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# A Biovoltine Silkworm Variety, *Huayuan* × *Dongshen*, That is Resistant to Fluoride Contamination

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**The major dominant fluoride-endurance (*Dfe*) gene was introduced into the commercial varieties by crossing and pedigree selection to breed silkworm races that could normally develop in the area that polluted by fluoride. After backcrossed for two generations, the *Dfe* gene was made homozygous, and individuals with good economic characters were selected to generate next generation. After 8 generations of selection, their characters became stable, and the silkworm variety which is resistant to fluoride, *Huayuan* × *Dongsheng*, for spring rearing were bred.**

**Key words**: Fluoride-endurance gene; Silkworm (*Bombyx mori*); Commercial Variety

## Introduction

The air pollution becomes serious along with the fast growing of economy in China, especially the development of the township enterprises since 1980s.The content of fluoride in the atmosphere achieved such a degree that the mulberry leaves were polluted by it, and these mulberry leaves were toxic to silkworms. When silkworms ate such mulberry leaves, they would develop slowly and some of them could not make cocoons and die. It is reported that 73% of the mulberry area in *Huzhou* (*Zhejiang* province) was polluted by fluoride in 1986, and the harvest of spring cocoon decreased by 2900 tons (Ma *et al.*, 1987); 45% of the area in *Jiaxing* (*Zhejiang* province) was polluted too, and the harvest decreased by 1500 tons(Wu *et al.*, 1987). The losses achieved about 3 million Yuan (361445 US$) due to the fluoride pollution in *Huaiyin* and *Shuyang* (*Jiangsu* province) in 1995 (Qu *et al.*, 1999). Thus some researchers examined the fluoride-endurance ability of different silkworm races and analyzed the relationship between it and other characters, and studied the accumulation and excretion of fluoride in silkworm body (Zhang *et al.*, 1982; Bi *et al.*, 1988; Lin *et al.*, 1992). Lin *et al.* (1996) investigated the fluoride-endurance ability in 300 silkworm varieties and studied the relationship between it and other characters. The major dominant fluoride-endurance (*Dfe*) gene was discovered, and the silkworm hold that gene could endure 100 mg/kg of fluoride (Lin *et al.*, 1997; Li *et al.*, 2001). Then, the *Dfe* gene was introduced into the commercial silkworm varieties with lower fluoride-endurance ability, by backcrossin and then selfing to make homogenous and stable, to breed the fluoride-endurance silkworm variety, *Huayuan* × *Dongsheng*, for spring rearing.

## Materials and Methods

### Breeding materials

*T6*, was highly endurance to fluoride, harboring the *Dfe* gene, while *827* and *Xuesong*, which were Chinese bivoltine and Japanese bivoltine commercial silkworm race respectively, and were sensitive to fluoride.

### Methods of in investigation on fluoride-resistance

Mulberry leaves were aired after they were dipped in 100 mg/kg of NaF solution for five min. Silkworm larvae were fed with such leaves since 2$^{nd}$-instar until they began to spin silk. Mortality rate was checked in the course of feeding.

### Introducing of *Dfe*

*Dfe* gene in *T6* was introduced to *827* and *Xuesong* respectively by backcrossing methods. The homozygous

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**Table 1.** Genetic constituent and heredity component of backcross offspring.

| Backcross time | Offspring genotypes | Heredity component of T6 | Heredity component of *Huayuan* or *Dongsheng* |
|---|---|---|---|
| 0 | $Def/+^{Dfe}$ & $0+^{Dfe}/+^{Dfe}$ | 0.50 | 0.50 |
| 1 | $Def/+^{Dfe}$ & $1+^{Dfe}/+^{Dfe}$ | 0.25 | 0.75 |
| 2 | $Def/+^{Dfe}$ & $3+^{Dfe}/+^{Dfe}$ | 0.125 | 0.875 |
| 3 | $Def/+^{Dfe}$ & $7+^{Dfe}/+^{Dfe}$ | 0.0625 | 0.9375 |
| : | : | : | : |
| : | : | : | : |
| n | $Def/+^{Dfe}$ & $(2^n-1)+^{Dfe}/+^{Dfe}$ | $(1/2)^{n+1}$ | $1-(1/2)^{n-1}$ |

of *Dfe* genotype was set as *Dfe/Dfe* and fluoride-sensitive genotype was set as *+Dfe/+Dfe*. Based on the population genetics, the *Dfe* genotype and genetic constitution of each generation are listed in Table-1. The $BF_1$, $BF_2$, $BF_3$ population were fed with NaF from 2nd to 4th instar. The individuals with *Dfe/+Dfe* genotype (survived individuals after feeding with NaF) were chosen and the individuals with good quality of commercial characters were selected to bacrossed to the recurrent parents, *827* or *Xuesong*. *Dfe* was made homozygous after $B_3$ generation for their commercial characters reached the recurrent parents' level, and the *Def/+Dfe* individuals were sib mated to establish strains having homozygous *Def*, batch selection was carried out since then.

## Results and Discussion

### Characteristics of *Huayuan*

The eggs color is celadon. The larvae are plain and bluish white in color. Cocoons are white and oval with medium grains. Larval duration is around 25 days. The cocoon shell ratio, raw silk percentage, filament length and neatness are good. The breeding process and economic characters of *Huayuan* were given in Table 2. It can endurance 100 mg/kg of fluoride without influencing its economical characters.

### Characteristics of *Dongshen*

The eggs color is gray purple. The larvae are plain and bluish white in color with normal marking (*+p/+p*). Cocoons are white and dumbbell with medium grains. Larval duration is around 25 days. The cocoon shell ratio, raw silk percentage, filament length and neatness are good. The breeding process of *Dongshen* were showen in Table-2. It can endurance 100 mg/kg of fluoride without influencing its economical characters.

### Characteristics of *Huayuan* × *Dongshen*

The larvae are bluish white and plain in color with normal marking (*+p/p*). Cocoons are white and intermediate shape between oval and dumbbell. Larval duration is around 25 days. The most prominent characters of this hybrid are endurance to fluoride, high survival, high cocoon shell ratio, longer filament length and high quality raw silk. The hybrids are robust. This makes them easy rearing at farmers level, especially in the areas where fluoride pollution are serious. The hybrids can endurance 100 mg/kg of fluoride without influencing its economical characters.

In 2003 and 2004 spring, the hybrids of *Huayuan* × *Dongshen* were tested in three centers located at different regions. Most of the *Jingsong* × *Haoyue* individuals (widely used in Chinese sericulture), the control hybrids, could not molt at the 2nd instar. Their growth was extremely ununiform and were all died in the 3rd instar. *Huayuan* × *Dongsheng* hybrids could develop well for all instars, and their economical characters were similar to the normal rearing ones. The hybrid of their parents, *827* × *Xuesong*, developed more slowly then *Huayuan* × *Dongshen* when they were fed with 100 mg/mL of NaF, only about 19% of them could spin cocoon., and their economic characters were significantly lower than *Huayuan* × *Dongshen* too. Its economic characters were above the control hybrid combination - *Jingsong* × *Haoyue* too (Table 3).

*Huayuan* and *Dongshen* were tested for homozygosity for *Dfe* gene by crossing them with 827 and Xuesong respectively, and their offspring could all develop normally when they were fed with 100 mg/mL of NaF. But only 75 – 80% of $F_2$ individuals of them could survive when they were fed with 100 mg/mL of NaF (Data not shown). These phenomena proved that the *Dfe* gene was homozygous in *Huayuan* and *Dongshen*.

In recent years, fluoride pollution had damaged seri-

**Table 2.** Breeding process of *Huayuan*, *Dongshen* and their characters in each generation.

| Generation (rearing year and season) | Race | Duration of feeding period (D:H) | Survival rate* (%) | Percentage of pupation (%) | Cocoon weight (g) | Cocoon shell weight (g) | Cocoon shell ratio (%) | Filament length (m) | Reela-bility (%) | Length of non-broken cocoon filament (m) | Neatness (P) | Filament size (D) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parents (1997Sp) | T6 | 25:0 | 100 | 96.00 | 1.610 | 0.33 | 20.50 | 671.0 | 83.30 | 559.20 | 94 | 2.22 |
| | 827 | 26:12 | 6.3 | 92.4 | 1.92 | 0.44 | 23.0 | 1430 | 71.0 | 1016 | 96.2 | 2.28 |
| | Xuesong | 27:6 | 19.2 | 90.0 | 1.82 | 0.42 | 23.0 | 1180 | 83.1 | 980 | 95.3 | 2.79 |
| $F_1$ (1997Ma) | $HyF_1$ | 24:12 | 100 | 95.4 | 1.65 | 0.37 | 22.42 | 852 | 62.3 | 531 | 94 | 2.23 |
| | $DsF_1$ | 25:6 | 100 | 93.6 | 1.6 | 0.35 | 21.88 | 763 | 67.1 | 512 | 94.5 | 2.54 |
| $BF_1$ (1998Sp) | $HyBF_1$ | 25:06 | 54.2 | 96.1 | 1.76 | 0.40 | 22.73 | 1185 | 80.3 | 952 | 98 | 2.29 |
| | $DsBF_1$ | 26:12 | 59.3 | 95.2 | 1.71 | 0.38 | 22.22 | 982 | 83.0 | 815 | 93 | 2.67 |
| $BF_2$ (1998Ma) | $HyBF_2$ | 24:18 | 53.1 | 96.4 | 1.66 | 0.37 | 22.29 | 1137 | 71.4 | 812 | 98 | 2.21 |
| | $DsBF_2$ | 25:12 | 58.5 | 95.2 | 1.68 | 0.36 | 21.43 | 952 | 69.6 | 663 | 93 | 2.71 |
| $BF_3$ (1999Sp) | $HyBF_3$ | 25:12 | 55.6 | 94.6 | 2.03 | 0.48 | 23.65 | 1428 | 78.8 | 1125 | 93 | 2.77 |
| | $DsBF_3$ | 26:6 | 60.5 | 96.4 | 1.84 | 0.43 | 23.37 | 1193 | 85.8 | 1023 | 96.2 | 2.62 |
| $BF_3S_1$ (1999Ma) | $HyBF_3S_1$ | 25:0 | 75.8 | 93.8 | 1.76 | 0.42 | 23.86 | 1295 | 76.1 | 985 | 94 | 2.31 |
| | $DsBF_3S_1$ | 25:22 | 79.7 | 92.5 | 1.74 | 0.4 | 22.99 | 976 | 74.3 | 725 | 94.5 | 2.76 |
| $BF_3S_2$ (2000Sp) | $HyBF_3S_2$ | 25:00 | 98.3 | 98.1 | 1.76 | 0.42 | 24.0 | 1380 | 75.2 | 1038 | 94 | 2.28 |
| | $DsBF_3S_2$ | 25:18 | 98.7 | 98.6 | 1.74 | 0.40 | 22.7 | 981 | 75.7 | 744 | 94.5 | 2.70 |
| $BF_3S_3$ (2000Ma) | $HyBF_3S_3$ | 25:05 | 97.1 | 96.1 | 1.67 | 0.38 | 22.9 | 1355 | 76.5 | 1.32 | 98 | 2.21 |
| | $DsBF_3S_3$ | 26:08 | 98.4 | 95.2 | 1.68 | 0.36 | 21.5 | 981 | 86.5 | 848 | 93 | 2.75 |
| $BF_3S_4$ (2001Sp) | $HyBF_3S_4$ | 24:20 | 98.0 | 98.6 | 1.74 | 0.40 | 23.1 | 1196 | 84.7 | 1012 | 96.2 | 2.64 |
| | $DsBF_3S_4$ | 25:17 | 97.8 | 93.2 | 1.90 | 0.42 | 22.4 | 1212 | 62.6 | 1118 | 92 | 2.73 |
| $BF_3S_5$ (2001Ma) | $HyBF_3S_5$ | 26:08 | 99.5 | 97.1 | 1.89 | 0.46 | 24.2 | 1445 | 61.0 | 882 | 95.8 | 2.23 |
| | $DsBF_3S_5$ | 26:10 | 99.5 | 97.4 | 1.95 | 0.44 | 22.5 | 1160 | 79.4 | 921 | 94.3 | 2.75 |
| $BF_3S_6$ (2002Sp) | $HyBF_3S_6$ | 24:16 | 98.98 | 98.4 | 1.99 | 0.51 | 25.36 | 1554.2 | 68.5 | 1064.5 | 97.5 | 2.61 |
| | $DsBF_3S_6$ | 24:21 | 98.93 | 96.0 | 1.92 | 0.46 | 24.16 | 1176.6 | 80.65 | 948.8 | 95 | 2.93 |

*Feeding with mulberry leaves which had been dip in 100 mg/kg of NaF

Note: the achievements of the parents were the individuals which had not been fed with fluoride.

D=day, H=Hour, Hy=Huanyuan, Ds=Dongshen, Sp=Spring, Ma=Middle autumn.

**Table 3.** Performance of *Huayuan* × *Dongshen* in three centers located at different regions, (average achievements for 2003 spring and 2004 spring)

| District | Hybrid | Duration of feeding period (D:H) | Survival rate (%) | Rate of dead worm cocoons (%) | Cocoon weight (g) | Cocoon shell weight (g) | Cocoon shell ratio (%) | Cocoon yield per $10^4$ larvae (kg) | Cocoon shell weight per $10^4$ larvae (kg) | Raw silk percentage of cocoons (%) | Cocoon filament length (m) | Reela-bility (%) | Length of non-broken cocoon filament (m) | Size (D) | Neat-ness (P) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zhenjiang (Jiangsu province) | *Hy* × *Ds** | 25:15 | 97.4 | 1.3 | 2.12 | 0.47 | 22.3 | 21.3 | 4.68 | 17.66 | 1133 | 77.64 | 881 | 3.19 | 97.5 |
| | *827* × XS* | 29:20 | 21.3 | 17.5 | 1.65 | 0.32 | 19.39 | | | | | | | | |
| | *Hy* × *Ds* | 24:06 | 97.615 | 1.9 | 2.23 | 0.475 | 21.325 | 20.26 | 4.32 | 18.72 | 1155 | 82.7 | 955 | 3.17 | 97.5 |
| | Js × Hy | 27:00 | 97.67 | 1.4 | 2.08 | 0.487 | 23.41 | 17.93 | 4.195 | 18.87 | 1150 | 66.45 | 764 | 3.06 | 95 |
| Hefei (Anhui province) | *Hy* × *Ds** | 23:06 | 94.89 | 0 | 2.129 | 0.503 | 23.57 | 12 | 2.83 | 23.05 | 1404 | 79.37 | 1114.3 | 2.96 | 94.2 |
| | *827* × XS* | 29:14 | 22.5 | 16.8 | 1.68 | 0.33 | 19.64 | | | | | | | | |
| | *Hy* × *Ds* | 22:06 | 94.6 | 9.54 | 2.104 | 0.492 | 23.39 | 13.68 | 3.19 | 22.28 | 1423.4 | 96.15 | 1368.6 | 2.82 | 97.5 |
| | Js × Hy | 23:23 | 88.25 | 2.08 | 1.988 | 0.478 | 24.04 | 11.82 | 2.84 | 22.7 | 1363.7 | 81.97 | 1117.8 | 3.0 | 95 |
| Hangzhou (Zhejiang province) | *Hy* × *Ds** | 23:16 | 95.31 | 1.82 | 1.965 | 0.46 | 23.34 | 19.12 | - | 18.53 | 1310 | 93.17 | 1220 | 2.86 | 94 |
| | *827* × XS* | 30:6 | 19.8 | 19.7 | 1.53 | 0.28 | 18.30 | | | | | | | | |
| | *Hy* × *Ds* | 22:19 | 96.9 | 1.24 | 2.150 | 0.501 | 23.33 | 21.24 | - | 19.05 | 1415 | 92.02 | 1302 | 2.90 | 95 |
| | Js × Hy | 24:17 | 98.67 | 0.42 | 1.900 | 0.466 | 24.54 | 19.04 | - | 19.97 | 1296 | 93.17 | 1230 | 2.77 | 95 |
| Average | *Hy* × *Ds** | 24:04 | 82.39 | 1.04 | 2.071 | 0.478 | 23.07 | 17.47 | 3.76 | 19.75 | 1282 | 83.39 | 1072 | 2.97 | 95.4 |
| | *Hy* × *Ds* | 23:02 | 83.04 | 4.23 | 2.161 | 0.489 | 22.68 | 18.39 | 3.76 | 20.02 | 1331 | 90.29 | 1208 | 2.96 | 96.7 |
| | Js × Hy | 25:05 | 84.86 | 1.3 | 1.989 | 0.477 | 24 | 16.26 | 3.52 | 20.49 | 1270 | 81.12 | 1037 | 2.97 | 95 |

Annotate: 1. *The larvae were fed with mulberry treated with 100 mg/kg NaF solution.

2. *Hy* × *Ds* = *Huayuan* × *Dongshen*; *Js* × *Hy* = *Jingsong* × *Haoyue*; *XS* = *Xuesong*, parent of *Dongshen*; D = day, H = Hour.

culture heavily in some provinces of China. Rearing silkworm hybrids that can endurance fluoride is a good way to avoid the economic loss in the sericultural area. These silkworm races could be used in China but also in other countries where air pollution is serious.

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