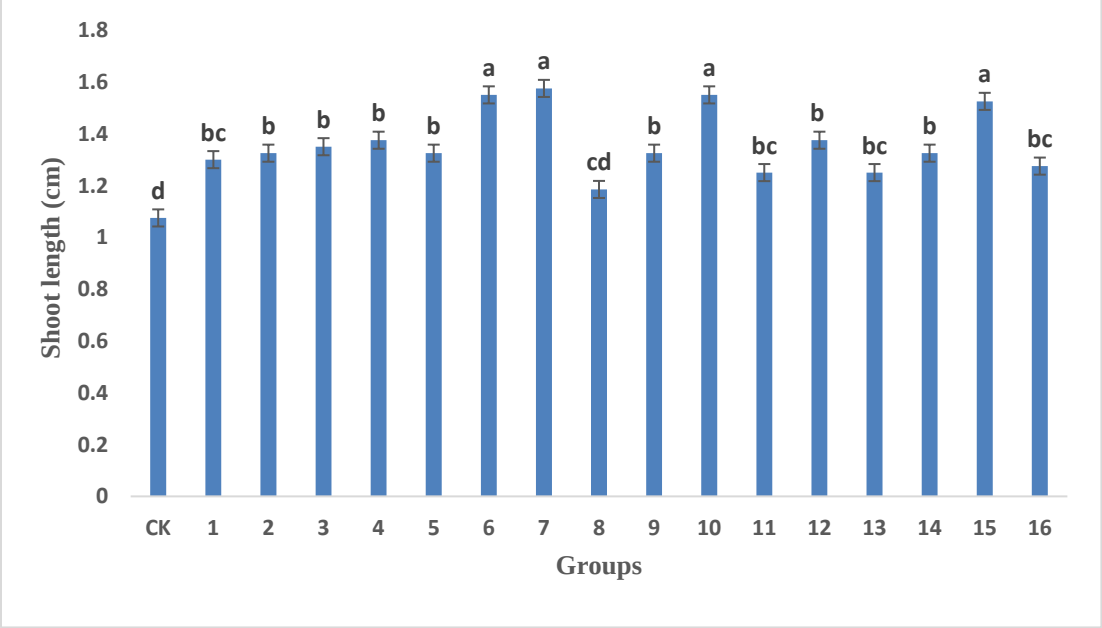

Figure 10. Effect of compound treatments on shoot length.

the 16 kinds of compound treatments as well as control experimental data were converted into txt format data set, imported into R language software for clustering analysis and heat map drawing (Figure 11). The results showed that the 8 germination index clusters could be divided into 3 types including shoot fresh weight (SFW), shoot length (SL) in one group; root length (RL) as a group; germination rate (GP), germination vigor (GV), root dry weight (RDW), root fresh weight (RFW), shoot dry weight (SDW) in one group.

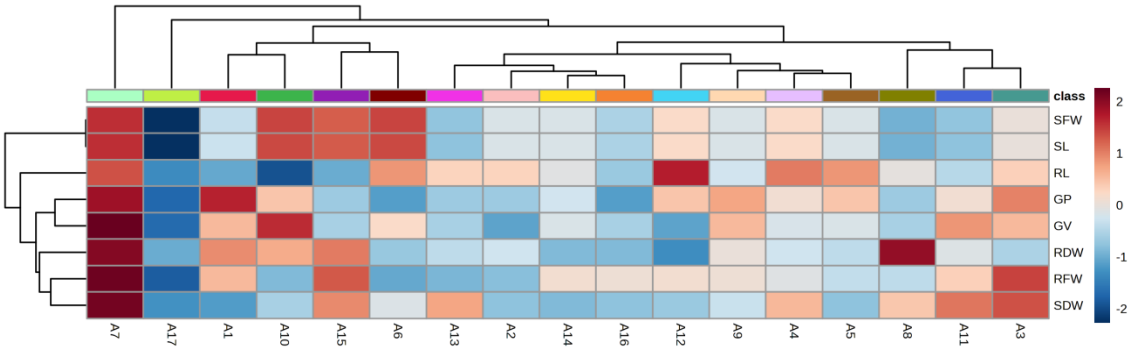

Figure 11. Cluster analysis of germination traits of compound treatments. GP: germination percentage. GV: germination vigor. RL: root length. SL: shoot length. RDW: root dry weight. RFW: root fresh weight. SFW: shoot fresh weight. SDW: shoot dry weight.

The clustering of the 17 sprouting conditions could be categorized into 5 main groups including optimal sprouting conditions (A7), natural sprouting conditions (A17, the control group), good sprouting conditions (A1, A6, A10, and A15), normal sprouting conditions (A3, A8, and A11), and non-promoted sprouting conditions (A2, A4, A5, A13, A14, A16, A9, and A12). The heat map demonstrated that the highest values of germination indexes were obtained under the A7 treatment, and the germination conditions of the treatments in the middle part of the figure were poorly germinated.

According to previous research on pepper seed germination, the use of 2.5% sodium carbonate degreasing treatment combined with hormone soaking could well promote the seed germination. The seed germination rate of *Zanthoxylum armatum* v. novemfolius could be improved by soaking the seeds in 2.5% sodium hydroxide for 12 h followed by soaking them in water for 24 h [15]. Another study showed that germination rate of *Zanthoxylum dissitum* Hemsl. could be improved by breaking the seed coat soaked them with gibberellin and then mixed the soil with grass charcoal [16]. The promotional effect of kinetin on pepper seeds was similar to that of other crop seeds, and appropriate concentrations of kinetin could increase the germination rate of many kinds of seeds, such as *Oryza sativa* L., *Zea mays* L., and *Vallisneria natans* [17-20]. The thick and hard seed coat leads to low seed germination rate, while the hormone can effectively improve the seed coat permeability and activate the seed embryo cells to make the seeds germinate. The results of this study showed that 2.5% sodium carbonate degreasing treatment combined with kinetin soaking could significantly improve the many indicators of germination, which was similar to the results of previous studies.

Conclusion

This study designed 16 groups of compound treatment with 2.5% sodium carbonate degreasing for different time, different concentrations of kinetin for different seed soaking time, and incubation under different temperatures. The results showed that group 7 had a significant effects on germination rate, germination vigor, germination index, vitality index, root length, and shoot length with 2.5% sodium carbonate degreasing for 12 h, seed soaking with 50 mg/L kinetin for 48 h, and incubating at constant temperature of 25°C. Group 16 had a significant promotion effects on shoot fresh weight and shoot dry weight with 2.5% sodium carbonate degreasing for 48 h, seed soaking with 100 mg/L kinetin for 12 h, and incubating at 25/35℃. Group 6 showed a significant promotion effect on the root fresh weight with 2.5% sodium carbonate degreasing for 12 h, 25 mg/L kinetin soaking for 12 h, and incubating at 18/25℃. Group 15 demonstrated a significant promotion of root dry weight with

2.5% sodium carbonate degreasing for 48 h, 50 mg/L of kinetin soaking for 24 h, and incubating at 25℃. Cluster analysis showed that the best overall performance was achieved in group 7. However, the seed germination rate was still relatively low in this study because of not applying stratification treatment. The effects of different stratification treatments on the seeds germination of *Zanthoxylum armatum* DC. should be studied in the future. In addition, to have a more comprehensive understanding of the factors affecting the seeds germination, temperature changes and light should be added to the subsequent research.

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