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Full Length Research Paper

Effects of plant growth regulators and temperature on seed germination of yellow nut-sedge (*Cyperus esculentus* L.)

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Effects of temperature, concentrations of plant growth regulators on germination of yellow nut-sedge seeds was studied under controlled environmental conditions. Yellow nut-sedge seeds were placed in Petri dishes with filtration papers and the germination and radical development followed during eleven days periods. Germination development of radical length was significantly influenced by both light and temperature. Germination was highest at 35°C and poor germination was observed at other temperatures (27 and 45°C). The plant growth regulators enhanced the seeds germination and radical length to different degree. The results were consistent with yellow nut-sedge seeds being recalcitrant species, needing a certain environment condition to germinate.

Key words: Yellow nut-sedge, growth regulator, temperature, germination rate, tissue culture.

INTRODUCTION

Yellow nut-sedge (*Cyperus esculentus* L.), a perennial herb and a potential medicinal plant with high value, has tubers with 20 to 25% even up to 36% oil. It is a highly adaptable crop and grows well under a wide range of climatic and soil condition (Anon, 1962). It is grown in Africa, South America, Europe and Asia. The total planting area of yellow nut-sedge in China is small, but is widely covering south and north of china, as Guangxi, Guangdong and the Xinjiang Autonomous Region, where it is cultivated as an annual oilseed crop (Anon, 1977). In various provinces of China, yields of 2250 to 3000 kg/ha of tubers at storage and moisture content have been reported for medium fertility soils. The tuber grows from tubers per plant and one tuber weighs 2 to 2.6 g. There the underground stalk of yellow nut-sedge with 50 to 250 are about 2000 to 2600 individual tubers/kg. The oil extracted from yellow nut-sedge can be used as food oil as well as for industrial and medicinal purposes. Nut-sedge tuber has high nutritive value. It contains 3 to 15% protein, 15 to 20% sugar, 20 to 25% starch, 4 to 14% cellulose and trace amounts of natural resin. Yellow nut-sedge produced from seed from eight California fields sampled during 1974 to 1976. There was a linear relationship between seed weight and germination percentage (Jthullen, 1979; Ma et al., 2011).

However, those studies on different responding mechanisms of Yellow nut-sedge under plant growth regulators and temperature is scanty. Thus this paper seeks to further show that NAA, IAA and NaCl have obvious promotion functions on seed germination in improving germination energy and germination rate and drought stress and provide valuable information for the introduction and popularization of Yellow nut-sedge from Guangxi to Hainan in China.

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Abbreviations: NAA, 1-naphthlcetic acid; **IAA,** indole-3-acetic acid; **CK,** control treatment.

MATERIALS AND METHODS

Yellow nut-sedge seeds were collected from the field where it was

Table 1. Germination percentage and contaminated numbers from tissue culture.

Factor	Germination percentage	Contaminated number
A	75	9
B	60	6
C	56.6	3
D	55	3
E	60	5

extensively cultivated Guangxi in China. Seeds with similar size were selected for the germination experiment. All seeds were dry in shade under normal temperature and preserved, which were sterilized with 70% alcohol and 1 g L-1 mercuric chloride. Seeds displaying radical emergence greater than 1 mm were considered germination. All experiments had three replicates. NAA, IAA and NaCl were treating reagents Table 1.

The interactive effects of temperature were 27, 35, 45, 55 and 65°C. Thirty seeds with 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 under dark at all times by wrapping the dishes in aluminum foil and placing the Petri dish in aluminum trays. The dark treatments ended until the light treatments had finished germination (after eleven days). In the light treatment (12 h light and 12 h dark), all the seeds were checked daily for germination during eleven days after which no seeds germinated. After the seventh day the lengths of primary shoots 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. The effects of three different plant growth regulators, IAA I, II, III, IV, V (gibberellic acid, 5, 10, 15, 20 ,and 25 mg L^{-1}), NAA I, II, III, IV, V (Indole-3-acetic acid, 5, 10, 25, 35 and 50 mg L^{-1}) and NaCl I, II, III, IV, V (gibberellic acid, 0.2, 0.4, 0.8, 1.2 and 1.8%), were tested in a controlled environment system. Thirty seeds with uniform size were immersed in the corresponding resolution of growth regulators for 24 h, with distilled water as the 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 28°C. All the seeds were checked daily for germination during seven days until when no seeds germinated. After seven days, the length of primary shoots was measured.

Yellow nut-sedge seeds with similar size were washed in soap water and then were immersed in distilled water for two days, which were soaked, sterilized with 70% alcohol and 0.2% mercuric chloride and washed by distilled water, respectively. The seeds were placed on a modified MS medium (Reynolds and Murashigue, 1978) to induce shoots calli. The pH is adjusted to 5.6 before sterilization. Each seed was cultured in one Petri dish at 27±2°C.

Basal medium: MS macro+ MS micro+ vitamins+ 0.1 g/L-1 myo-inositol+ 0.1 g/L-1 L-glutamine+ 3% sucrose 30 g.

Hormone: A, MS+ NAA1 mg/L-1; B, MS+NAA1 mg/L-1+6-BA0.2 mg/L-1; C, MS+ NAA1 mg/L-1+KT0.2 mg/L-1; D, MS+ NAA0.5 mg/L-1; E, MS+ NAA 0.2 mg/L-1+6-BA 0.5mg/L-1.Germination percentage = (n/N) ×100％ (n was the amount of seeds germinating normally, N was the total amount of seeds used in the experiment); (2) Germinability was the percent of the normal germination amount to total used amount.

Statistical analysis

The results are presented as mean ± standard errors obtained from at least three replicates. Significant differences between the treated and control plants are determined using ANOVA test ($P<0.05$). Statistical analysis was conducted using the statistical software package SPSS13.0 for Windows.

RESULTS

Effects of temperature

Temperature had a significant effect on both radical length and percentage germination ($P < 0.001$). Less germination was observed at the higher temperatures (65°C). The maximum germination rate was observed at 35°C. They were significantly greater than other treatments. However, the germination percent was not significantly different among all treatments at 27, 45 and 55°C. The growth of radicals was significantly affected by temperature radical length was highest at 35°C. The radical length was generally higher in the light at all temperatures. At 35°C, they took one more day. The highest germination percentage was 56% at 35°C. The temperature increased, percentage germination reduced slowly with time and completed within seven days in all treatments (Figures 1-4)

Effects of plant growth regulators

Different concentration had different effects on the seeds germination. The seeds germination was obviously enhanced by IAA 10 and IAA 20, whilst it was reduced by the other concentration. Germination of CK was 23.33% and that of seeds treated by IAA 10 and IAA 20 was 96.67 and 100% respectively, but only 20, 63.33 and 6.67% for IAA 5, IAA 15 and IAA 25. Thus, IAA 20 was the best concentration for seeds germinating (Justice et al., 1946) (Figures 5-8). NaCl treatment enhanced the seeds germination obviously. Much higher than the 23.33% for CK, 66.67, 83.33, 100, and 76.67% seeds germinated for NaCl 0.2, 0.4, NaCl 0.8, 1.2 and 1.8, respectively. The best treatment was NaCl 0.8 and NaCl 1.2, in which all the seeds germinated. NAA had relatively less effect on the seeds germination than IAA and NaCl.NAA had different effect, similarly to the IAA effects above. NAA5 and NAA 10 reduced the seeds germination. In NAA5 and NAA 10 had 10.00 and 3.33% seeds germination, less than 23.33% for CK whilst in NAA 25 and NAA 50, 70.00 and 56.67% seeds germinated. In NAA 35, the seeds

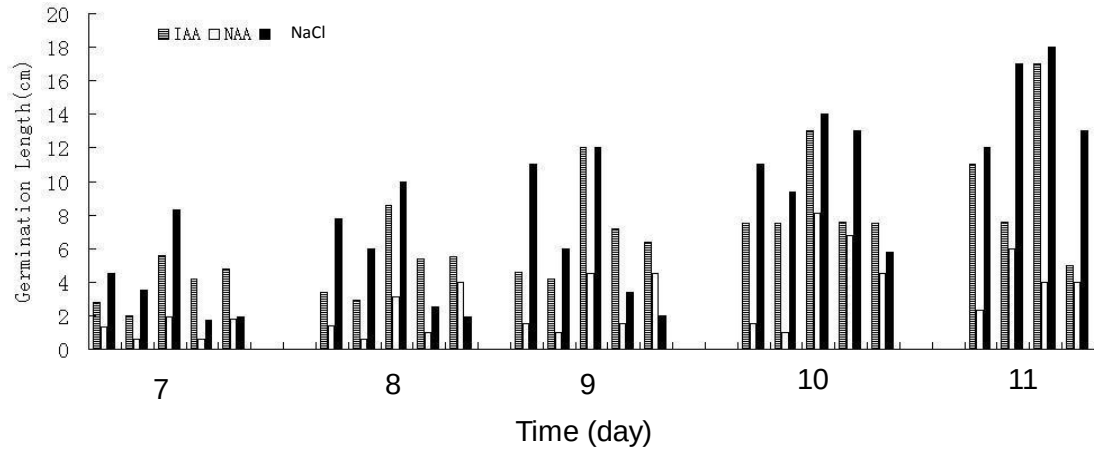

Figure 1. Radical length of Yellow nut-sedge seeds from different plant growth regulators treatments during 7th-11th day. IAA，NAA, and NaCl followed the order of I, II,III, IV, V, respectively.

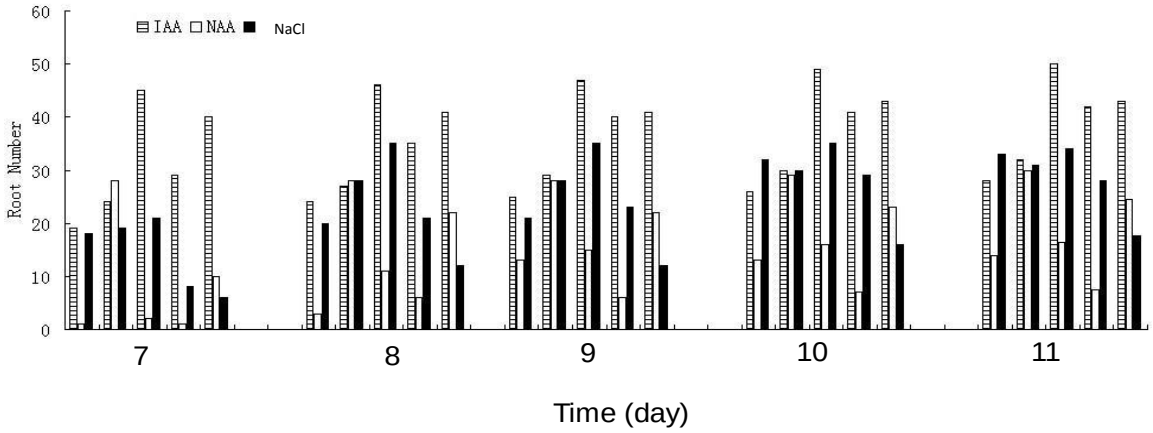

Figure 2. Root numbers of Yellow nut-sedge seeds from different plant growth regulators treatments during 7th-11th day. IAA，NAA, and NaCl followed the order of I, II,III,.IV, V., respectively.

germination was 26.67%, which was similar to CK treatments. The radical development was obviously enhanced (Figure 1). In different treatments, the radical length increased with the concentration of plant growth regulators. Radical length of Yellow nut-sedge seeds increase process during, 7^{th} to 11^{th} day (Figure 3). In the NaCl III treatment, the radical length was 18 cm, much longer than that in IAA III and NAA III. However, effect of NAA during 7^{th} to 11^{th} day was limited. Likewise, the effect of NaCl and 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.

It was observed that the number of root was enhanced (Figure 2) during the 7^{th} to 11^{th} day, when the root length was longer in IAA III than in NaCl and IAA. The number of root had 50 pieces, in that seeds root slowly germinated.

Effects of tissue culture

For shoot development, the basal medium was further supplemented with 1 mg/L^{-1} NAA and 0.2 mg/L-1 6-BA (Figure 8). Percent germination from tissue culture was 75%.

Conclusion

It took 10 or 11 days to germinate for Yellow nut-sedge seeds in almost all treatments. And the germination rate was low (23.33%). Yellow nut-sedge seeds germination were significantly affected by temperature. The optimum germination temperature was 35°C. Seeds germinated hardly when the temperature was higher than 45°C or lower than 27°C. Temperature may affect either the initial processes of water uptake by seeds or the following

Figure 3. Germinating process for radical length of yellow nut-sedge seeds.

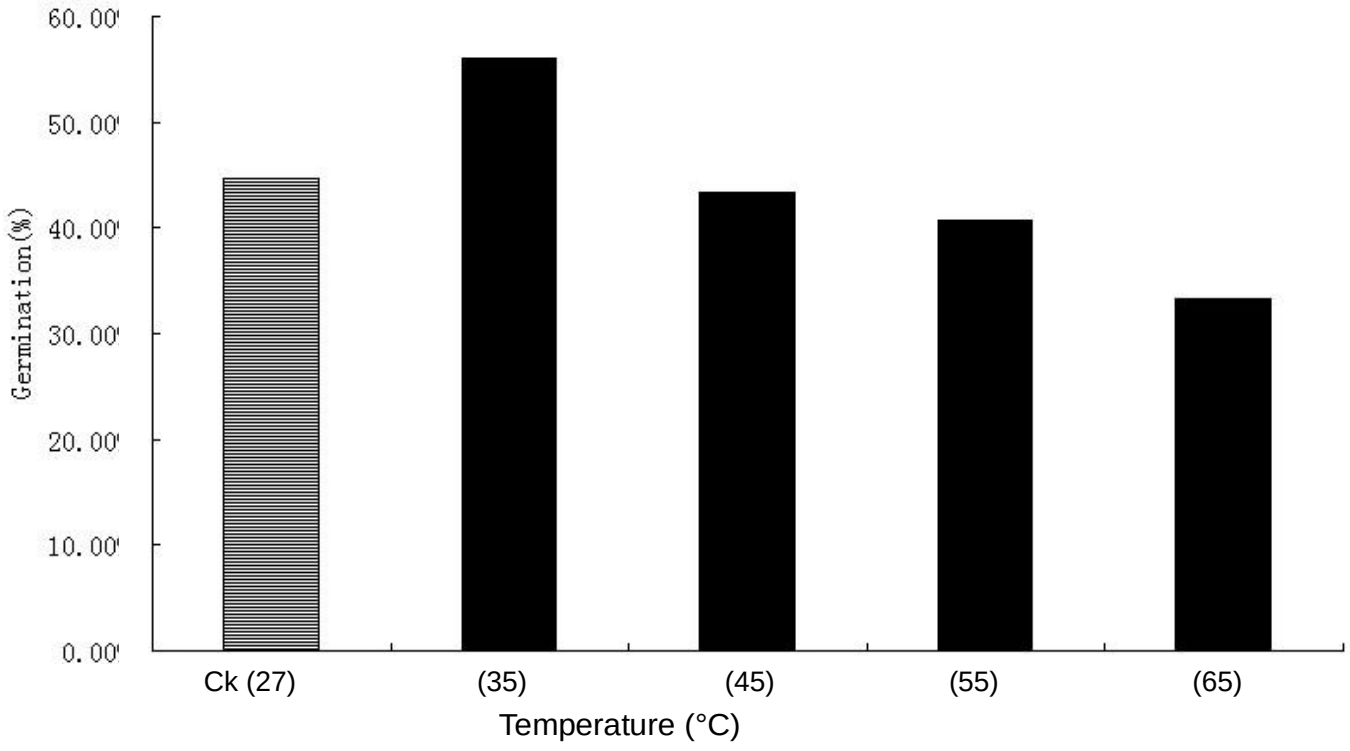

Figure 4. Germination percentages of yellow nut-sedge seeds under different temperature.

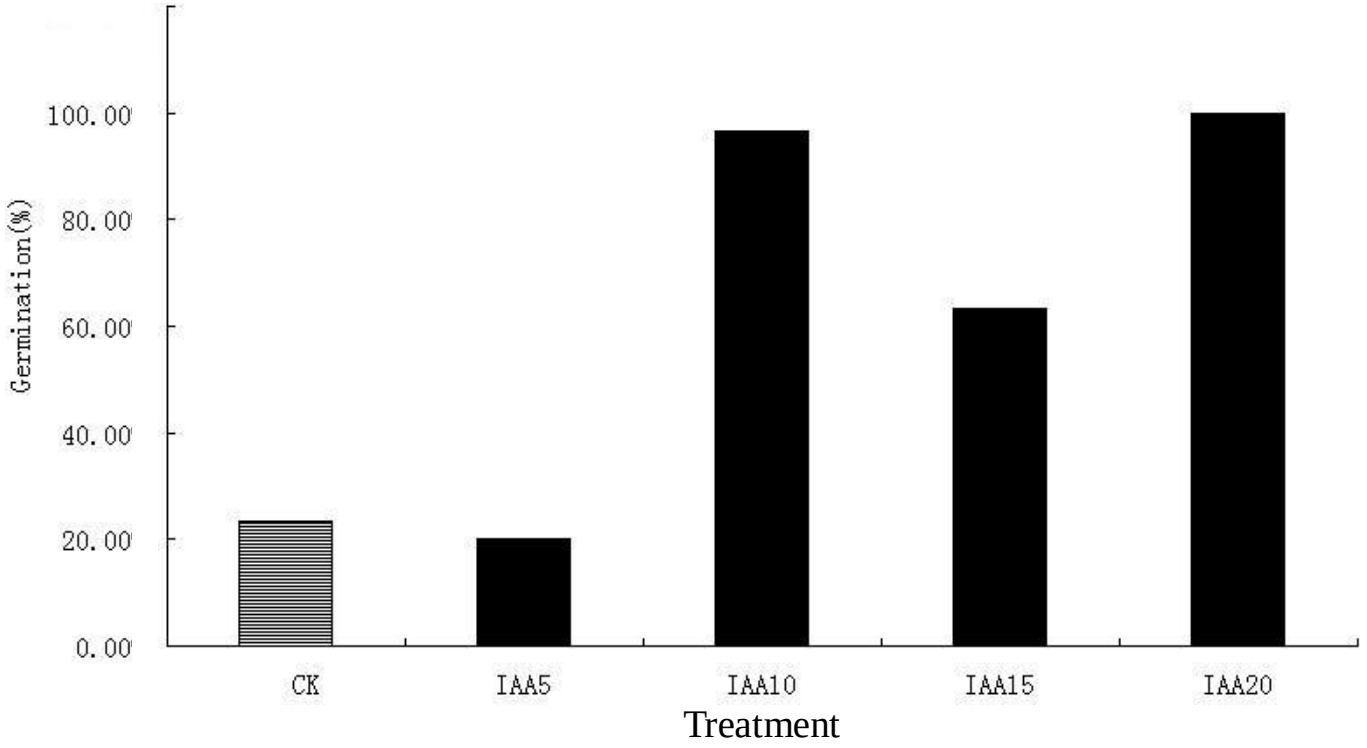

Figure 5. Germination of yellow nut-sedge seeds from different plant growth regulators treatments.

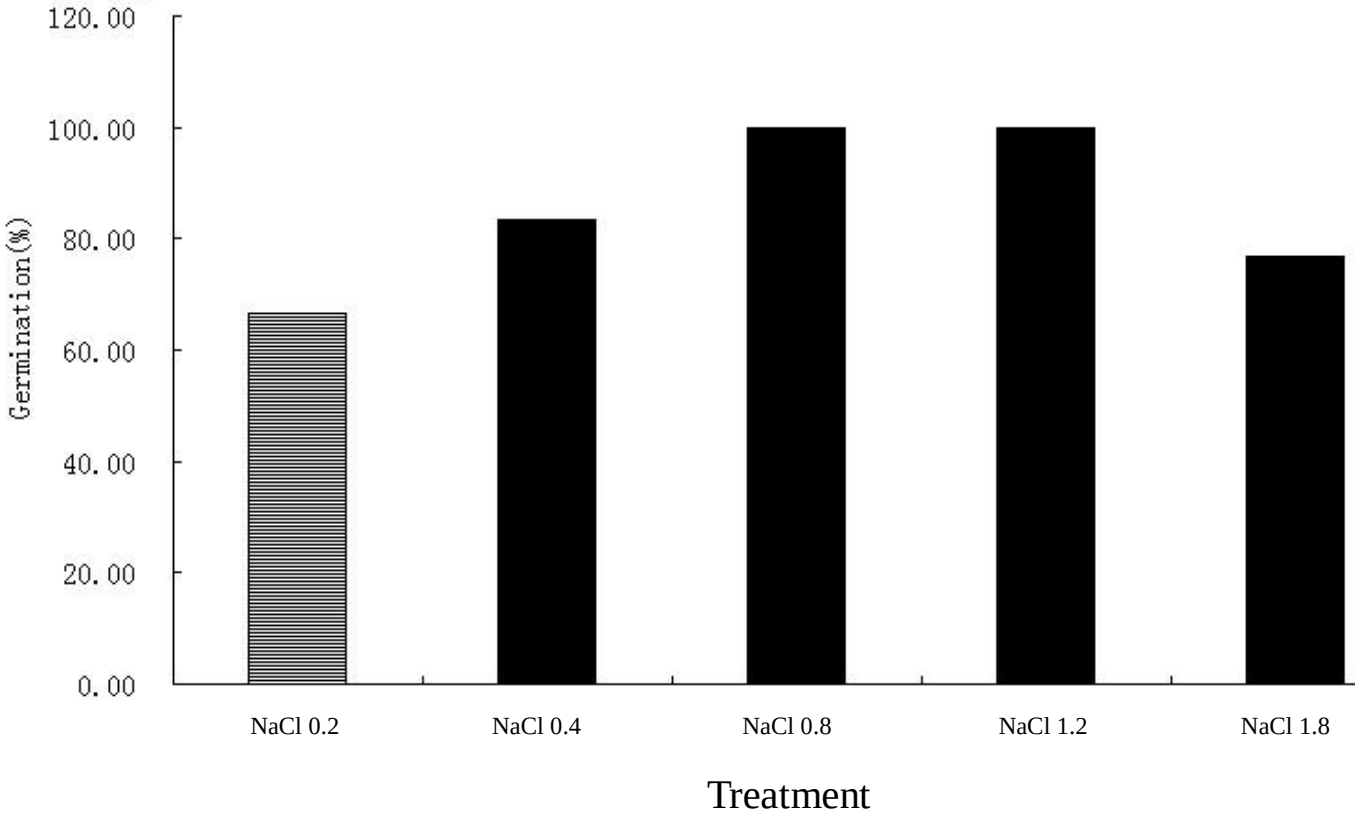

Figure 6. Germination of yellow nut-sedge seeds under different plant growth regulators treatments.

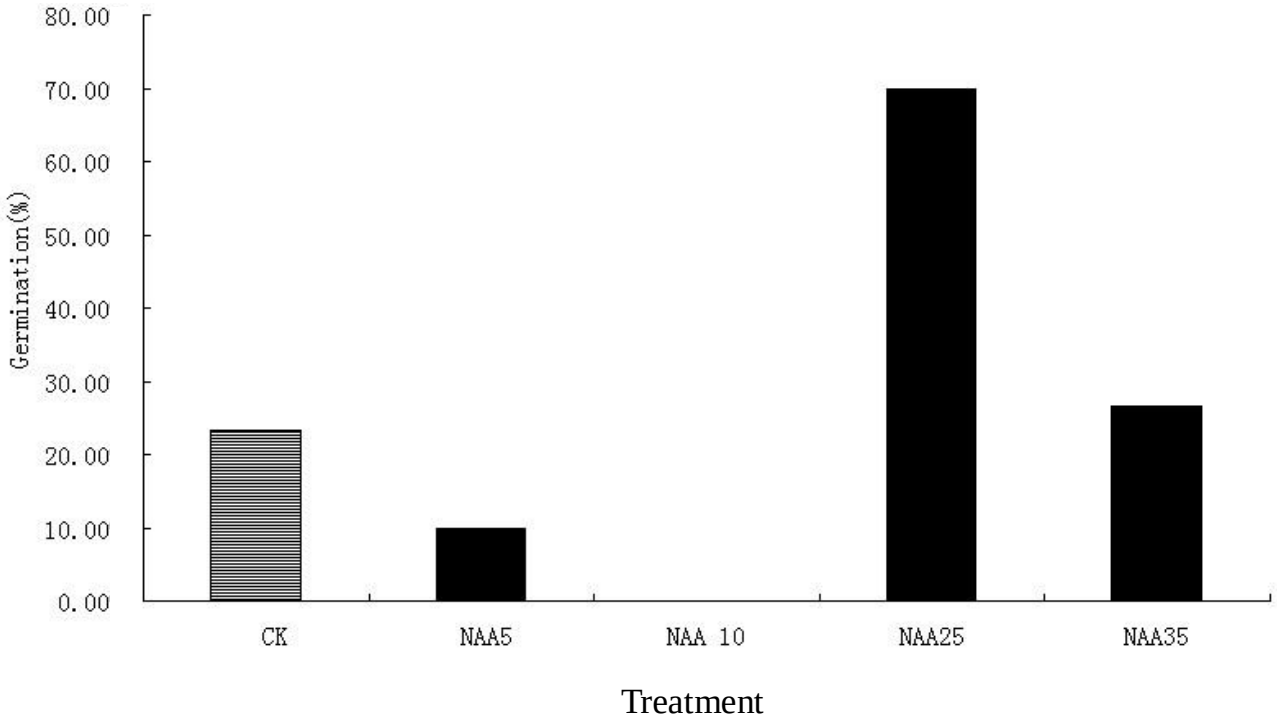

Figure 7. Germination of yellow nut-sedge seeds under different plant growth regulators treatments.

Figure 8. Effects of concentration strength of modified MS medium on germination of yellow nut-sedge seeds.

biochemical processes that result in cell division (Bewley and Black, 1978; Kermode, 1990).

Germination could be enhanced with different plant growth regulators germination occurred at highest percentages when growth regulators were between NaCl III, NaCl IV and AA IV. Seed germination was low (not more than 50%) as it was inhibited by IAA I and NAA I treating (Phani et al., 2011, Yaser et al., 2011). However, Yellow nut-sedge seeds germinated at 100% from NaCl treatment in continuous light conditions. However, some yellow nut-sedge seeds were dormant, while others had a semi dormant to non-dormant status (Lapham and Drennan, 1990; Mero-Macias, 1996; Mulligan and Junkins, 1976). Once they overwinter a high percentage of sprouting has been observed (95%) (Anderson, 1999; Mero-Macias, 1996; Mulligan and Junkins, 1976). Within the first 6 months after sowing, proportions of dormant seed were reduced rapidly at all sown depths (Lapham and Drennan, 1990; Mero-Macias, 1996). 79% of harvested Yellow nut-sedge seeds germinated within 4 months (Mero-Macias, 1996). There may be one or more substances present in or on the epidermis of the tuber which inhibits the buds from sprouting on a tuber (Tumbleson, 1960, 1962; Shao et al., 2005, 2009).

The development of radical length was significantly influenced by different plant growth regulators at 35°C. The radical length was longer in the dark treatments than light. This is related to the auxin content in the embryo. In this experiment, radical length was the highest in the NaCl III treatment. So, the optimum condition for the radical development of yellow nut-sedge seeds is in the NaCl III treatment and IAA III, obviously. The root length was enhanced and the effect was the best. The longest root numbers had 50 pieces from IAA III.

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