

## Development of closed-type transplant production system and discussion of its application mode for flue-cured tobacco

Jiang Hou-Long<sup>1\*</sup>, Li Na-Jia<sup>1,2</sup>, Xu An-Ding<sup>1</sup>, Yang Chao<sup>1</sup>, Wang Hong-Feng<sup>1</sup>, Chen Hai-Tao<sup>1</sup>, Shan Pei-Xiang<sup>1,3</sup>, Ding Wei<sup>1</sup>

<sup>1</sup>Chongqing Tobacco Science Research Institute, College of Plant Protection, Southwest University, Postdoctoral Research Station of Plant Protection, Chongqing 400716, China

<sup>2</sup>College of Agronomy Biotechnology of Southwest University, Chongqing 400716, China

<sup>3</sup>Dali Branch of Yunnan Province Tobacco Company, Dali 671000, Yunnan, China

\*Corresponding author: [jhl513@163.com](mailto:jhl513@163.com)

### Abstract

To overcome difficulties in seedling breeding in low temperature (the average temperature is 14.65 °C in March and April) areas and in areas receiving a low amount of sunlight (the average sunlight hour is 93.55 h in March and April), a closed-type transplant production system was designed and studied using flue-cured tobacco as the plant material. The system was used for breeding the tobacco seedlings and was compared with the floating system. The closed-type transplant production system shortened the breeding period, with the tobacco seedlings reaching the 5-leaf growth stage at approximately 25 d sooner than in the floating system. The stem diameter, dry weight of the roots, stems and leaves, root/shoot ratio, and rate of healthy seedlings were higher by 35%, 160%, 49%, 8%, 74%, and 84%, respectively, in the closed-type system than in the floating system. The costs of seedling breeding in the closed-type system with and without heeling-in (direct planting) were reduced by 70% and 21%, respectively, compared with the floating system. Our results showed that the closed-type transplant production system effectively mitigated the problems of long seedling age and low rate of healthy seedlings in low temperature areas and in areas receiving a low amount of sunlight without lowering the agronomic qualities of seedlings. Furthermore, this closed-type transplant system reduced the consumption of wetland peat, which is a non-renewable resource, thereby significantly reducing the cost of seedling breeding. This system can potentially solve transplant crop production problems encountered in low temperature areas and in areas receiving a low amount of sunlight in China.

**Keywords:** closed-type seedling production system, floating seedling production system, flue-cured tobacco, seedling.

### Introduction

Floating system is widely applied in China for production of tobacco transplants (Ma et al., 2003). This system allows the cultivation of robust tobacco seedlings with well-developed root system, strong resistance, and uniformity (Yang et al., 1997). Furthermore, the floating system eliminates soil-borne diseases, which occur during the seedling stage, and minimizes the cost and time of transplant production (Shi and Wang, 1999). Therefore, the floating system is particularly suitable for specialized and large-scale tobacco production (Lv and Yi, 2006). However, this system has several disadvantages including the low ratio of healthy seedlings in cold areas and in areas with constantly changing climate during the seedling stage. In addition, viral infection caused by the leaf removal threatens tobacco production (Liu and Duan, 2007; Li et al., 2008). In the seedling stage, the average daily temperature and sunlight hours are the major factors affecting the seedling age and quality (Jin et al., 2014). However, most tobacco production areas in China have low temperature and low sunlight conditions during the early stage of tobacco seedlings; such conditions delay seedling growth, reduce seedling quality, and postpone the transplanting stage (Li et al., 2011; Xiong et al., 2012). Long seedling age and low seedling quality could lead to less effective leaves, poor stress resistance, and low economic benefits (Zhu and Sun, 2007). The long period of

transplant production in low temperature areas and in areas receiving a low amount of sunlight cannot guarantee the timeliness of transplantation.

Wetland peat is a special soil that accumulates partially decayed plant matter, which is a highly organic material found in marshy or damp regions. Wetland peat is composed of partially decayed vegetable matter. Some features of wetland peat include unique light weight, water retention, permeability, and richness in organic matter. Thus, wetland peat is an important material for the floating nursery seedling system. As of this writing, the supply of wetland peat, which is a non-renewable resource (Shi et al., 2001; Yang et al., 2008), is nearly exhausted in China. Therefore, new technologies must be used to grow tobacco seedlings to economize on the use of wetland peat. The objectives of this study were (i) to design and build a closed-type nursery room, (ii) to verify the feasibility of nursery seedling production using the closed-type system, and (iii) to compare the efficiency of a closed-type nursery seedling production with that of the floating system. Our findings provide helpful insights into overcoming difficulties encountered in the production of optimally aged and stuggy seedlings in low temperature areas and in areas receiving a low amount of sunlight in the spring and in northern cold areas of China.

**Table 1.** Effects of different seedlings by two seedling systems on growth period of tobacco seedling

Seedling system	Seedling emergence age (day)	2-leaf growth stage (day)	4-leaf growth stage (day)	5-leaf growth stage (day)	Mature seedling age (day)
Closed-type system	7.00 A	10.75 A	19.25 A	22.00 A	22.00 A
Float system	21.00 B	30.00 B	41.50 B	46.75 B	76.25 C
Heeled-in seedlings of closed-type	7.00 A	10.75 A	19.25 A	22.00 A	45.00 B

Mean values in each column with the same letter(s) are not significantly different using Duncan tests at 5% of probability.

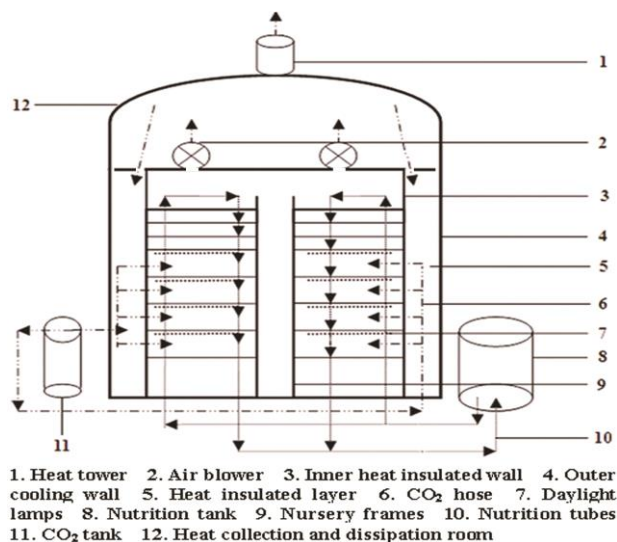
## Results

### *Effects of different seedling systems on the growth period*

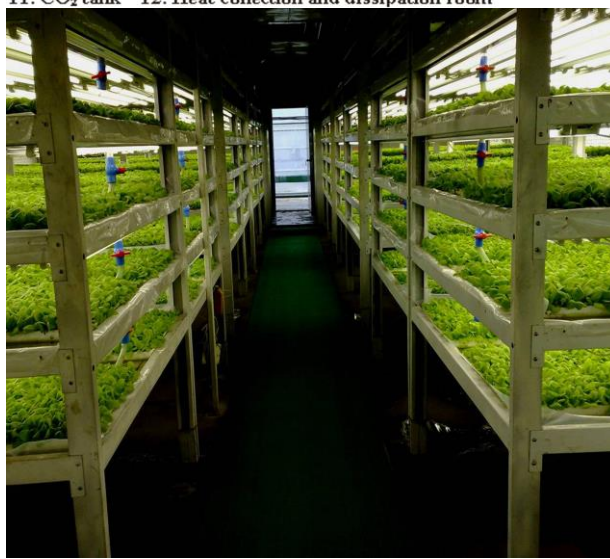
Table 1 shows that the closed-type transplant production system only needed 7 d to progress from sowing to germination, whereas the floating system required 14 d. The seedlings in the closed-type nursery room required only 22 d to reach the 5-leaf growth stage, whereas those in the floating system in a greenhouse required 47 d. The closed-type nursery considerably reduced the duration of the tobacco growth stage. Seedlings at the 5-leaf growth stage were prepared for "transplanting under film" or "transplanting of well cellar type" without leaf removal (Luo, 2012), thereby preventing possible viral infections. The seedlings were also prepared for heeling-in in the nutritive soil with or without a substrate before transplanting. After heeling-in, the seedlings at the 5-leaf growth stage in the closed-type nursery system required only 48 d to grow further, and such duration was shorter by 26 d to 30 d than in the floating system. Out of the four batches sown, the seedlings from the first batch were used at early planting sites or on-site. Meanwhile, the seedlings from the second and third batches were used for transplanting in large areas and those of the fourth batch were used for late or complementary planting. Thus, all four batches bred in one season via this system were appropriately utilized. This result illustrates the advantage of the closed-type system in overcoming the difficulties in transplant production and long seedling period in low temperature areas and in areas receiving a low amount of sunlight.

### *Influence on seedling quality*

Table 2 shows a comparison of quality at the 5-leaf growth stage between seedlings cultured by heeling-in and those cultured by the floating system in a greenhouse. The stem diameter and dry weights of the roots, stems, and leaves of seedlings in the closed-type nursery increased by 35.29%, 8.81%, 49.61%, and 154.34%, respectively, compared with the control group. The root-shoot ratio of the seedlings grown in the closed-type nursery increased by 74%, and the rate of healthy seedlings (Zhang et al., 2004) also significantly increased by 84% compared with the control. Thus, seedlings grown in the closed-type nursery room had stronger stems and roots and higher dry weight accumulation than those grown in the floating system. Seedlings grown under the closed-type system were assumed to have a shorter recovery period and a higher survival rate upon transplantation to the fields than those grown in the floating system. The leaf number and height of the seedlings from the two methods were not significantly different. However, the dry weight, root-shoot ratio, and proportion of the healthy seedlings were significantly higher for seedlings grown in the closed-type nursery room than those grown in the floating system in a greenhouse. Furthermore, the root development in the closed-type system was better than that in the control seedlings. Thus, the closed-type transplant production system effectively addresses the problem of low healthy seedling rate in low temperature areas and in areas



1. Heat tower 2. Air blower 3. Inner heat insulated wall 4. Outer cooling wall 5. Heat insulated layer 6. CO<sub>2</sub> hose 7. Daylight lamps 8. Nutrition tank 9. Nursery frames 10. Nutrition tubes 11. CO<sub>2</sub> tank 12. Heat collection and dissipation room



**Fig 1.** The sketch map (up) and real picture (down) of closed-type transplant production system room.

receiving a low amount of sunlight, such as Chongqing.

### *Effects of different seedling systems on traits in open fields*

The characteristics of the tobacco seedlings grown using three different methods are shown in Table 3. After field transplantation, the seedlings from closed-type nursery rooms were significantly higher than the other two types of seedlings. The difference in the open field traits between the heeled-in seedlings from closed-type nursery room and those from the floating system was significant. However, the effective leaf numbers and yields of the three types of seedlings were not significantly different. The yield of the heeled-in seedlings from closed-type nursery rooms was the highest, with a 5%

**Table 2.** Effects of three seedlings on agronomic characters and dry matter of tobacco seedling ready stage.

Seedling system	Number (piece)	Height (cm)	Stem circumference (cm)	Leaf dry weight (mg)	Stem dry weight (mg)	Root dry weight (mg)	Root-shoot ratio	Healthy index
Heeled-in seedlings of closed-type	7.31 B	11.9 B	2.3 C	188.00 C	97.65 C	65.34 C	0.23 C	0.07 C
Seedlings of float system	7.41 C	12.15 B	1.70 B	173.26 B	65.27 B	25.69 B	0.11 B	0.04 B
Seedlings of closed-type	5.00 A	3.55 A	0.42 A	33.67 A	11.06 A	4.61 A	0.10 A	0.01 A

**Note:** ①Healthy index= (stem circumference/height)/dry weight of seedling. ②The samples of heeled-in seedling of closed-type and seedlings of float system were collected in mature seedling age, and the sample of seedlings of closed-type was collected in 5-leaf growth stage. ③Mean values in each column with the same letter(s) are not significantly different using Duncan tests at 5% of probability.

**Table 3.** Effects of the three seedlings on agronomic characters.

Seedling system	Height (cm)	Stem circumference (cm)	Number (piece)	Yield (kg/hm <sup>2</sup> )	Output value (\$/hm <sup>2</sup> )
Heeled-in seedlings of closed-type	91.00 a	8.89 a	16.47 a	3368.05 a	7506.66 c
Seedlings of float system	91.39 a	9.09 b	16.47 a	3325.95 a	7138.67 b
Seedlings of closed-type	94.56 b	9.13 b	16.67 a	3378.90 a	7087.34 a

Mean values in each column with the same letter(s) are not significantly different using Duncan tests at 5% of probability.

**Table 4.** Costs of the three seedlings. (\$/667m<sup>2</sup>).

Seedling system	Facility cost	Operation cost	Total cost
Heeled-in seedlings of closed-type	44.80 A	233.76 B	278.56 B
Seedlings of float system	135.04 B	216.48 B	351.52 C
Seedlings of closed-type	44.80 A	63.52 A	108.48 A

Mean values in each column with the same letter(s) are not significantly different using Duncan tests at 1% of probability.

increase in yield compared with the control group. The yield of the seedlings from closed-type nursery room was slightly higher than the seedlings of the floating system. In terms of output value, the heeled-in seedlings from closed-type system showed the best results, and the seedlings from the float system showed the worst results. The heeled-in seedlings or seedlings from closed-type nursery room were not inferior compared with those in the control group in terms of their adaptability and growth in the open field.

#### Cost analysis of transplant production

The cost of transplant production in the present study included the facility and operation costs. The facility cost was calculated using a 10 year amortization. The costs of breeding heeled-in seedlings of the closed-type system and those in the closed-type and floating systems are shown in Table 4. The total cost of the breeding heeled-in seedlings in the closed-type nursery room was lower by 20.8% compared with the floating system. The cost of direct planting in closed-type nursery room was even lower (30.1% below that of the control group). Thus, adoption of the closed-type transplant production system can significantly reduce production cost. Widespread application of this method is highly promising.

#### Discussion

The too-long growth period of tobacco seedling because of low sunlight and low temperature conditions in China is a major problem. The long growth period of tobacco seedling will lead to less effective leaves, poor stress resistance, and low economic benefits (Zhu and Sun, 2007). The growth period of tobacco seedling was significantly shortened in the closed-type nursery system. Light, temperature, water, gas, fertilizer, and other environmental factors are key factors of tobacco seedling growth (Liu et al., 2003) that must be considered in implementing a comprehensive and integrated management technique to optimize a control system, such as the closed-type

nursery system. The tobacco seedling quality is important for shortening the tobacco rejuvenation period, increasing the number of effective leaves, and enhancing stress resistance. Good quality tobacco seedlings lead to excellent tobacco leaf quality and high economic efficiency (Zhu and Sun, 2007). The seedlings grown in the closed-type nursery room had stronger stems and roots and higher dry weight accumulation than those grown in the floating system. The dry weight, root-shoot ratio, and proportion of healthy seedlings were significantly higher in seedlings grown in the closed-type nursery room compared with those grown in the floating system in a greenhouse. The abovementioned results may be due to the optimal environmental conditions in the closed-type nursery system. Moreover, the traits and output values of closed-type system seedlings in open fields were better than those of floating system seedlings. Closed-type system seedlings adapted well to the open environment. With the development of flue-cured tobacco seedling technology and application of new technologies, the cost of nursery facilities continue to increase, whereas the total cost of seedling nursery and labor costs continue to decrease (La et al., 2011). The results of the present study suggest that the total costs of breeding heeled-in seedlings and direct planting in a closed-type nursery room were lower by 20.8% and 30.1%, respectively, than the total costs of breeding seedlings in the floating system. Some of the abovementioned results were due to the larger supply capacity (high-density, multi-layer, and multi-batch characteristics) of the seedlings in the closed-type system. The supply capacity of seedling was at least 60 times higher in the closed-type system than in the floating system. The closed-type transplant production system have the following limitations: the design of the nursery frame is complicated; the operation process is difficult; heat production and electricity consumption by regular fluorescent lamps are too high; the corresponding seeder and transplanter for high-density seedlings are lacking; the formula of the nutrient solution is not sufficiently reasonable; and the sensibility of the automatic control system is poor. Given these limitations, the system needs refinement

before it can be widely used. Furthermore, the application of the system to vegetables, flowers, trees, Chinese herbal medicines, and other crops, as well as its comprehensive utilization during non-seedling period, require further investigation. With further validation and improvements, this system has the potential to be highly useful in low temperature areas and in areas receiving a low amount of sunlight, such as the areas in southern China and the cold areas of northern China.

## Materials and Methods

### Plant material

The experiments were conducted on flue-cured tobacco (*Nicotiana tabacum*).

### Treatments

Given that seedlings generally require strict temperature, humidity, and illumination, a closed-type nursery room was designed. This nursery room was separated from the outside environment and was equipped with an illumination system, a CO<sub>2</sub> supply system, a temperature and humidity regulation system, and a nutrient solution supply and drainage system for transplant production. The nursery room also featured multi-layered, high density, and multi-batch production (Fig. 1). The experiments were conducted in 2012 and 2013 with flue-cured tobacco as the research material in four closed-type transplant production systems. The transplant production experiment was conducted in four batches in a nursery room with an area of 96 m<sup>2</sup> and a transplant production area of 59.47 m<sup>2</sup>. The first batch of seeds was sown on 10 March in the seedling layer, which is a layer of nursery frames for nursery tobacco seedling. Warm air was blown into the nursery to accelerate germination. After germination, the nutrient solution (nutrient-supplemented water) was added. On the 7<sup>th</sup> day before the first batch of the seedlings reached the 5-leaf growth stage, the second batch was planted in the germination layer, which is layer of nursery frames for accelerating germination. After seedling emergence, the seedlings were transferred to seedling layers. When the first batch of tobacco seedlings reached the 5-leaf growth stage, they were transferred out of the closed-type nursery room for transplanting or were floated in a plastic shelter in preparation for the transplanting season. By transplanting time, the seedlings in the germination layer had already emerged for transfer to the seedling layer. The 3<sup>rd</sup> and 4<sup>th</sup> batches were sown on 14 and 28 April, respectively. The control group was produced via the floating system, in which all the seedlings were sown on 10 March. The closed-type nursery room was equipped with a floating nursery tray with 504 pores (49 cm × 32 cm), and the floating system in the greenhouse had a floating nursery tray with 200 pores (67 cm × 34 cm). The wetland peat used in the pores in the closed-type system was only 15% of that used in the floating system. Thus, the closed-type system consumed less wetland peat than the floating system.

### Closed-type transplant production system

#### Greenhouse facilities

The closed-type nursery room had double-layered walls comprising an inner heat-insulated layer and an outer cooling layer. In the nursery room, 14 nursery frames were installed, each with dimensions of 1.4 m × 3.4 m and composed of 8 layers. Layers 1 to 4 were seedling layers (with an interval of

25 cm) with fluorescent lamps equipped at the top of each layer to supply heat and light. The germination layers were from layer 5 to layer 8 (with an interval of 20 cm) and unequipped with fluorescent lamps. The germination layers used residual heat in the nursery for germination. Every layer accommodated 26 trays with 504 pores.

### Illumination system

An artificial light system comprising T5-type fluorescent lamps (28 W) was installed in the nursery room. At the top of the seedling layers, fluorescent lamps were installed (9 lamps/m<sup>2</sup>) to maintain maximum light intensity at 12,000 lux to 15,000 lux. The fluorescent lamps were divided into three groups. Before the 2-leaf growth stage, only one group of fluorescent lamps was turned on. From the seedling 2-leaf growth stage to the 4-leaf growth stage, two groups of lamps were turned on. After the 4-leaf growth stage, all three groups of lamps were turned on. This system reversed the "light period" and "night period" for the transplant production. The "light period" started from 6:00 PM to 8:00 PM and from 7:00 AM to 9:00 AM. The purpose of this lighting system was to better regulate the temperature inside the room.

### CO<sub>2</sub> system

The closed-type nursery system was equipped with a special CO<sub>2</sub> supply system to satisfy the requirement for photosynthesis of the tobacco seedlings. A CO<sub>2</sub> tank was used as the CO<sub>2</sub> source to supply CO<sub>2</sub>, which was delivered through a plastic hose into the heat-insulated layer and into the nursery room through apertures in the heat-insulated wall. The CO<sub>2</sub> supply was regulated with an automatic CO<sub>2</sub> regulation system. The blower provided power for CO<sub>2</sub> recycling. CO<sub>2</sub> supply started at 1 h after the illumination system was turned on and stopped at 1 h before the light was turned off. The amount of supplied CO<sub>2</sub> increased with seedling growth. CO<sub>2</sub> density was maintained at 800 ppm before the 2-leaf growth stage. From the 2- to 4-leaf growth stages, the density of CO<sub>2</sub> was maintained at 1,200 ppm. After the 4-leaf growth stage, the CO<sub>2</sub> density was maintained at 1,500 ppm. When the light period ended, the heated air windows were opened, and the blower was turned on for 30 min to remove the exhaust air. At night, the heated air windows were kept open to remove the warm air in the heat collection and dissipation room.

### Temperature and humidity regulation system

The temperature and humidity regulation system in the closed-type nursery room comprised fluorescent lamps, an air blower, an air conditioner, and an automatic control system. The temperature in the nursery room was maintained by the residual heat of the fluorescent lamps. Too high temperature during the light period resulted in an automatic increase in the air exhaust capacity of the air blower. In this process, the warm air was drawn to the heat collection and dissipation room for cooling. The cooled air subsequently entered the closed-type nursery room through the apertures in the heat-insulated wall. Too high temperature at night would automatically turn the air conditioner on to reduce the temperature. Evaporated water from the nutrient solution was replenished to maintain humidity. When the humidity was too high during the light period, the air blower drew wet air to the heat collection and dissipation room for plastic film condensation at the top. Dry air was then delivered back to the closed-type nursery room through the apertures in the heat-insulated wall. When the humidity was too high during the night period, the air conditioner was turned on

for dehumidification. Before the fluorescent lamps were turned on, dehumidification was started for 30 min to reduce humidity in the room, thereby preventing the wet air from damaging the lamps. The air blower and apertures in the heat-insulated wall were closed at night to prevent hot air from entering the closed-type nursery room. Thus, the consumption of the photosynthesis products caused by excessive heat was minimized.

### **Nutrition system**

The closed-type transplant production system is essentially a type of floating system. Its nutrition supply system is composed of nutrition pools, tubes, and pumps. Nutrient solution and clear water were pumped into the top of the seedling and germination layers and transferred to the next layer through the valves. When the nutrient solution reached an appropriate depth on every seedling and germination layer, the remaining nutrient solution or clear water flowed back to the nutrition pool. The germination layers were supplied only with clear water, whereas the seedling layers were supplied with nutrient solution in addition to clear water.

### **Conclusion**

The closed-type transplant production system is a new system that integrates heating, humidification, light compensation, and seedling nursing in shallow water. In this study, transplant production experiments in four batches were conducted using tobacco plants in a closed-type nursery room with an area of 96 m<sup>2</sup>. The new system reduced the seedling age of tobacco in low temperature areas and in areas receiving a low amount of sunlight. Furthermore, the new system significantly enhanced the quality of the seedlings and optimally supplied aged seedlings for transplantation. The cost of the closed-type transplant production was considerably less than that of the floating system in a greenhouse. The direct transplantation of seedlings at 5-leaf growth stage prevented viral infection caused by leaf removal and was economical. Furthermore, the closed-type system efficiently reduced the consumption of wetland peat, which is a non-renewable resource used in the floating system for breeding tobacco transplants.

### **Acknowledgments**

The authors thank the Chongqing Tobacco Company for funding this research. The authors also thank Shen Zheng, Wang Zhijian, Wang Hongye, Li Huachuan for their assistance in sampling the flue-cured tobacco seedling.

### **Reference**

- Jin X, Tian BW, Chen FL (2014) Comparative study on intensive three-dimensional breeding and floating seedling raising of flue-cured tobacco. *J Henan Agric Sci.* 43(5):62-65.
- La GX, Xiao JG, Xie DP (2011) Cost analysis of three different seedling systems for flue-cured tobacco. *Acta Tabacaria Sinica.* 17(4):74-77.

- Li CJ, Chen YG, Wang HT (2008) Screening of disinfectants for clipping tools in tobacco floating seedbed system [J]. *Tobacco Sci Technol.* (8):63-64.
- Li PZ, Luo ZB, Hu W (2011) Effects of vinyl house with sunlight conditioning film on the growth and physiology of tobacco seedling with floating system. *Chinese Tobacco Sci.* 32 (6):63-66.
- Liu GS, Xi XY, Shi XD (2003) Studies on basal growth rules of flue-cured tobacco seedlings in the floating system. *Acta Agrice Boreali-Sinica.* 18(3):36-40.
- Liu Y, Duan YQ (2007) Spread characteristics of tobacco mosaic virus through clipping in tobacco floating-seedling system. *J Yunan Agric Univ.* 22(1):45-48, 60.
- Luo HB (2012) The transplanting technology of well cellar type for Flue-cured tobacco. *Agric Technol Service.* (3):344.
- Lv F, Yi JH (2006) The emphasis and difficulty in the process of flue-cured tobacco floating seedling. *J Yunan Agric.* (6):9-12.
- Ma JM, Cheng L, Song SY (2003) The new technology of floating system in tobacco. *Chinese Agric Sci Bulletin.* 19(6):110-112.
- Shi HZ, Wang J (2001) The industrialized floating seedling for tobacco of America. *World Agric.* (11):38-40.
- Shi XD, Liu GS, Chen JH (2001) Effects of medium on tobacco transplant growth and development in floating system. *Acta Tabacaria Sinica.* (1):18-22.
- Yang CL, Hu YX, Chen GH (1997) Study on the technique of tobacco seedling production with the direct-seeded float system. *Acta Tabacaria Sinica.* 3(4):53-59.
- Yang YH, Ji Y, Wu YP (2008) Effect of available iron content in matrix of flue-cured tobacco floating system on germination rate and seedling growth. *Tobacco Sci Technol.* (11):63-66.
- Zhang K, Wei MZ, Chen QY (2004) Study on the healthy index of the plug seedlings of the cucumber. *J Huazhong Agric Univ.* 35(Supplement): 240-244.
- Xiong Y, Chen JJ, Lin RF (2012) Effects of defense technique for low-temperature seedling production on enzymatic activity and economic characters of flue-cured tobacco. *Acta Tabacaria Sinica.* 18(3):55-60.
- Zhu XX, Sun ZJ (2007) Effect of seedling quality on agronomic trait after transplanting. *J Anhui Agri Sci.* 35(4):1061-1062.