Leaf Coloration and Photosynthetic Characteristics of Hybrids between *Forsythia* ‘Courtaneur’ and *Forsythia koreana* ‘Suwon Gold’

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Abstract. Yellow-leaved cultivars usually do not grow as vigorous as their green-leaved counterparts, which affect their use in landscapes. To breed *Forsythia* cultivars with both yellow leaves and vigorous growth, crosses between *F.* ‘Courtaneur’ (♀) and *Forsythia koreana* ‘Suwon Gold’ (♂) were conducted, and 52 F₁ hybrid progenies with different leaf colors (green, chartreuse, and yellow) were obtained. The progenies were categorized into three groups [Yellow Group (YG), Chartreuse Group (CG), and Green Group (GG)] based on leaf colors. The growth index (GI) and the number of branches and leaves of YG progenies were significantly lower at 2%, 35%, and 34% of GG progenies. As the leaves changed from green to chartreuse and to yellow, chlorophyll content, leaf thickness, and chlorophyll fluorescence parameters decreased and the chloroplast structures were disintegrated gradually, which influenced the leaf photosynthetic activity and led to weak growth. Compared with yellow-leaved progenies, the leaf chlorophyll content and leaf thickness of chartreuse-leaved progenies were significantly higher at 71% and 9%. The chloroplast structure of stroma lamella of chartreuse-leaved progenies was relatively intact. Carboxylation efficiency (CE), photochemical efficiency of PS II (F_v/F_m), and the number of branches and leaves of GG progenies were significantly higher than YG progenies; however, they have no significant difference with CG progenies. The results were promising for breeding new forsythia cultivars from moderate growth and chartreuse leaves.

Yellow-leaved plants, with leaves showing different shades of yellow, are widely used in landscapes (Yu and Zhang, 2000). Mutations in both nuclear and organelle genomes reportedly cause leaf-leaved variegation (Gao et al., 2016; Liu et al., 2016;

Moseyko et al., 2002; Xiao et al., 1995). There are significant differences between yellow-leaved plants and green-leaved ones in terms of pigment contents, photosynthesis, and leaf structure (Hu et al., 2007; Zhang et al., 2015). Total leaf chlorophyll content and the ratio of chlorophyll to carotenoid content in yellow-leaved plants are generally lower than that of their green-leaved counterparts (Hu et al., 2007). Photosynthetic apparent quantum yield (AQY) of yellow-leaved plants is lower than that of green-leaved ones in *Eupatorium makinoi* (Funayama et al., 1997). The net photosynthetic rate of yellow-leaved *Sophora japonica* var. *golden* is lower than that of the green-leaved species

S. japonica (Liu et al., 2003). There is a significant difference in chloroplast structure between yellow-leaved and green-leaved plants, and the decrease in the number of granum and granum lamellae results in the reduction of chlorophyll content and the incomplete development of photosynthetic system, which then results in yellow leaves with lower photosynthetic capacity and weak growth in *Brassica napus* and *Ulmus pumila* ‘Jinye’ (Xiao et al., 2013; Zhu et al., 2014).

Forsythia koreana ‘Suwon Gold’ is a beautiful cultivar with bright yellow leaves on the outside of the canopy throughout the growing season. Compared with green-leaved cultivars, ‘Suwon Gold’ is less vigorous, and leaf scorch often occurs under high light intensity during summer. To breed forsythia cultivars with both bright yellow leaves and vigorous growth, interspecific crosses between *F.* ‘Courtaneur’ (female parent with green leaves) and ‘Suwon Gold’ (male parent) were carried out, and F₁ progenies with different leaf colors were obtained in the study. The growth, leaf pigment contents, photosynthetic parameters, leaf structure, and chloroplast structure were investigated to lay a foundation for understanding the formation of yellow leaves in *Forsythia*.

Materials and Methods

Plant materials. The parent plants used in hybridization were 3 years old and were planted in the nursery of National Engineering Research Center for Floriculture (NERCF) (40°02’N, 115°50’E) in Beijing, China. Pollinations were performed in Apr. 2013. The dehiscent and undersized buds of ‘Courtaneur’ were removed, whereas the flowers which could dehisce in 1 d were emasculated and pollinated at 11:00 AM to noon with freshly collected pollens of ‘Suwon Gold’. The pollinated flowers were then bagged and tagged immediately. A total of 351 hybrid seeds were obtained on ‘Courtaneur’ plants in October. The seeds were sowed in 50-cell plug trays (the top and bottom diameter is 50 and 22 mm, respectively, and the depth is 50 mm) in a greenhouse on the campus of Beijing Forestry University on 15 Nov. 2013. Fifty-two seedlings were obtained and then planted in the nursery of NERCF on 18 May 2014.

Morphological characteristics. The plant height, crown widths, and the number of branches and leaves of hybrid progenies were measured every 15 d from 20 Apr. to 18 Oct. 2014. Growth index was calculated using the following equation: $GI (m^3) = \pi(w/2)^2 \times h$ (w : the average two plant widths, one measured at the widest point and the other one perpendicular to the first; h : the plant height; Debalin et al., 2015). The colors of fully expanded leaves of the third to fourth nodes from the top of current growth of every progeny were confirmed using the fifth edition of Royal Horticultural Society Color Chart.

Leaf pigment content. Five plants randomly chosen from parent and progeny

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groups were used to measure leaf pigment contents. Mature leaves of the third to fourth nodes from the top of shoots under full sun were collected and frozen in liquid nitrogen and then preserved at -80°C . The contents of chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), and carotenoid were extracted and measured with a BioMate 3S ultraviolet-visible spectrophotometer (Thermo Fisher Scientific, Shanghai, China) following Wang's (2006) protocol.

Microstructure of leaf and ultrastructure of leaf chloroplast. Mature leaves selected from the third fully expanded leaf were collected from the parent plants, three groups of progenies were cut into pieces of $\approx 2 \times 4$ mm, and then dehydrated pieces were obtained. To observe the microstructure of leaf and ultrastructure of leaf chloroplast, semithin sections and ultrathin sections were made. The methods for obtaining semithin sections and ultrathin sections were in accordance with the method of Zhang et al. (2011). The dehydrated samples were embedded in epoxy resin and were sliced with a LEICA DC6 ultramicrotome (Leica Microsystems, Wetzlar, Germany). Ultrathin sections and semithin sections (1 μm thick) were made according to Ruppel et al. (2011). The 1- μm -thick sections were stained with toluidine blue (Zhang et al., 2011) and observed under a microscope. Three different vision fields under the microscope were randomly chosen and each vision field was repeated three times to measure the thickness of the whole leaf, upper epidermis, lower epidermis, and palisade and spongy parenchyma. The ratio of the palisade thickness to leaf thickness (CTR = palisade thickness/leaf thickness) was calculated (Jiang et al., 2012; Kong et al., 2016). The ultrathin sections were examined under a JEM-1010 electron microscope (JEOL, Tokyo, Japan) for starch grains, osmiophilic granules (OG), and the structure of stroma lamellae in chloroplasts.

Photosynthetic parameters. The light-response curves and CO_2 -response curves were determined with an LI-6400XT portable photosynthetic instrument (LICOR Biosciences, Lincoln, NE). Three healthy plants were randomly selected from each group, and the mature leaves of the third to fourth nodes from the top of shoots were used to measure the net photosynthetic rate between 9:00 and 10:00 AM. The photosynthetic active radiance (PAR) intensity was set at 2000, 1800, 1500, 1200, 1000, 800, 600, 400, 200, 180, 150, 100, 50, 20, and 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at a temperature of 25°C and CO_2 concentration maintained at 400 $\mu\text{L}\cdot\text{L}^{-1}$ in the chamber. Net photosynthesis (P_n) was recorded automatically at 120 s intervals for each PAR. The AQY, light compensation point (LCP), light saturation point (LSP), and maximum net photosynthetic rate (P_{max}) were calculated in accordance with the method of Zhuang et al. (2007). The CO_2 -response curve was determined after the leaves were adapted to 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 20 min, and CO_2 concentration was set as a gradient-intensity: 400, 300,

200, 150, 100, 80, 50, 30, 50, 80, 100, 150, 200, 400, 600, 800, 1000, 1200, 1500, and 2000 $\mu\text{L}\cdot\text{L}^{-1}$. Each CO_2 concentration was maintained for 120 s and P_n recorded automatically. The photosynthetic rates under the CO_2 concentration of 50–300 $\mu\text{L}\cdot\text{L}^{-1}$ were fitted into a linear equation, and the slope of the fitted line was CE. The values of AQY, P_{max} , CE, and maximum CO_2 concentration (C_{max}), CO_2 compensation point (CCP), CO_2 saturation point, LCP, LSP, and dark respiratory rate were calculated using the Photosyn Assistant software (Dundee Scientific, Scotland, UK).

Chlorophyll fluorescence parameter measurements. Minimal fluorescence (F_0) and maximum fluorescence (F_m) of the leaves (same as those selected for photosynthetic parameters measurement) were measured using PAM-2500 photosynthesis analyzer (MINI-PAM; Walz, Effeltrich, Germany). The variable fluorescence (F_v), F_v/F_m , and potential activity of PS II (F_v/F_0) were calculated by applying the formula of $F_v = F_m - F_0$ (Olaf and Snel, 1990). To determine the dark-adaption time, the fluorescence parameters of 'Courtaneur' and 'Suwon Gold' were measured after 20 and 30 min in the darkness, respectively, and the data of F_0 and F_m both have no significant differences. So, 20 min dark-adaption was used in our research.

Data analysis. The design was a one factor (leaf color) complete randomized one with three (photosynthetic characteristics) or five (growth and leaf characteristics) replications. The experiment was conducted in July and Aug. 2014. The data were analyzed by Duncan analysis of variance using SPSS 18.0 (IBM, Armonk, NY).

Results

Morphological traits of hybrid progenies. The leaf color of 52 progenies ranged from

yellow to green in the hybrid population (Fig. 1). Based on leaf colors, the progenies were divided into YG (9 plants), CG (25 plants), and GG (18 plants). The Royal Horticultural Society Color Chart color codes for YG, CG, and GG plants were Yellow Green Group 151 to 153, Yellow Green Group 144 to N144, and GG 137 to 143, respectively.

Growth of hybrid progenies. There were significant differences in growth between YG and GG (Table 1). The GI and the number of branches and leaves of YG progenies were 2%, 35%, and 34% of GG progenies at the end of the growth period, respectively. The growth of CG progenies was between that of GG and YG, being 23%, 71%, and 66% of GG progenies.

Leaf pigment contents of progeny groups. In F_1 population, the contents of Chl *a*, Chl *b*, total chlorophyll, and carotenoid gradually decreased as the leaves changed from green to yellow, and there were significant differences among the three groups. In general, the pigment contents of YG and CG progenies were close to 'Suwon Gold', whereas the pigment contents of GG progenies were close to 'Courtaneur'. The ratio of Chl *a/b* in YG progenies was greater than that in GG (Table 2).

Microstructure of leaf and ultrastructure of chloroplast. The palisade tissue (PT) of YG progenies has one layer of cells (Fig. 2A), whereas the PT of CG (Fig. 2B) and GG progenies (Fig. 2C) has two layers of cells. The PT of *F. koreana* 'Suwon Gold' (Fig. 2D) has one or two layers of cells. The PT of *F. 'Courtaneur'* (Fig. 2E) has more than two layers of cells. The chloroplasts are mainly distributed in spongy tissue (ST) and PT. Table 3 shows that the thickness of ST has not been significantly different between GG and CG, CG, and YG, whereas the thickness of PT and CTR has been significantly different among the three group

Fig. 1. Different leaf shapes and colors in *F. 'Courtaneur'* $\times$ *F. koreana* 'Suwon Gold' hybrid population (examined on 22 July 2014; bar = 3 cm).

progenies. As leaf color changes from green to yellow among F₁ progenies, PT, ST, and CTR decrease gradually, and the leaves become thinner mainly because of the changes in the thickness of PT. Meanwhile, the thickness of upper epidermis (U) has no significant differences among progenies,

whereas the thickness of the lower epidermis of CG is significantly higher than that of YG and GG (Table 3).

Most chloroplasts disintegrate in the yellow leaves of *F. koreana* ‘Suwon Gold’ (Fig. 3A), whereas the green leaves of *F. koreana* ‘Suwon Gold’ have normal chloro-

plasts (Fig. 3B). There is no visible structure of grana lamellae (G) in the yellow leaves of YG progenies (Fig. 3C and D). Grana lamellae may have disintegrated to small vesicle structures filled in chloroplasts (Fig. 3C). There are few or no visible starch granules (S), but the OG increased (Fig. 3D). The

Table 1. The annual changes of growth index (GI), branch number, and total leaf number of three groups of progenies in *F. ‘Courtaneur’* × *F. koreana* ‘Suwon Gold’ hybrid population.

Phenotype	No. of hybrid progenies	GI ² (cm ³)	No. of branches	No. of leaves
Green Group	18	124.4e3 ± 8.7 a	3.4 ± 2.6 a	200.4 ± 124.0 a
Chartreuse Group	25	28.1e3 ± 7.6 b	2.4 ± 2.3 ab	132.0 ± 94.2 ab
Yellow Group	9	2.7e3 ± 0.4 c ³	1.2 ± 1.0 b	67.8 ± 51.7 b

²GI was calculated using equation: $GI (m^3) = \pi(w/2)^2 \times h$.

³Means ± SE followed by the same letter in the same column are not significantly different at the 0.05 level based on Duncan’s test.

Table 2. The contents of photosynthetic pigments of three groups of progenies in *F. ‘Courtaneur’* × *F. koreana* ‘Suwon Gold’ hybrid population, in comparison with parent plants.

Group	Chl a (mg·g ⁻¹)	Chl b (mg·g ⁻¹)	Chl ² (mg·g ⁻¹)	Chl a/b	Carotenoid (mg·g ⁻¹)
<i>Forsythia</i> Courtaneur (♀)	0.9 ± 0.3 b ³	0.3 ± 0.1 b	1.2 ± 0.4 b	3.5 ± 0.2 bc	0.3 ± 0.1 b
Green Group	1.4 ± 0.3 a	0.4 ± 0.1 a	1.9 ± 0.4 a	3.4 ± 0.1 c	0.5 ± 0.1 a
Chartreuse Group	0.6 ± 0.2 c	0.1 ± 0.0 c	0.7 ± 0.2 c	7.0 ± 1.0 a	0.2 ± 0.1 c
Yellow Group	0.2 ± 0.1 d	0.1 ± 0.0 c	0.2 ± 0.1 d	4.7 ± 2.5 b	0.1 ± 0.0 d
<i>F. koreana</i> Suwon Gold (♂)	0.5 ± 0.2 c	0.1 ± 0.0 c	0.5 ± 0.2 c	7.9 ± 0.9 a	0.1 ± 0.0 cd

²Total chlorophyll content = Chl a + Chl b.

³Means ± SE followed by the same letter in the same column are not significantly different at the 0.05 level based on Duncan’s test.

Fig. 2. The leaf microstructure of three groups of progenies in *F. ‘Courtaneur’* × *F. koreana* ‘Suwon Gold’ hybrid population, in comparison with parent plants. (A) Yellow Group; (B) Chartreuse Group; (C) Green Group; (D) ‘Suwon Gold’ (♂); (E) ‘Courtaneur’ (♀). L = lower epidermis; PT = palisade tissue; ST = spongy tissue; and U = upper epidermis.

Table 3. The leaf anatomical structure of the three groups of progenies in *F. ‘Courtaneur’* × *F. koreana* ‘Suwon Gold’ hybrid population, in comparison with parent plants.

Group	Upper epidermis thickness (μm)	Palisade thickness (μm)	Spongy thickness (μm)	Lower epidermis thickness (μm)	Leaf thickness (μm)	CTR ² (%)
Courtaneur (♀)	22.4 ± 3.1 a ³	170.6 ± 31.4 a	189.0 ± 27.5 a	17.6 ± 3.1 a	336.5 ± 58.1 a	0.32 ± 0.0 a
Green Group	19.4 ± 1.9 b	84.7 ± 10.0 b	160.7 ± 14.5 b	15.4 ± 1.7 b	280.1 ± 9.7 b	0.30 ± 0.0 a
Chartreuse Group	18.2 ± 3.1 b	67.8 ± 22.8 c	148.3 ± 28.6 bc	15.2 ± 2.9 c	249.5 ± 44.1 c	0.27 ± 0.1 b
Yellow Group	19.1 ± 3.2 b	44.5 ± 11.2 d	146.8 ± 16.4 c	15.9 ± 2.6 b	226.2 ± 8.9 d	0.20 ± 0.1 d
Suwon Gold (♂)	21.0 ± 3.3 a	60.2 ± 15.9 c	156.5 ± 25.9 bc	15.5 ± 2.4 b	253.1 ± 39.3 c	0.24 ± 0.0 c

²CTR= palisade thickness/leaf thickness.

³Means ± SE followed by the same letter in the same column are not significantly different at the 0.05 level based on Duncan’s test.

chloroplast structure with visible G in CG progenies (Fig. 3E) is between GG (Fig. 3F and G) and YG progenies (Fig. 3C and D); however, the layers of G are less than those in GG progenies (Fig. 3F and G). Meanwhile, the S are visible or intact and the OG are less than those in YG progenies (Fig. 3C and D). In green leaves of GG progenies (Fig. 3F and G) and *F. 'Courtaneur'* (Fig. 3H and I), there

Fig. 3. The chloroplast ultrastructure of *F. koreana* ‘Suwon Gold’ (♂; **A** and **B**), three hybrids of different leaf colors (yellow—**C** and **D**; chartreuse—**E**; green—**F** and **G**), and *F. 'Courtaneur'* (♀; **H** and **I**). G = grana lamellae; OG = osmiophilic granules; and S = starch granules.

Table 4. The photosynthetic parameters of three groups (YG = yellow group; CG = chartreuse group; and GG = green group) of progenies in *F. 'Courtaneur'* (♀) × *F. koreana* ‘Suwon Gold’ (♂) hybrid population, in comparison with parent plants.

Group	AQY (mmol·mol ⁻¹)	LCP (μmol·m ⁻² ·s ⁻¹)	LSP (mmol·m ⁻² ·s ⁻¹)	<i>P</i> _{max} (μmol·m ⁻² ·s ⁻¹)	Rd (μmol·m ⁻² ·s ⁻¹)	CE (mmol·m ⁻² ·s ⁻¹)	CCP (μL·L ⁻¹)	CSP (mL·L ⁻¹)	<i>C</i> _{max} (μmol·m ⁻² ·s ⁻¹)
Courtaneur	26.4 ± 3.1 b ²	63.0 ± 14 b	1.2 ± 0.1 b	12.6 ± 2.0 b	1.6 ± 0.2 b	19.4 ± 2.3 b	65.0 ± 0.7 b	2.0 ± 0.2 a	23.8 ± 0.5 b
GG	51.1 ± 12.8 a	56.0 ± 4.5 b	1.3 ± 0.1 ab	18.9 ± 1.6 a	2.9 ± 1.0 a	48.6 ± 9.4 a	86.8 ± 19.3 b	1.6 ± 0.2 bc	44.2 ± 8.5 a
CG	30.0 ± 8.6 b	48.4 ± 15.2 b	1.4 ± 0.1 ab	11.7 ± 2.3 b	1.3 ± 0.1 bc	36.9 ± 7.8 a	67.9 ± 8.1 b	1.6 ± 0.1 bc	25.6 ± 4.3 b
YG	8.1 ± 2.3 c	135.8 ± 13.6 a	1.4 ± 0.1 ab	2.7 ± 1.2 c	1.1 ± 0.3 bc	5.4 ± 3.0 c	481.0 ± 323.8 a	1.9 ± 0.0 ab	2.6 ± 0.1 c
Suwon Gold	10.2 ± 0.2 c	42.8 ± 3.8 b	1.5 ± 0.2 a	3.1 ± 0.8 c	0.5 ± 0.1 c	13.8 ± 0.8 bc	70.8 ± 0.9 b	1.4 ± 0.1 c	3.6 ± 0.2 c

AQY = apparent quantum yield; LCP = light compensation point; LSP = light saturation point; *P*_{max} = maximum net photosynthetic rate; Rd = dark respiratory rate; CE = carboxylation efficiency; CCP = carbon dioxide compensation point; CSP = carbon dioxide saturation point; *C*_{max} = maximum CO₂ concentration.
²Means ± SE followed by the same letter in the same column are not significantly different at the 0.05 level based on Duncan’s test.

are many chloroplasts with normal structure of G, and the G are surrounded by mostly oval-shaped S, and S completely fill the chloroplasts.

Photosynthesis and chlorophyll fluorescence of parents and hybrid progenies. Photosynthetic parameters, including AQY, $P_{\max}$, and

$C_{\max}$ of the yellow-leaved ‘Suwon Gold’ and YG progenies were significantly lower than those of the other two hybrid groups and ‘Courtaneur’ (Table 4; Fig. 4). However, there was no significant difference between ‘Suwon Gold’ and YG progenies. There was no difference in CE between

‘Suwon Gold’ and ‘Courtaneur’, GG and CG progenies. However, the CE of CG progenies was significantly higher than that of YG progenies. Among the hybrids, YG progenies had significantly higher LCP, CCP, and lower $P_{\max}$ and $C_{\max}$ than CG and GG progenies. There was no significant

Fig. 4. (A) Net photosynthetic rate (P_n)-light response curves and (B) P_n -CO₂ response curves of three groups of progenies in *F. 'Courtaneur' (♀) × F. koreana 'Suwon Gold' (♂)* hybrid population. Symbols represent the mean of replications and error bars represent the least significant difference between the means at $P \leq 0.05$.

difference between YG and GG progenies, but the CO₂ and light energy use efficiency of CG progenies were significantly higher than those of YG progenies.

All the chlorophyll fluorescence parameters (F_o , F_m , F_v , F_v/F_o , and F_v/F_m) of yellow-leaved forsythia were significantly lower than those of the other two groups and 'Courtaneur' (Table 5).

Discussion

Pigment contents and leaf structure in different leaf color performance. Leaf pigments mainly included chlorophyll and carotenoid. The difference in pigment contents is the main reason for different leaf colors, and the higher chlorophyll content is the reason why normal leaves are green. In our research, the contents of Chl *a*, Chl *b*, and total Chl content in 'Suwon Gold' and YG progenies were significantly lower than those of the green-leaved forsythia. Among interspecific progenies, carotenoid contents decreased significantly with leaf yellowing, which was similar to the results from the researches on golden *U. pumila* (Zhu et al., 2014). The ratio of Chl *a/b* of YG progenies was significantly higher than that of CG progenies, which means that the lack of Chl *b* is higher than that of Chl *a* and was similar to the researches on *Ligustrum lucidum* cv. *Aurea* (Li, 2005) and *Capsicum annuum* (Ma et al., 2011). The yellow-leaved forsythia might be a leaf color mutant of total Chl and Chl *b* deficient type (Falbel and Staehelin, 1996). The contents of Chl *a* and carotenoid of CG progenies were higher than those of yellow-leaved forsythia.

For 'Suwon Gold' and YG progenies, the layers of PT were less than that in green leaves, and chloroplasts disintegrated, which may be one reason for yellow leaves. This phenomenon was also found in chartreuse-leaved chrysanthemum mutant (Chang et al., 2008). Meanwhile, the stacked granum lamellae in chloroplasts decreased, which led to the decrease of contents in a variety of pigments, causing the leaves to turn yellow (Zhu et al., 2014).

Pigment contents and photosynthetic capacity. The photosynthetic performance is an important factor affecting plant growth, and the plants with strong photosynthetic capacity can accumulate more photosynthates for growth. It was generally found that the growth of yellow-leaved plants was usually weaker and smaller than the green-leaved plants.

In our research, LCP and LSP of the YG progenies were higher than that of GG progenies, whereas AQY was lower, which was similar to the yellow-leaved mutant of *Rosa beggeriana* (Wei, 2013). The plants with higher AQY have higher growth capacity (Gao, 2013; Wang, 2010; Zhang et al., 2010), and thus the higher AQY of the GG progenies in our research may be one factor for their strong growth. The parameter AQY of 'Courtaneur' and CG progenies had no difference; however, it was significantly higher than 'Suwon Gold' and CG progenies. Therefore, breeding new forsythia cultivars with moderate growth and chartreuse leaves is promising. We also found that both CE and CCP of YG progenies were lower than that of green-leaved progenies, which is consistent with the results of Zhuang et al. (2007). The results indicated that the CO₂ and light use efficiency significantly decreased with leaves turning yellow, which resulted in the decrease in photosynthetic capacity, and the P_{max} and C_{max} of the yellow-leaved forsythia were less than a third of the values of the other groups. The destruction of chloroplast structure was probably the main reason for the decrease in photosynthesis capacity of YG progenies.

Lack of significant difference between the chartreuse-leaved and green-leaved progenies indicated that their photosynthetic capacity was similar (Zhang et al., 2015) and that was consistent with the results of photosynthesis measurements. We concluded that the incomplete development of PS II caused the decrease in photochemical efficiency and potential activity and ultimately decreased the P_n of yellow-leaved forsythia. The CG progenies obtained in our research have more normal chloroplasts, and their photosynthetic capacity and growth potential were both significantly higher than those of the yellow-leaved forsythia, which will be important materials for breeding forsythia cultivars with moderate growth and chartreuse leaves.

From the differences in both photosynthetic pigment contents and leaf structure, we concluded that the decrease in photosynthetic pigments content in yellow-leaved forsythia, especially serious deficiencies of chl *b*, resulted in a significant decrease in light energy absorption and growth. The CTR and chloroplast structure of yellow leaves appeared visibly different from the green leaves, which was consistent with findings in leaf color mutants of *B. napus* (Dong et al., 2000). Decrease in chloroplasts and disin-

tegration of chloroplast granum lamellae affected light energy absorption of yellow-leaved forsythia and resulted in a decrease in the AQY and CE. This decrease contributed to the decrease in photosynthetic ability, which explained why the growth of yellow-leaved forsythia 'Suwon Gold' was significantly lower than that of green-leaved forsythia 'Courtaneur'. In our experiment, the contents of Chl *a*, carotenoid, Chl *a/b*, and CTR of CG progenies were higher than that of YG progenies. Previous studies showed that only Chl *a*, the center pigment, had the ability to convert light energy into electrical energy and then into chemical energy (Zheng et al., 2012). Higher CTR or more developed PT is correlated to a stronger photosynthetic ability (Jiang et al., 2012), which explained why chartreuse forsythia had higher photosynthetic ability.

Photosynthesis is closely related to plant organic matter accumulation, which will directly affect the growth of plants. Light reaction (PS I and PS II) occurs on thylakoid membrane of chloroplast. In this study, fluorescence parameters F_v/F_m of yellow-leaved forsythia were significantly lower than the other two groups and 'Courtaneur'. Under stressed conditions, the value of F_v/F_m will decrease rapidly (Woo et al., 2008). In terms of YG and 'Suwon Gold', the data suggest that natural light intensity may cause light stress. This study also found that the chloroplast structure of yellow-leaved forsythia is abnormal with less thylakoid membrane and high Chl *a/b*, which could result in poorer light capture capability (Zhou et al., 1999). Therefore, it is speculated that the formation of chloroplast and the structure of thylakoid membrane are closely related to pigment content and photosynthetic characteristics, which in turn affect the color and growth of leaves.

Feasibility of improving leaf color in yellow-leaved plants through hybridization. Yellow-leaved plants usually grow significantly weaker than their green-leaved counterparts, which limit their use in landscapes. So, the main aim of yellow-leaved plant breeding programs is to breed yellow-leaved cultivars with strong growth. In our interspecific population of 'Courtaneur' × 'Suwon Gold', there were visible leaf color segregation and chartreuse-leaved progenies with leaf color between both parents had stronger growth than the yellow-leaved parent 'Suwon Gold'. Our results have shown that yellow-leaved forsythia may have been caused by the incomplete chloroplast structure, which

Table 5. The chlorophyll fluorescence parameters of three groups of progenies in *F. 'Courtaneur' × F. koreana 'Suwon Gold'* hybrid population, in comparison with parent plants.

Group	F_o	F_m	F_v	F_v/F_o	F_v/F_m
Courtaneur(♀)	0.034 ± 0.003 a [*]	0.111 ± 0.002 a	0.076 ± 0.004 b	2.23 ± 0.28 a	0.69 ± 0.03 a
Green Group	0.028 ± 0.001 a	0.123 ± 0.008 a	0.094 ± 0.008 a	3.34 ± 0.32 a	0.77 ± 0.02 a
Chartreuse Group	0.026 ± 0.005 a	0.089 ± 0.004 b	0.064 ± 0.006 b	2.62 ± 0.79 a	0.71 ± 0.06 a
Yellow Group	0.004 ± 0.002 b	0.008 ± 0.004 c	0.003 ± 0.003 c	0.71 ± 0.35 b	0.39 ± 0.12 b
Suwon Gold (♂)	0.009 ± 0.006 b	0.018 ± 0.016 c	0.008 ± 0.009 c	0.56 ± 0.48 b	0.30 ± 0.21 b

^{*}Means ± SE followed by the same letter in the same column are not significantly different at the 0.05 level based on Duncan's test.

could lead to reduction in the content of photosynthetic pigment, photosynthetic capacity, and growth potential in sequence. Our efforts provided an excellent method and materials for breeding new forsythia cultivars with moderate growth and chartreuse leaves.

Literature Cited

- Chang, Q.S., F.D. Chen, N.J. Teng, S.M. Zhang, J.G. Lu, and S.M. Chen. 2008. Chlorophyll contents and chloroplast microstructure and ultrastructure of leaves in chartreuse mutant of *Chrysanthemum*. *Acta Bot. Bor-Occid. Sin.* 28:1772–1777.
- Debalin, S., I. Suat, L.L. John, Z.K. Stevan, and J.J. Amit. 2015. Effect of water stress on the growth and fecundity of common waterhemp (*Amaranthus rudis*). *Weed Sci.* 64:42–52.
- Dong, Z., J.Y. Liu, H.M. Ma, C.K. Xu, H. Sun, and J.D. Zhang. 2000. Chlorophyll contents and chloroplast ultrastructure of chlorophyll deficient mutant in *B. napus*. *Chinese J. Oil Crop Sci.* 22(3):27–34.
- Falbel, T.G. and L.A. Staehelin. 1996. Partial blocks in the early steps of the chlorophyll synthesis pathway: A common feature of chlorophyll b-deficient mutants. *Physiol. Plant.* 97:311–320.
- Funayama, S., K. Hikosaka, and T. Yahar. 1997. Effects of virus infection and growth irradiance on fitness components and photosynthetic properties of *Eupatorium makinoi* (Compositae). *Amer. J. Bot.* 84:823–829.
- Gao, J.Z. 2013. Scanning index and evaluation method of herbaceous peony cultivars under protected cultivation condition. Beijing Fore Univ., Beijing, China, PhD Diss.
- Gao, M., L. Hu, and Y. Weng. 2016. The chlorophyll-deficient *golden leaf* mutation in cucumber is due to a single nucleotide substitution in *CsChlI* for magnesium chelatase I subunit. *Theor. Appl. Genet.* 129:1961–1973.
- Hu, H.Z., R. Zhang, A.Q. Shan, L.J. Zhao, and Z.M. Lu. 2007. Response of pigment content of yellow-leafed plants to light intensity. *Acta Hort. Sin.* 34:717–722.
- Jiang, J.F., Y. Zhang, Z.S. Tian, X.C. Fan, and H.C. Liu. 2012. Observation on microstructure and ultrastructure of leaves in *Vitis* L. *Acta Bot. Bor-Occid. Sin.* 32:1365–1371.
- Kong, D.X., Y.Q. Li, M.L. Wang, M. Bai, R. Zou, H. Tang, and H. Wu. 2016. Effects of light intensity on leaf photosynthetic characteristics, chloroplast structure, and alkaloid content of *Mahonia bodinieri* (Gagnep.) Laferr. *Acta Physiol. Plant.* 38:120.
- Li, B.Y. 2005. Study on the chlorophyll content in the leaves of *Ligustrum vicaryi* and *L. quihoui*. *For. Sci. Tech.* 30(3):63–65.
- Liu, C., A. Chang, and C. Du. 2016. Genetic, physiological and biochemical analysis of the formation of yellow-green leaf color of burley tobacco (*Nicotiana tabacum*). *Intl. J. Agr. Biol.* 18:192–197.
- Liu, G.L., H.Y. Liang, and X.J. Liu. 2003. Study on photosynthesis characteristic of *Sophora japonica* L. *J. Agr. Univ. Hebei.* 26(4):68–70.
- Ma, Z.H., H.T. Wang, W.Q. Wei, and Y.H. Yan. 2011. Change of photosynthetic pigments and enzyme activities of hot pepper (*Capsicum annuum*) CMS line leaves with chartreuse marker. *China Cucurbits Veg.* 24(5):20–24.
- Moseyko, N., T. Zhu, H.S. Chang, X. Wang, and L.J. Feldman. 2002. Transcription profiling of the early gravitropic response in *Arabidopsis* using high-density oligonucleotide probe microarrays. *Physiol. Plant.* 130:720–728.
- Olaf, V.K. and J.F.H. Snel. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.* 25:147–150.
- Ruppel, N.J., C.A. Logsdon, and C.W. Whippon. 2011. A mutation in *Arabidopsis* seedling plastid development affects plastid differentiation in embryo-derived tissues during seedling growth. *Plant Physiol.* 155:342–353.
- Wang, N.N. 2010. Responses of photosynthetic characteristics of *Davidia involucre* Baill seedlings to drought stress, shading and carbon dioxide elevation. Beijing Fore Univ., Beijing, China, Master Diss.
- Wang, X.K. 2006. Principles and techniques of plant physiological biochemical experiment. 2nd ed. Higher Education Press, Beijing, China.
- Wei, J.J. 2013. Leaf morphological ultrastructure, chlorophyll biosynthesis and photosynthesis of the yellow-leafed mutant of *Rosa beggeriana*. Chinese Acad. Agr. Sci., Beijing, China, Master Diss.
- Woo, N.S., R.B. Murray, and J.P. Barry. 2008. A rapid, non-invasive procedure for quantitative assessment of drought survival using chlorophyll fluorescence. *Plant Methods* 4(1):27.
- Xiao, H.G., H.W. Yang, Y. Rao, B. Yang, Y. Zhu, and W.L. Zhang. 2013. Analysis of chloroplast ultrastructure, stomatal characteristic parameters and photosynthetic characteristics of chlorophyll reduced mutant in *Brassica napus* L. *Sci. Agr. Sin.* 46:715–727.
- Xiao, S.H., T.Z. Zhan, and J.J. Pan. 1995. Inheritance of virescent mutants and their utilization in heterosis of upland cotton. *J. Nanjing Agr. Univ.* 18(3):28–33.
- Yu, X.N. and Q.X. Zhang. 2000. Progress on the formation of colorful colored-leaf plants. *Acta Hort. Sin.* S1:533–538.
- Zhang, S., H. Jiang, S.M. Peng, H. Korpelainen, and C.Y. Li. 2011. Sex-related differences in morphological, physiological, and ultrastructural responses of *Populus cathayana* to chilling. *J. Expt. Bot.* 62:675–686.
- Zhang, X.M., S.F. Sun, F.H. Li, J. Wang, and Z.S. Shi. 2015. Photosynthesis of a yellow-green mutant line in maize. *Photosynthetica* 53:499–505.
- Zhang, Z.W., B.Y. Zhang, H.F. Tong, and L. Fang. 2010. Photosynthetic LCP and LSP of different grapevine cultivars. *J. Northwest For. Univ.* 1:24–29.
- Zheng, J., Z.H. Hu, S.H. Guo, T.T. Qu, and P.S. Leng. 2012. Photosynthetic pigments characteristics and photosynthesis abilities in *Ligustrum vicaryi*. *Nonwood Forest Res.* 30:13–18.
- Zhou, Y., H.G. Zhou, and X.L. Zhang. 1999. The study on variegation and chimerism in ornamental plants. *J. Guangxi Agr. and Biol. Science.* 18(4):304–309. (in Chinese with English abstract).
- Zhu, X.J., A.Q. Shang, M.S. Yang, and Q.Q. Yu. 2014. Photosynthetic characteristics and leaf coloration mechanism of the next generation of Golden *Ulmus pumila*. *Acta Bot. Bor-Occid. Sin.* 34:950–956. (in Chinese with English abstract).
- Zhuang, M., W.B. Jiang, M.L. Weng, G. Li, and J. Cao. 2007. Comparative study of the photosynthetic characteristics of *Ligustrum vicaryi* and *Ligustrum quihoui* Carr. *J. Nanjing Agr. Univ.* 30(2):39–43. (in Chinese with English abstract).