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RESEARCH ARTICLE

Additive insect-resistant effects of transgenic triploid Chinese white poplar against *Clostera anachoreta*

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Abstract **Two generations of *Clostera anachoreta* (Fabricius) larvae were fed continuously with leaves of selected clones of transgenic (Bt + API) triploid Chinese white poplar in order to evaluate additive insect-resistant effects of transgenic triploid Chinese white poplar. When the two generations were subcultured with the high and medium level insect-resistant transgenic poplar, the high and medium level insect-resistant transgenic poplar manifested its obvious additive resistance to *C. anachoreta* (Fabricius), with higher mortality of the second generation *C. anachoreta* (Fabricius) larvae and longer larvae growth period of the second generation than those of the first generation. Furthermore, the weight of pupa and the fecundity of the second generation were lower than those of the first generation. However, there was no significant difference between both generations when subcultured with low level insect-resistant transgenic poplar. These results implied that the population of *C. anachoreta* (Fabricius) would decline gradually to under the economic threshold even when planting the medium level insect-resistant transgenic poplars in the environment.**

Keywords transgenic triploid Chinese white poplar, *Clostera anachoreta*, additive effect, insect resistance

Introduction

The triploid Chinese white poplar, a breed of pollen radiation and sexual hybridization developed by ZHU Zhiti, an academician at the Beijing Forestry University, in 1984, was derived from the cross (*Populous tomentosa* Carr. × *P. bolleana*) × *P. tomentosa* Carr. (Kang et al., 1999; Kang et al., 2000; Zhang et al., 2000), growing well in the areas of Hebei, Henan, Beijing, Shaanxi, and Gansu after many years of regional tests. However, the plant was seriously damaged by leaf-eating pests. To reduce the environmental pollution and human input, 29 clones of transgenic poplar were acquired by transforming the insect-resistant gene (*Bt cry1Ac* gene and arrowhead proteinase inhibitor (*API*) gene) into the triploid Chinese white poplar using *Agrobacterium*-mediated transformation technique (Liang and Chen, 1995; Yang et al., 2006). In the present study, six clones of all the 29 insect-resistant transgenic poplar were selected to detect the additive insect-resistance to *Clostera anachoreta* (Fabricius).

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Materials and methods

Materials

High level insect-resistant transgenic poplar clone 1 (1H) and clone 7 (7H), medium level insect resistant clone 10 (10M) and clone 28 (28M), low level insect-resistant clone 16 (16L) and clone 26 (26L), and the non-transgenic poplar (CK) were obtained from the Laboratory of Forest Genetics and Biotechnology of Agricultural University of Hebei.

Samples of *C. anachoreta* (Fabricius) were collected from Raoyang County, Hebei Province of China, and reared in the laboratory with non-transgenic poplar leaves until egg stage.

Methods

Insect feeding

More than one hundred *C. anachoreta* (Fabricius) larvae were fed with the fresh leaves of different levels of insect-resistant clones respectively, with the leaves of non-transgenic poplar as control (CK). Twenty larvae were fed in each rearing tank, and maintained under the optimum rearing conditions of 25±1ºC and 60%–70% relative humidity. The fresh leaves were replaced daily and the development stage and the other indices of *C. anachoreta* (Fabricius) were noted. After eclosion of the first generation of *C. anachoreta* (Fabricius), 5 pairs of adults were selected randomly to record their fecundity (Gao et al., 2002; Gao et al., 2004; Liu et al., 2004). After the larvae hatched, the second generation larvae of *C. anachoreta* (Fabricius) were fed with fresh leaves daily using the corresponding clones of transgenic triploid poplar, and developmental index was noted.

Data analysis

Data analysis was carried out by using the DPS operating system (Tang and Feng, 2002) and the following equations.

$$\text{Total mortality rate} = \text{Total death number of larvae}/\text{Total number of larvae raised} \times 100\%,$$

$$\text{Corrected mortality rate} = (\text{Mortality of tested insects} - \text{mortality of CK insects})/(1 - \text{mortality of CK insects}),$$

$$\text{Death index} = (\sum \text{Day Number} \times \text{Day mortality})/(\text{Total mortality} \times \text{Total day number}).$$

Results and analysis

Influence of different transgenic poplar clones on the mortality and growth period of the second generation larvae

The first generation larvae of *C. anachoreta* (Fabricius) were fed with the fresh leaves of all the selected clones respectively, which all died when fed with 1H and survived till the 2nd generation when fed with other clones. The corrected mortality rate of the first generation larvae fed with 7H, 10M, 28M, 26L and 16L was 79.79, 65.96, 54.26, 25.53 and 9.57, respectively while that of the second generation fed with 7H, 10M and 28M was 100%. However, the corrected mortality rate of the second generation fed with 7H, 10M and 28M was higher than that of the first generation (Table 1).

The first generation larvae fed with highly resistant 7H had the longest growth period among those fed with all different clones, with the lowest growth rate among those fed with 7H, 10M, 28M, 26L and 16L. The growth period of the first instar larvae of the second generation fed with 10M was approximately 3 d, which was longer than that of the first generation. The similar growth period of the second generation fed with 28M and 10M appeared at the first instar, with larvae all dead at the second instar and the third instar respectively. The growth period of each instar larvae of the second generation was similar to that of the first generation when fed with 26L but longer than that of the first generation when fed with 16L. Obviously, the growth period of each instar of the 2nd generation larvae was longer than that of the first generation while the growth rate was lower than the 1st generation larvae feed with the same clones (Table 2).

Influence of different transgenic poplar clones on the pupa weight and fecundity of the 1st and 2nd generation

Pupa weight and fecundity of the first generation of *C. anachoreta* (Fabricius) fed with all the clones and CK were assessed and the average weight of 20 pupae was calculated. The average pupa weight of *C. anachoreta* (Fabricius) fed with 7H was significantly different from that fed with 26L, 16L and 28M. Likewise, that fed with CK was significantly different from that fed with 7H, 10M, 28M, and 26L, but not significantly different from that fed with 16L.

Only some larvae fed with 26L and 16L could become pupae and the average pupa weight of the second generation fed with 26L was extremely lighter than that of the first generation while that fed with 16L was similar to that of the first generation (Table 3).

Five pairs of adult *C. anachoreta* (Fabricius) from each treatment were selected to study the fecundity by multiple comparison (Tables 3 and 4). The adult fecundity of *C.*

Table 1 Mortality and death index of the 1st and 2nd generation larvae of *C. anachoreta* (Fabricius)

Generation		Result						
		1H	7H	10M	28M	26L	16L	CK
1st	Number of larvae	104	98	100	100	99	96	100
	Death index	0.54	0.55	0.64	0.68	0.73	0.75	0.79
	Mortality rate (%)	100	81	68	57	30	15	6
	Corrected mortality rate(%)	100	79.79	65.96	54.26	25.53	9.57	–
2nd	Number of larvae	–	100	102	96	97	99	100
	Death index	–	0.26	0.38	0.46	0.65	0.74	0.83
	Mortality rate (%)	–	100	100	100	46.9	16.3	9.2
	Corrected mortality rate (%)	–	100	100	100	41.25	7.82	–

Table 2 Development status of each instar of the 1st and 2nd generation larvae of *C. anachoreta* (Fabricius)

Generation		Growth period (day)					
		7H	10M	28M	26L	16L	CK
1st	1st instar	2.5146	2.4938	2.3496	2.0313	1.9984	1.9952
	2nd instar	4.0012	3.92	4.2571	3.1182	2.6371	2.5873
	3rd instar	5.3501	5.1203	4.7939	4.7103	4.0667	4.0032
	4th instar	9.0124	8.8496	7.9936	6.105	5.3967	5.3107
	5th instar	8.9511	8.4317	7.6453	6.8074	6.192	6.135
2nd	1st instar	–	3.2103	2.9274	2.6226	2.3838	2.3496
	2nd instar	–	–	4.0783	3.1104	2.7996	2.7693
	3rd instar	–	–	–	4.7596	4.3122	4.2499
	4th instar	–	–	–	6.006	5.5991	5.5096
	5th instar	–	–	–	6.8587	6.5703	6.566

Table 3 Multiple comparison of pupa weight of the 1st and 2nd generation *C. anachoreta* (Fabricius)

Clone	Average pupa weight of 1st generation (g/head)	xi-x6	xi-x5	xi-x4	xi-x3	xi-x2	Average pupa weight of 2nd generation (g/head)	xi-x3	xi-x2
CK	0.1653	0.0792**	0.053**	0.0401**	0.0246	0.0124	0.1628	0.0473**	0.0006
16L	0.1529	0.0668**	0.0406**	0.0277	0.0122	–	0.1634	0.0479**	–
26L	0.1407	0.0546*	0.0284	0.0155	–	–	0.1155	–	–
28M	0.1252	0.0391*	0.0129	–	–	–	–	–	–
10M	0.1123	0.0262	–	–	–	–	–	–	–
7H	0.0861	–	–	–	–	–	–	–	–

Note: * and ** in a column represent significant difference at 0.05 and 0.01 probability levels, respectively.

Table 4 Multiple comparison of fecundity of the first generation *C. anachoreta* (Fabricius)

Clone	Average fecundity (zooid/head)	xi-x6	xi-x5	xi-x4	xi-x3	xi-x2
CK	291	225.2**	156.6**	143.4**	66.4*	–6.4
16L	297.4	231.6**	163**	149.8**	72.8*	–
26L	224.6	158.8**	90.2**	77*	–	–
28M	147.6	81.8*	13.2	–	–	–
10M	134.4	68.6	–	–	–	–
7H	65.8	–	–	–	–	–

Note: * and ** in a column represent significant difference at 0.05 and 0.01 probability levels, respectively.

anachoreta (Fabricius) fed with 7H, 10M and 28M was significantly different from that of CK. Likewise, the fecundity of *C. anachoreta* (Fabricius) when fed with 26L was significantly different from that of CK, while 28M showed an extremely significantly different fecundity from 7H and 10M. Both fecundity and pupa weight fed with 7H and 10M were not significantly different. The high and intermediate insect-resistance clones affected the pupa weight obviously, and were significantly different from CK.

Discussion and conclusions

The high and medium level insect-resistant transgenic poplar clones showed higher resistance to the larvae of the second generation when subcultured with the transgenic poplar. The mortality of the first generation *C. anachoreta* (Fabricius) larvae was lower than that of the second generation. The low level insect-resistant transgenic poplar clones had no obvious influence on the change of the two generations. A corresponding decline in fecundity occurred with the decline of the pupa weight which depends on the resistance level of transgenic poplar. In other words, the high and medium level insect-resistant clones had stronger resistance to *C. anachoreta* (Fabricius).

The high and medium level insect-resistant transgenic poplar clones had apparently additive insect-resistance. These transgenic poplars possessed more obvious influences on the growth and development of the subcultured generation and reduced the insect population density. These results imply that the population of *C. anachoreta* (Fabricius) would decline gradually to under the economic threshold even when only planting the medium level insect resistant transgenic poplars in the environment.

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