Ma Taiguang, Li Haiping, Guo Xiuxia, et al. “Effects of Humic Acid on Hypocotyl and Root Growth and Antioxidant Properties of Cucurbita Pepo under NaCl Stress.” [J]. *Jiangsu Agricultural Sciences*, 2016, 44(9): 189-192.

Doi: 10.15889/j.issn.1002-1302.2016.09.054

Effects of Humic Acid on Hypocotyl and Root Growth and Antioxidant Properties of Cucurbita Pepo under NaCl Stress

Ma Taiguang[1], Li Haiping[1], Guo Xiuxia[1], Zhang Ruiteng[1], Li Lingzhi[1], Zhou Kejie[2]

(1. College of Horticulture, Shanxi Agricultural University, Taigu 030801, Shanxi; 2. Xinyi Sumeng Fertilizer Co., Ltd., Xinyi 221400, Jiangsu)

Abstract: The effects of humic acid on hypocotyl and root growth and antioxidant properties of Cucurbita pepo under NaCl stress was studied with Nongyuan No.1 Cucurbita pepo as the studied material. The findings revealed that seed soaking in an appropriate concentration of Highly Active Humic Acid diluent could mitigate the inhibitory effects of NaCl stress on the hypocotyl and root of Cucurbita pepo, and the optimal mitigation effect was observed when the Highly Active Humic Acid was diluted 450 times. Treatment with Highly Active Humic Acid diluent could significantly increase the germination percentage, germination index, vigor index, fresh weight, root length, hypocotyl length, first-order lateral roots and superoxide dismutase and peroxidase activity in the hypocotyl and root of Cucurbita pepo seeds while reducing the malondialdehyde (MDA) content in the hypocotyl and root under NaCl stress. The results showed that Highly Active Humic Acid could mitigate the inhibitory effects of NaCl stress on the hypocotyl and root growth of Cucurbita pepo and enhance the salt resistance of Cucurbita pepo seeds.

Keywords: Humic acid; Cucurbita pepo; Seeds; NaCl; Stress; Antioxidant enzyme; Germination

Chinese Library Classification No.: S642.601 **Document Code:** A **Article No.:** 1002-1302(2016)09-0189-03

In recent years, with the rapid development of facility vegetable production in China, the long-term unreasonable application of chemical fertilizers and excessive pesticide spraying within the facilities have led to an increase in soil salinization, a decline in fertility, and a significant rise in pest and disease occurrences. Consequently, there has been a gradual decrease in both the quantity and quality of vegetable yield, which severely hampers the sustainable development of facility vegetable cultivation[1]. Cucurbita pepo, the primary facility melon vegetable in China, is greatly affected by salt damage in terms of yield and quality. Therefore, the research on cultivation techniques that can address facility soil salinization and enhance the resistance of Cucurbita pepo to salt damage has become a production issue of common concern[2].

Humic acid, an organic macro-molecular substance widely found in nature, possesses the activation property that can not only enhance the activity and metabolic functions of oxidase in plants but also improve the structural properties of soil, ultimately boosting the ability of plant roots to absorb water and nutrients[3-5]. The research conducted by Guo Wei et al. showed that seed soaking in humic acid could effectively mitigate the effect of saline-alkali stress on wheat[6]. However, so far the effect of humic acid on the germination of Cucurbita pepo seeds under salt stress has not been reported yet. Therefore, this test aims to study the effects of seed soaking in humic acid on the antioxidant systems of the root and hypocotyl of Cucurbita pepo under NaCl stress by measuring changes in the seed germination and the root and hypocotyl growth of Cucurbita pepo under NaCl stress through the treatment of seed soaking in humic acid. The objective is to provide theoretical insights to uncover the salt stress tolerance mechanism and improve the salt stress resistance path of Cucurbita pepo. Furthermore, this test will also provide a theoretical basis and practical guidance for enhancing the production of Cucurbita pepo in saline-alkali regions.

1. Material and Method

1.1 Test Material

The test material was Nongyuan No.1 Cucurbita pepo; NaCl had the purity of >99% and was produced by Life Science Products & Services; the Highly Active Humic Acid liquid was supplied by Xinyi Sumeng Fertilizer Co., Ltd. in Jiangsu.

Date of Reception: March 14, 2016

Fund Project: Shanxi Provincial Project for Science and Technology Development (No. 20130311008-4); National Fund for Agricultural Transformation of Scientific and Technological Achievements (No.: 2014GB2A300017); Shanxi Provincial Demonstration Action Project of Agricultural Technology Promotion (No.: SXNKTG11).

About the Author: Ma Taiguang (1991-), male, born in Xiaoyi, Shanxi, is a postgraduate specialized in vegetable cultivation and physiology. E-mail: matg1717@163.com.

Corresponding Author: Li Haiping, holder of master’s degree, is an associate professor specialized in organic vegetable cultivation and vegetable cultivation physiology. E-mail: lihp0205@163.com.

1.2 Test Design

The test involved 6 treatments as follows: (1) CK, without seed soaking in Highly Active Humic Acid liquid and NaCl; (2) T1, without seed soaking in Highly Active Humic Acid liquid but with NaCl; (3) T2, with humic acid 50 + NaCl (seed soaking in Highly Active Humic Acid liquid diluted 50 times + NaCl); (4) T3, with humic acid 250 + NaCl (seed soaking in Highly Active Humic Acid liquid diluted 250 times + NaCl); (5) T4, with humic acid 450 + NaCl (seed soaking in Highly Active Humic Acid liquid diluted 450 times + NaCl); (6) T5, with humic acid 650 + NaCl (seed soaking in Highly Active Humic Acid liquid diluted 650 times + NaCl).

Full and intact Cucurbita pepo seeds of relatively uniform size were chosen, sterilized with a 0.1% potassium permanganate solution for 15min, washed repeatedly with deionized water, and dried with absorbent paper. These Cucurbita pepo seeds were then soaked in the Highly Active Humic Acid solution (diluted 50, 250, 450 and 650 times) and deionized water for 8h [(26±1)℃]. 25 seeds were placed in each of the culture dishes lined with double layers of sterile filter paper for germination. The same process was replicated three times. Each culture dish was added with 8mL 200mmol/L NaCl solution (equivalent deionized water was added to CK), and no solution should pool at the bottom of the dish when the filter paper was moistened and tilted. The culture dishes were kept in a dark environment provided by artificial intelligence climate incubators [(26±1)℃]. During the test process, 1-2mL deionized water and NaCl solution were alternately added every other day, and the concentration of NaCl solution in each treatment was kept basically constant. The germinated seeds were counted from Day 2, with radicle length equal to 1/2 of the seed length as the criterion for germination [7]. Rotten seeds were excluded in a timely manner. The test was concluded on Day 7, when the germination percentage, germination index and vigor index were calculated and the superoxide dismutase (SOD) and peroxidase (POD) activity and the malondialdehyde (MDA) contents were measured.

1.3 Measurement Items and Method

Germination Percentage (*GP*) = Number of germinated seeds on Day 7/total number of tested seeds x 100%;

Germination Index (*GI*) = $\sum(G_t/D_t)$ (G_t represents the number of germinated seeds at *t*, and D_t represents the number of days when the seeds are germinated);

Vigor Index (*VI*) = *GI*x*S* (*GI* represents germination index, and *S* represents the fresh weight of seedlings measured on Day 7);

Ten seedlings were chosen at random from each treatment. A ruler was used to measure their radicle lengths and hypocotyl lengths; an analytical balance was used to measure their fresh weight; then, the MDA contents in the hypocotyl and root of Cucurbita pepo were measured with the thiobarbituric acid method[8] and were expressed as μmol/g; an ultraviolet spectrophotometer was used to measure the antioxidant enzyme activity; the SOD activity was measured with the nitroblue tetrazolium (NBT) reduction method[8] and expressed as U/(g•min); the POD activity was measured with the guaiacol method[8] and expressed as Δ_{D470nm}/(g•min).

SAS and Microsoft Office Excel 2013 software were used for variance analysis and charting of the test results.

2. Result and Analysis

2.1 Effect of Humic Acid Treatment on the Germination of Cucurbita Pepo Seeds under NaCl Stress

Under NaCl stress, the germination percentage, germination index, vigor index, first-order lateral roots and seedling fresh weight of Cucurbita pepo seeds were significantly lower than those in CK (P<0.05), which indicated that NaCl stress inhibited the germination of Cucurbita pepo seeds. When treated with Highly Active Humic Acid liquid diluted 450 times, the germination percentage, germination index, vigor index, first-order lateral roots and seedling fresh weight of Cucurbita pepo seeds were maximum and significantly different from those at T1; however, treatment with Highly Active Humic Acid liquid diluted 50 times aggravated the inhibitory effect (Table 1, Figure 1, and Figure 2). The results showed that an appropriate concentration of Highly Active Humic Acid diluent could facilitate the germination of Cucurbita pepo seeds under NaCl stress.

2.2 Effect of Humic Acid Treatment on Hypocotyl and Root Lengths of Cucurbita Pepo under NaCl Stress

Under NaCI stress, the hypocotyl and root lengths of Cucurbita pepo seedlings were significantly less than those in CK, and treatment with an appropriate concentration of Highly Active Humic Acid diluent effectively mitigate the inhibitory effect of NaCI stress on the hypocotyl and root growth of Cucurbita pepo. In this test, the optimal effect was observed when the Cucurbita pepo seeds were treated with Highly Active Humic Acid liquid diluted 450 times, with hypocotyl and root lengths 1.51cm and 1.85cm more than those at T1, and the differences were significant (Table 1).

Table 1 Germination of Cucurbita Pepo Seeds Treated with Humic Acid under NaCI Stress

Treatment	Germination Percentage (%)	Germination Index	Vigor Index	First-order Lateral Roots (Piece)	Fresh Weight (g)	Hypocotyl Length (cm)	Root Length (cm)
CK	94.67a	34.81a	31.80a	9.03a	0.92a	10.59a	11.56a
T1	70.67c	14.41d	3.61d	5.07b	0.25d	1.09cd	3.46c
T2	57.33d	7.61e	1.88e	1.43c	0.25d	0.53d	1.55d
T3	76.00bc	17.61cd	4.66d	2.63c	0.27cd	1.53c	3.04c
T4	86.67ab	22.63b	9.89b	2.77c	0.44b	2.60b	5.31b
T5	77.33bc	19.03bc	6.39c	3.07c	0.34c	1.5c	4.31bc

Note: Different lowercase letters behind the same column of data indicate significant differences (P<0.05) between the treatments.

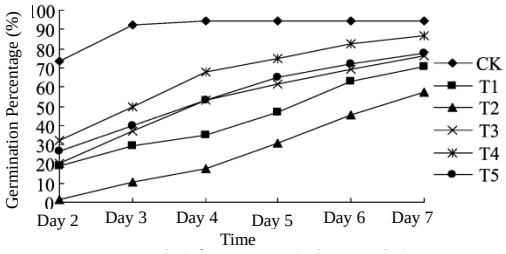


Figure 1 Effects of Different Concentrations of Highly Active Humic Acid on Germination Percentage of Cucurbita Pepo Seeds under NaCI Stress

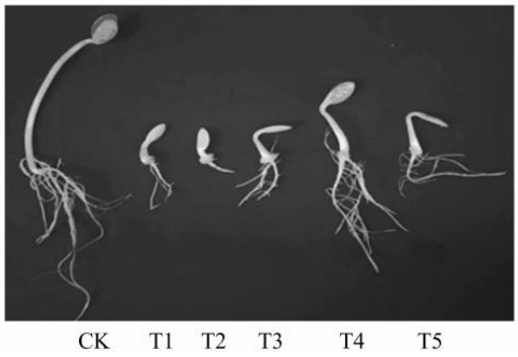
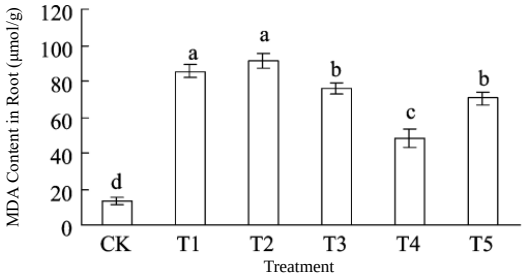


Figure 2 Effects of Different Concentrations of Highly Active Humic Acid on Growth of Cucurbita Pepo Seeds under NaCI Stress

2.3 Effect of Humic Acid Treatment on MDA Contents in Hypocotyl and Root of Cucurbita Pepo under NaCl Stress

Under NaCl stress, the MDA contents in the hypocotyl and root of Cucurbita pepo were significantly increased when compared with those in CK, while seed soaking in Highly Active Humic Acid diluent dragged down such increases and the MDA contents first decreased and then increased as the dilution ratio of Highly Active Humic Acid increased. Compared with those at T1, the MDA contents in the hypocotyl and root of Cucurbita pepo treated with Highly Active Humic Acid liquid diluted 450 times decreased by 39.10% and 43.60%, respectively, resulting in the optimal effect (Figure 3 and Figure 4).



Different lowercase letters on the bars indicate significant differences (P<0.05) between the treatments, the same below.

Figure 3 Effect of Humic Acid on MDA Content in the Root of Cucurbita Pepo under NaCI Stress

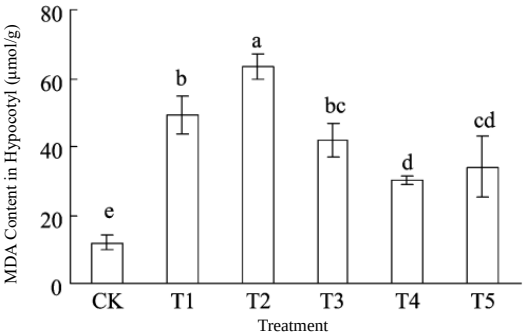


Figure 4 Effect of Humic Acid on MDA Content in the Hypocotyl of Cucurbita Pepo under NaCI Stress

2.4 Effect of Humic Acid Treatment on SOD Activity in Hypocotyl and Root of Cucurbita Pepo under NaCl Stress

Compared with that in CK, the SOD activity in the hypocotyl and root of Cucurbita pepo was significantly inhibited at T1. Under

NaCI stress, the SOD activity in the hypocotyl and root of Cucurbita pepo treated with Highly Active Humic Acid liquid diluted 450 times was increased by 35.05% and 55.30% from T1, respectively, resulting in a significant effect (Figure 5 and Figure 6).

2.5 Effect of Humic Acid Treatment on POD Activity in Hypocotyl and Root of Cucurbita Pepo under NaCl Stress

NaCI treatment significantly inhibited the POD activity in the hypocotyl and root of Cucurbita pepo, while treatment with Highly Active Humic Acid liquid diluted 450 times could significantly reduce the inhibitory effect of NaCI stress on the protective enzyme POD activity in the hypocotyl and root of Cucurbita pepo and its effect was similar to the other indicators stated above, with the optimal mitigation effect observed when Highly Active Humic Acid liquid was diluted 450 times (Figure 7 and Figure 8).

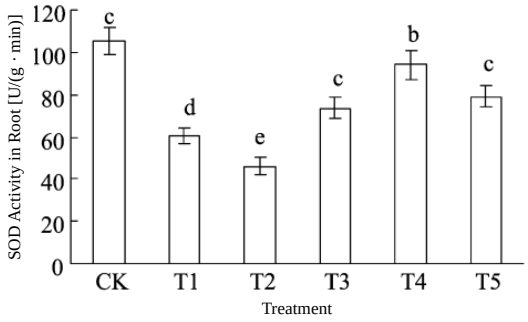


Figure 5 Effect of Humic Acid on SOD Activity in the Root of Cucurbita Pepo under NaCI Stress

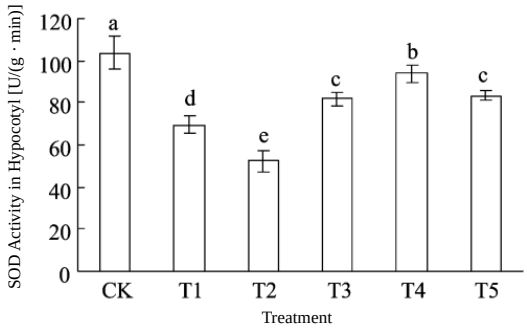


Figure 6 Effect of Humic Acid on SOD Activity in the Hypocotyl of Cucurbita Pepo under NaCI Stress

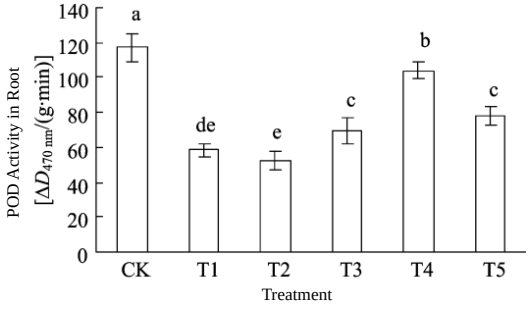


Figure 7 Effect of Humic Acid on POD Activity in the Root of Cucurbita Pepo under NaCI Stress

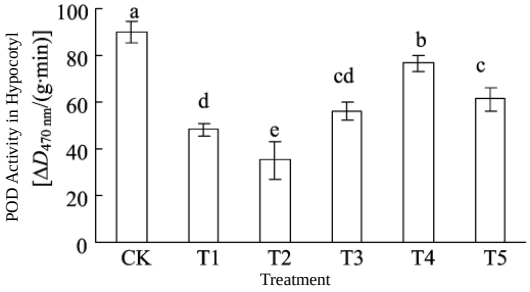


Figure 8 Effect of Humic Acid on POD Activity in the Hypocotyl of Cucurbita Pepo under NaCI Stress

3. Discussion

Seed germination is a crucial stage in crop development as it directly influences the growth and yield of crops in later stages. The presence of salt stress during seed germination can cause damage to the structure and function of cell membranes, leading to disruptions in seed physiology and metabolism, ultimately resulting in reduced germination ability[9]. The results in this test indicated that the treatment of Cucurbita pepo seeds with 200mmol/L NaCl significantly inhibited the germination of Cucurbita pepo seeds. This could be attributed to the detrimental effect of the treatment with 200mmol/L NaCl on the integrity of the cell plasma membrane, which resulted in decreased selectivity permeability of the cell membrane, imbalance of ions inside and outside cells, and reduced water potential. This impeded the water absorption by seed sprouts and thus affected seed germination and sprout growth. These results were consistent with those of previous studies[10-15]. The research conducted by Sun Xiaofang et al. also showed that the absorption of substantial Na^+ and Cl^- during the germination season made the seeds vulnerable to osmotic stress and ion poisoning as they germinated[16]. Seed soaking in humic acid under NaCl stress could significantly increase the germination potential, germination percentage, germination index and vigor index of Cucurbita pepo seeds and facilitated the root and hypocotyl elongation of Cucurbita pepo seedlings. Research has shown that the application of humic acid under NaCl stress could stimulate the α-amylase activity in wheat seeds, increase the contents of total soluble sugar, sucrose and fructose, and further improve the germination percentage of seeds[17-18]. Therefore, it can be presumed that the effect of seed soaking in humic acid on facilitating the germination of Cucurbita pepo seeds could be related to changes in the α-amylase activity

and fructose content in Cucurbita pepo seeds. Further research is needed to uncover more specific findings.

The most significant change observed in plants under salt stress was the inhibition of growth[19]. The membrane system is injured due to the peroxidation of membrane lipids caused by reactive oxygen species (ROS) that would not be eliminated easily. SOD and POD, as endogenous ROS scavengers in plants, act as protective enzyme systems. Only plants with higher enzyme activity under stressful conditions can effectively get rid of ROS and keep them at a level low enough to prevent membrane peroxidation, thereby reducing damage to the membrane structure and enhancing the plants' resistance to stress[20-22]. In this test, treatment with NaCl could significantly increase the MDA contents in the hypocotyl and root of Cucurbita pepo. This indicated that the oxidative stress on the sprouts of Cucurbita pepo had caused damage to cell membrane, which was consistent with the findings of precious research[23-24]. The research conducted by Guo Wei et al. showed that seed soaking in humic acid could enhance the POD and SOD activity in wheat seedlings and root system and facilitate GSH synthesis[6,25]. The results of this research showed that treatment with Highly Active Humic Acid liquid significantly reduced the NaCl-induced oxidative stress, and this mitigation effect was closely related to the enhanced antioxidant enzyme activity. These results were basically consistent with the research findings of Guo Wei et al.

In summary, treatment with Highly Active Humic Acid liquid diluted 450 times could increase the germination ability of Cucurbita pepo seeds under NaCl stress, enhance the antioxidant enzyme activity in Cucurbita pepo seedlings, reduce the accumulation of oxygen free radical and MDA content, and mitigate membrane lipid oxidation. This could further enhance the selective permeability of the cell membrane in Cucurbita pepo seedlings under NaCl stress. As a result, soaking Cucurbita pepo seeds in humic acid before sowing in saline areas can increase the germination rate and boost the salt tolerance of seedlings. Nonetheless, research in this regard was started relatively late, and further investigation is needed to understand the effects of humic acid on other physiological and metabolic mechanisms of plants.

References:

[1] Liu Zhiyuan, Zhu Zhujun, Qian Yarong, et al. "Effect of Iso-osmotic $Ca(NO_3)_2$ and NaCl on Growth of Tomato Seedlings." [J]. *Acta Horticulturae Sinica*, 2001, 28(1): 31-35.

[2] Li Haiyun, Wang Xiufeng, and Xing Yuxian. "Research Progress on Salt Accumulation in the Greenhouse Soil and Control Methods." [J]. *Journal of Shandong Agricultural University (Natural Science Edition)*, 2001, 32(4): 535-538.

[3] Niu Yuhua, Li Zhongjin, Hao Mingde, et al. "Research Development of Humic Acid." [J]. *Journal of Anhui Agricultural Sciences*, 2008, 36(11): 4638-4639, 4651.

[4] Cheng Liang, Zhang Baolin, Wang Jie, et al. "Research Progress of Humic Acid Fertilizer." [J]. *Soil and Fertilizer Sciences in China*, 2011(5): 1-6.

[5] Zhang Min, Hu Zhaoping, Li Xinzhu, et al. "Research Progress and Prospect of Humic Acid Fertilizer." [J]. *Phosphate & Compound Fertilizer*, 2014, 29(1): 38-40.

[6] Guo Wei, and Wang Qingxiang. "Effects of Humic Acid on Activity of Antioxidation System in Wheat Root under Salinity-Alkalinity Stress." [J]. *Humic Acid*, 2012, 29(2): 47-47.

[7] Liu Weiping, Zhang Zhimin, Wang Shikui, et al. "Influence of Glucose Oligosaccharide Acids on Seeds Germination Ability and Seedling Growth of Tomato." [J]. *Northern Horticulture*, 2011(1): 49-51.

[8] Gao Junfeng. *Plant Physiology Experiment Guidance.* [M]. Beijing: Higher Education Press, 2006: 210-217.

[9] He Junmin, She Xiaoping, and Zhang Jian. "Mitigative Effects of Hydration-Dehydration Treatment on Salt Stress-induced Injuries to Tomato Seed Germination." [J]. *Acta Horticulturae Sinica*, 2000, 27(2): 123-126.

[10] Zhu Zhihua, Hu Ronghai, Song Jingzhi, et al. "Effects of Salt Stress on Seed Germination of Different Wheat Cultivars." [J]. *China Seed Industry*, 1996(4): 25-29.

[11] Ding Shunhua, Qiu Nianwei, Yang Hongbing, et al. "Physiological Index Selection for Salt-tolerance of Wheat." [J]. *Plant Physiology Communications*, 2001, 37(2): 98-102.

Du Biyun, Cui Huilin, Cui Qunxiang, et al. “Plant Regeneration from Sola-num Melongena L. Anther Induction Through Embryogenesis Pathway.” [J]. *Jiangsu Agricultural Sciences*, 2016, 44(9):192-195.
Doi:10.15889/j.issn.1002-1302.2016.09.055

Plant Regeneration from Sola-num Melongena L. Anther Induction Through Embryogenesis Pathway

Du Biyun[1], Cui Huilin[2], Cui Qunxiang[1], Zhou Xianzhong[1], Zhang Tianyu[1], Wu Feize[1], He Chengrong[1]
(1. Jinling Institute of Technology, Nanjing 210038, Jiangsu; 2. Anhui Agricultural University, Hefei 230036, Anhui)

Abstract: Nine Sola-num melongena L. anther samples were used as test materials for induction culture. The findings revealed that Sola-num melongena L. anther culture had the potential to regenerate plants through the embryogenesis pathway; all of the nine materials were induced to generate embryoids successfully, in spite o variations in the frequency of induction among different materials, with the highest induction rate of 17% found in the 15-Heiguan; out of the 91 embryoids, 12 were able to germinate into plants, with a germination rate of 13%; among these 12 plants, 7 were transplanted with bud appearing and flowering, including 5 haploid plants, 1 diploid plant, and 1 tetraploid plant; although the anther expansion rate was higher on Induction Medium 2 when compared with that on Induction Medium 1, Induction Medium 1 subsequently yielded more mature and uniform embryos. The results showed that promoting the maturation of embryoids, increasing the frequency of haploid doubling, and determining the cellular origin of embryoids are crucial constraints on the practical application of Sola-num melongena L. anther breeding.

Keywords: Sola-num melongena L.; Anther; Embryogenesis; Plant regeneration
Chinese Library Classification No.: S641.104^{+}.3 **Document Code:** A
Article No.: 1002-1302(2016)09-0192-04

Plant anther and pollen (microspore) culture is an effective method for producing haploid and double haploid plants, and it can significantly reduce the breeding time required in breeding research. Since Guha (1964) and Maheshwar (1982) used Datura in-noxia for anther culture to generate embryoids and regenerated plants, more than 200 plant species have been reported to achieve plant regeneration success through anther culture[1]. Sola-num melongena L. anther culture research was started in the early 1970s[2], and both anther and pollen culture has successfully generated haploid plants ever since [3]. In Sola-num melongena L. anther and pollen culture, plant generation mostly occurs via callus through the embryogenesis pathway[4-9], with only a few instances through embryonic germination reported[10-12]. Research has shown that Sola-num melongena L. anther culture could yield regenerated plants originating from pollen cells, while Sola-num melongena L. pollen culture typically involves the cultivation of free microspores from pre-cultured anthers, making the process more complicated than anther culture. As a result, domestic researchers of Sola-num melongena L. breeding based on haploid technology primarily focus on anther culture studies.

Date of Reception: March 13, 2016
Fund Project: Jiangsu Provincial Fund for Independent Innovation in Agricultural Science and Technology [No.: CX(14)2013]; Doctoral Fund Project of Jinling Institute of Technology (No.: 40610151).
About the Author: Du Biyun (1991-), female, born in Hangzhou, Zhejiang, is a postgraduate specialized in vegetable genetic breeding. E-mail: 492004647@qq.com.
Corresponding Author: Cui Qunxiang, PhD, is a professor specialized in teaching of horticultural crop genetic breeding and biotechnological breeding of Sola-num melongena L. E-mail: cqx@jit.edu.cn.

This research used the high-quality Sola-num melongena L. anthers collected by the Sola-num Melongena L. Breeding Research Group of Jinling Institute of Technology as the culture materials, with the aim of generating plants originating from pollen and establishing a stable and reliable regeneration system of Sola-num melongena L. anther culture to lay the foundation for the creation of new germplasm for Sola-num melongena L. genetic breeding.

1.Material and Method

1.1 Material

The test materials were the low-generation inbred lines of 8 commercial varieties of Sola-num melongena L., 1 offspring of F_1 generation variety through convergent cross, and plants of unknown varieties grown in the field collected by the Vegetable Breeding Research Group of Jinling Institute of Technology. The material numbers were 14-24, 14-25, 14-55, 14-84, 15-231, 15-232, 15-234, 15-Heiguan, and 15-Hybrid. Among them, 14-24, 14-25, 14-55 and 14-84 were bred in late 2013 and planted in 2014; the other materials were bred in late 2014 and planted in 2015; all of the plants were pruned and regenerated in August after cultivation in Spring. Following the bud appearing and flowering of the colonized plants or the pruned and regenerated plants, flower buds without blossoming and with a distance of ±2mm between petals and calyx lobes were selected for the anther culture test.

1.2 Method

1.2.1 Flower Bud Collection and Sterilization Qualified flower buds were used and stored in a refrigerator under 4℃.

[12] Lu Jingjun, Li Qiang, and Duo Li'an. "Effect of Salt Stress on Seed Germination of Lolium Perenne L. and Festuca Elata Keng." [J]. *Bulletin of Botanical Research*, 2002, 22(3): 328-332.

[13] Liang Yunmei, Li Yan, Duo Li'an, et al. "Effect of Salt Stress on Germination of Alfalfa Seeds." [J]. *Pratacultural Science*, 1998, 15(6): 22-26.

[14] An Shouqin, Yu Zhuo, Kong Lijuan, et al. "Study on Seed Germination and Salt Tolerance at Seedling Stage of Four Leguminous Plants Including Hedysarum Scoparium." [J]. *Grassland of China*, 1995(6): 29-32.

[15] Tursunay, Gao Huiyuan, An Shazhou, et al. "Comprehensive Evaluation of Salt Tolerance of Eight Forage Grasses." [J]. *Grassland of China*, 1995(1): 30-32, 29.

[16] Sun Xiaofang, Zheng Qingsong, and Liu Youliang. "Characteristics of Growth and Ion Absorbability in Different Genotypes of Cotton at Germinating and Seedling Stages under Salt Stresses." [J]. *Cotton Science*, 2001, 13(3): 134-137.

[17] Wang Guangyin, Han Shidong, Zhao Yipeng, et al. "Effects of NaCl Stress, Ca2+ and GA3 on Seed Germination of Three Vegetables in Cucurbita L." [J]. *Journal of Plant Resources and Environmen*t, 2005, 14(1): 26-30.

[18] Guo Wei, and Yu Lihe. "Effect of Seed Soaking with Humic Acid on Soluble Sugar Accumulation and Allocation in Germinated Wheat Seed under Salt Stress." [J]. *Journal of Triticeae Crops*, 2012, 32(1):90-96.

[19] Guo Wei, and Yu Lihe. "Effect of Seed Soaking with Humic Acid on Carbon and Nitrogen Metabolism in Wheat Cultivars with Different Salt Tolerances under Salt Stress." [J]. *Journal of Triticeae Crops*, 2013, 33(2): 344-349.

[20] Zhang Enping, Zhang Shuhong, Si Longting, et al. "Effects of NaCl Stress on the Membrance Lipid Peroxidation in Cotyledon of Cucumber Seedlings." [J]. *Journal of Shenyang Agricultural University*, 2001, 32(6): 446-448.

[21] Scandalios J G. "Oxygen Stress and Superoxide Dismutase." [J]. *Plant Physiology*, 1993, 101(1): 7-12.

[22] Foyer C H, Descourvieres P, Kunert K J. "Protection against Oxygen Radicals - An Important Defense - Mechanism Studied in Transgenic Plants." [J]. *Plant, Cell & Environment*, 1994, 17 (5): 507-523.

[23] Wang Suping, Guo Shirong, Li Jing, et al. "Effects of Salt Stress on the Root Growth and Leaf Water Use Efficiency of Cucumber Seedlings." [J]. *Chinese Journal of Applied Ecology*, 2006, 17(10): 1883-1888.

[24] Han Chunmei, Li Chunlong, He Yangdong, et al. "Effect of NaCl on Seed Germination, Root Physiological and Biochemical Index in Lettuce." [J]. *Journal of Changjiang Vegetables*, 2009(10): 21-23.

[25] Guo Wei, and Wang Qingxiang. "Effects of Seed Soaking with Humic Acid on Wheat Seedlings Antioxidant System under Salt-Alkali Stress." [J]. *Chinese Journal of Applied Ecology*, 2011, 22(10): 2539-2545.