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Full Length Research Paper

Influence of temperature, light and plant growth regulators on germination of black pepper (*Piper nigrum* L.) seeds

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Effects of temperature, light and different concentrations of plant growth regulators on germination of *Piper nigrum* L. seeds was studied under controlled environmental conditions. Black pepper seeds were placed in Petri dishes with filtration papers and the germination and radical development followed during eighteen days periods. The seeds generally germinated within six or seven days. There was no difference in percent germination between dark and light treatments, but the development of radical length was significantly influenced by both light and temperature. Germination was highest at 30°C, but seeds also germinated at 25 and 35°C. No germination was observed at low (20°C) and high (40 and 45°C) temperatures. The plant growth regulators enhanced the seeds germination and radical length different degree. The results are consistent with the *Piper nigrum L.* being recalcitrant species need a certain environment condition to germinate.

Key words: Temperature, light, plant growth regulator, black pepper, *Piper nigrum* L.

INTRODUCTION

Known as the "King" of spices, black pepper (*Piper nigrum* L.), a perennial crop of the tropics, is economically the most important and the most widely used spice crop of the world (Ravindran, 2000; Weiss, 2002). Black pepper use range from a simple dietary constituent to that of immense pharmacological benefits. Black pepper oil distilled from the fruits was used in perfumery (Dhanya et al., 2007).

Black pepper originated in the tropical evergreen forests of the Western Ghats. Early traders and travelers brought the black pepper to other countries. Vietnam, Brazil, Indonesia, China and Thailand have turned into main cultivating countries, besides India (Prabhakaran, 2004).

In China, people plant the black pepper by asexual reproduction technique, in order to maintain the excellent characters of a variety, but the long term asexual reproduction caused by toxin accumulation in the plant, results in susceptibility. Therefore, it is believed that, more and more work will be done on the sexual reproduction of black pepper. The seed germination is often the most sensitive stage in the life cycle of a plant. Germination of seeds may be affected by several environmental factors. In recent years, research on the black pepper concentrated on the extraction and effect of the medicinal and aromatic components (Francois et al., 2009; Asawalam, 2007; Vogler et al., 2006; Parthasarathy et al., 2008).
Studies on the species ecological adaptability, or response to the environmental factors were rarely reported. It is important to know seed germination requirements of black pepper, in order to guide further study research work of genetic breeding and high yield cultivation. This study was conducted to elucidate the effects of temperature, light, and plant growth regulators on black pepper seeds germination.

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Abbreviations: GA_3, Gibberellic acid; **IAA,** indole-3-acetic acid; **KT,** Kinetin; **CK,** control treatment.

Table 1. The result of a two-way ANOVA (F-ratios) for all experiments (Only data from 25, 30 and 35°C treatments are used in the test as no germination occurred at lower and higher temperatures).

Factor	Percent germination	Radical length
A (Temperature)	45.083***	177.462***
B (Light)	2.083	150.209***
A*B	1.583	11.715**
C(Regulators)	104.894***	258.899***
D(Concentration)	4.136*	79.002***
C*D	35.348***	20.131***

*P < 0.05; **P < 0.01; ***P < 0.001.

MATERIALS AND METHODS

Seed materials

Black pepper seeds were collected from field-grown black pepper (*P. nigrum* L.) c. v. Daye (Lampong Type), which is extensively cultivated in China (Liu et al., 2009), in the Xinglong tropical botanical garden in the Southeastern part of Hainan province in June, 2009. Seeds of similar size were selected for the germination experiment. All seeds were sterilized with 70% alcohol and 1 g L^{-1} mercuric chloride. Seeds displaying radical emergence greater than 1 mm were considered germinated. All experiments had three replicates.

Effects of temperature and light

The interactive effects of temperature (20, 25, 30, 35, 40 and 45°C) and light (dark and 220 µ mol s^{-1} m^{-2} PAR) were tested in a controlled environment system. Twenty seeds of uniform size were immersed in distilled water for 24 h, and then placed in a Petri dish with filter paper and 8 ml of distilled water. The dark treatments were kept completely dark at all times by wrapping the dishes in aluminum foil and placing the Petri dishes in aluminum trays. The dark treatments were not opened until the light treatments had finished germination (after 18 days). In the light treatment (12 h light and 12 h dark), all the seeds were checked daily for germination during 18 days after which no seeds germinated. After eighteen days the lengths of primary roots were measured and the percent germination was recorded for each treatment. Distilled water was added to the Petri dishes as necessary during the incubation to maintain saturation of the seeds.

Effects of plant growth regulators

The effects of three different plant growth regulators, GA_3 I, II, III (gibberellic acid, 10, 20 and 30 mg L^{-1}), IAA I, II, III (Indole-3-acetic acid, 10, 20 and 30 mg L^{-1}) and KT I, II, III (6-Furfurylamino-purine, Kinetin, 10, 20 and 30 mg L^{-1}), were tested in a controlled environment system. Twenty seeds of uniform size were immersed in the different concentrations of solution of three plant growth regulators for 24 h, distilled water for control treatment (CK). Then the seeds were rinsed with distilled water and placed in Petri dishes on filter paper and 8 ml of distilled water. The incubation temperature was 30°C. All the seeds were checked daily for germination during 18 days after which no seeds germinated. After eighteen days the lengths of primary roots were measured.

Statistical analysis

The effect of temperature, light and plant growth regulators on the seed germination and radical length increase were tested by a two-way analysis of variance (ANOVA) using SPSS 12.0 for windows (SPSS Inc. Illinois, USA).

RESULTS

Effect of temperature and light

Temperature had a significant effect on both radical length and percentage germination (P < 0.001), but light condition did not affect percent germination. However, light had a significant effect (P < 0.001) on radical length (Table 1). No germination was observed at the lowest temperature (20°C) and at the higher temperatures (40 and 45°C).

The maximum germination rate was observed at 30°C. There was no difference between light and dark treatment at 30°C, they were significantly greater than other treatments. However, the percent germination was not significantly different between all treatments at 25 and 35°C (Figure 1). The growth of radicals was significantly affected by temperature and light. Radical length was highest at 30°C and lowest at 25°C. The radical length was generally higher in the dark at all temperatures (Figure 2).

In the light treatments, black pepper seeds germinated at 30 and 35°C at the sixth day after treated. At 25°C, they took one more day. The highest germination percentage was 48.3% at 30°C. The germination increased slowly with time and completed within 12 days in all treatments (Figure 3).

Effect of plant growth regulators

The plant growth regulators enhanced the seeds germination and radical length to different extent (Table 2). Germination of CK was 48.3%. GA_3 significantly enhanced germination of black pepper seeds, whilst the effect of KT was not significant. In IAA III, 86.7% black pepper seeds germinated, 46.7 and 55.0% for IAA I and II, respectively (Figure 4).

The radical development was obviously enhanced by GA_3 (Figure 5). In GA_3 treatments, the radical length increased with the concentration of GA_3. In the GA_3 III treatment, the radical length was 1.92 cm, much longer than the 0.79 cm of CK. However, effect of KT and IAA was limited. Likewise, the effect of IAA was more obvious when the concentration was higher. Maybe, the effect would be much enhanced when the concentration increased to a certain amount that exceeds that in this experiment.

DISCUSSION

It took 6 or 7 days to germinate for black pepper seeds in

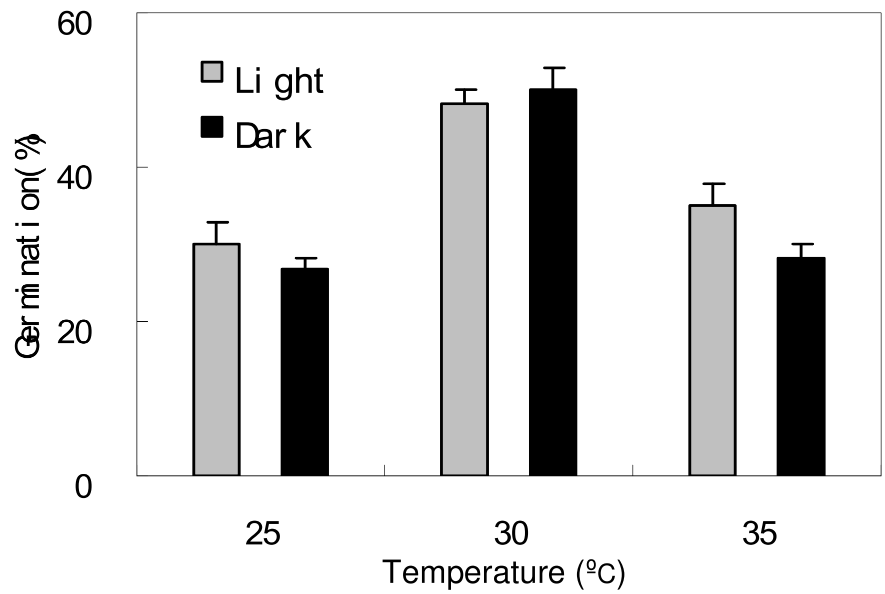

Figure 1. Germination percentage of Black pepper seeds from light and dark treatments at different temperature.

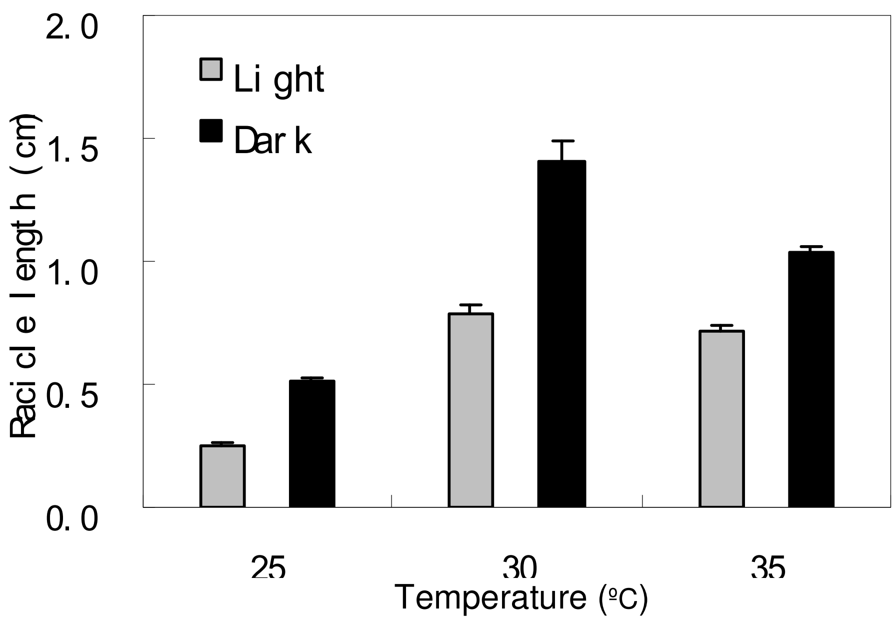

Figure 2. Radical length of Black pepper seeds from light and dark treatments at different temperature.

almost all treatments. And the germination rate is low (50.0% at 30℃), without exogenous regulation of substance. So, under natural environmental conditions, black pepper population formation in tropical area relieson the high seed setting rate, not the high seed germinating rate.

Black pepper seeds germination are significantly affected by temperature. The optimum germination temperature is between 30 and 35℃. Seeds do not germinate, when the temperature is higher than 40℃ or lower than 20℃. Temperature may affect either the initial processes of water uptake by seeds or the following biochemical processes that result in cell division (Bewley and Black, 1978; Kermode, 1990). There was no significant difference in the germination between light and dark treatments. This indicated that black pepper seeds can germinate

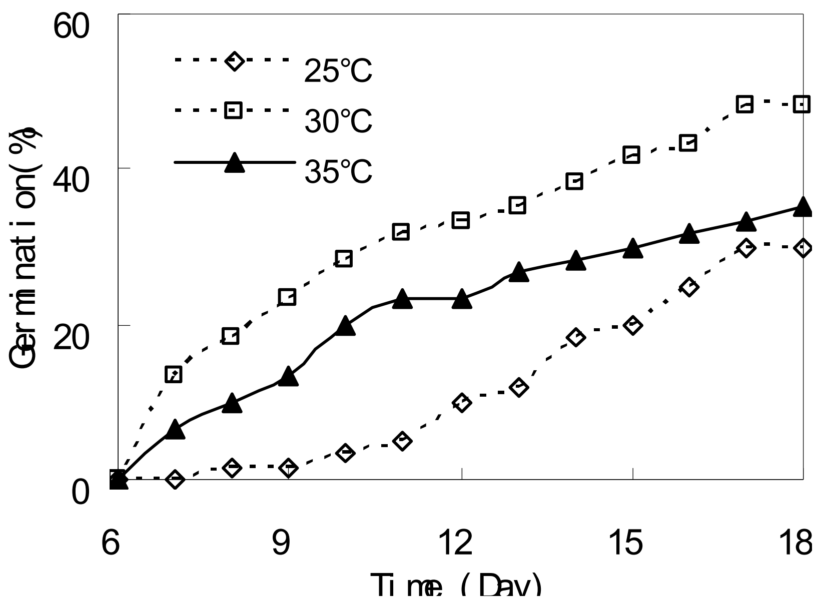

Figure 3. Average percentage germination of Black pepper seeds in the light treatments during 6th-18th day.

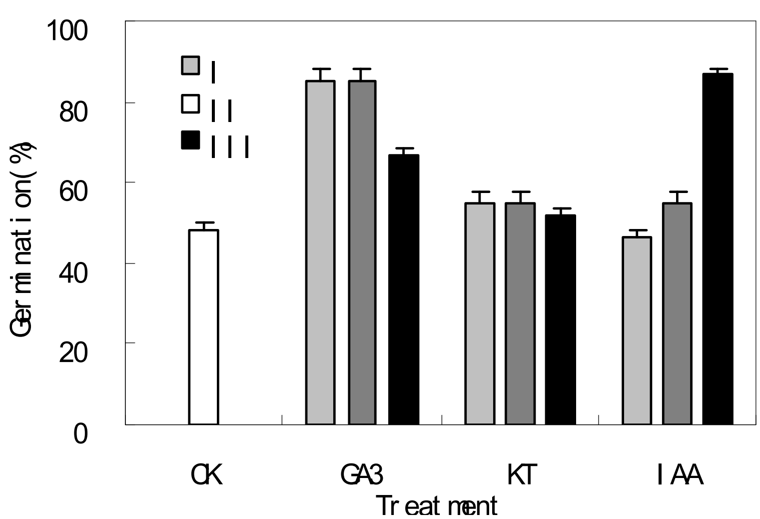

Figure 4. Percent germination of black pepper seeds from different plant growth regulators treatments.

without light.

The development of radical length was significantly influenced by both light conditions and temperature. At the same temperature, the radical length was longer in the dark treatments than light. That is related to the auxin content in the embryo. The light affects the distribution of auxin, as auxin is decomposed in the light. The growth of the primary radical is stimulated by high auxin levels (Taiz and Zeiger, 2006). In this experiment, radical length was highest at 30℃ and lowest at 25℃ in both light and dark treatments. So, the optimum condition for the radical development of black pepper seeds is at about 30℃ and without light.

The plant growth regulators enhanced the seeds germination and radical length in different degree. GA_3 promoted the radical elongation, effectively. And the effect was more obvious, the higher concentration applied. However, KT and IAA had less effect, at least for the certain concentration applied in this experiment.

Conclusion

Seeds of black pepper germinate hardly and slowly. The

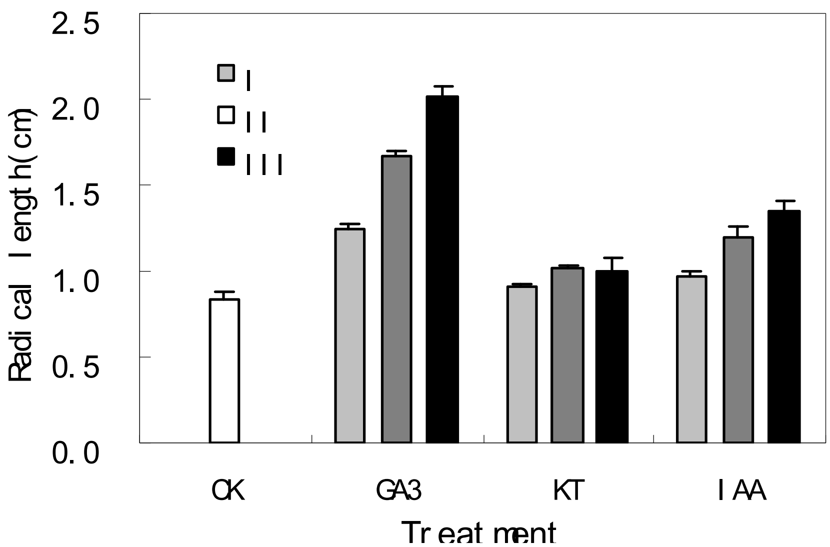

Figure 5. Radical length of black pepper seeds from different plant growth regulators treatments.

optimum germination temperature is 30 to 35℃. Black pepper seeds germinate both in the light and in the dark. No germination occurs at temperatures < 20 and > 40℃. Even in the optimum temperature, the germination rate is low (not more than 50%). However, GA_3 (20 mg L^{-1}) enhanced the germination and radical development obviously.

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