**Morphological and chemical studies of artificial Andrographis paniculata polyploids**

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Available online 20 Feb., 2018

**[ABSTRACT]** Andrographis paniculata (Burm. f.) Nees (AP) is commonly used for the treatment of many infectious diseases and has been cultivated widely in Asian countries, and has been included in United States Pharmacopoeia as a dietary supplement, but the cultivars of *A. paniculata* are not abundant due to its self-pollinated. With the aims to enrich AP resources and provide materials for after breeding we explored the polyploidy induction. Different explants, colchicine concentration, and treatment time were tested. After identification by flow cytometry, eleven polyploid plants with different morphologic traits were obtained. The agronomic traits and andrographolide concentration of the polyploids were improved greatly. One of the polyploids (serial 3-7) was chosen for further study. The traits of the second and third generation polyploids (serial 3-7) were stable. Compared with the normal plants, the seeds (2nd generation) weight increased by 31%, and the andrographolide concentration of the leaves increased by 14% (2nd) and 28% (3rd). In conclusion, AP autopolyploids with different morphologic traits were established successfully for the first time, and the polyploids induction might be effective for crop improvement of AP.

**[KEY WORDS]** Herba Andrographis; Crop improvement; Autopolyploid; Andrographolide; Cultivar; Colchicine

**[CLC Number]** Q944, R284

**[Document code]** A

**[Article ID]** 2095-6975(2018)02-0081-09

**Introduction**

Artificial induction of polyploids has been long developed and has become a common breeding method to improve the yield and quality of crops. Polyploidy provides increased allelic diversity and heterozygosity and permits generation of novel phenotypic variation. And compared with diploids, polyploids have stronger organs and higher content of some useful metabolites [1-3]. In China, some herbs, such as *Lonicera japonica* and *Isatis indigotica*, their polyploids have been induced for quality and yield improvements [4-5]. Among the reagents used for polyploid induction, colchicine is often used with good results. In the process of induction, using the cold treatment first can promote the agent to seep into the embryo and then transferring explants to heat treatment can promote the chemical reaction of agent [6].

Herba Andrographis (*Andrographis paniculata*, AP) has been widely used in China and other Asian countries for the treatment of many ailments, including fever, cough, tuberculosis, snake bites, active ulcerative colitis, and some animal diseases [7-9]. The therapeutic activities of this plant are mainly attributed to andrographolide, which has been found to have anti-cancer, anti-bacterial, anti-inflammatory, and anti-oxidative effects in target organs such as liver, lung, and bladder [10-13]. And there is a need to improve the yield and the content of effective components to enhance the therapeutic effect and meet the wide demand.

However, the cultivars of AP are not abundant because the plant is self-pollinated. The genetic diversity researches by RAPD have shown that the collected samples from various locations in Thailand or India are likely to belong to the same clusters [14-15], and AP germplasm from various regions in China have poor genetic diversity [16]. Focusing on these issues, many efforts have been put on the optimization of the
planting technology and application of chemical or biological agents to improve the AP yield and andrographolide content \cite{17}. But no research has been reported about the induction of polyploid AP.

The aims of the work described here were to induce AP polyploids by colchicine and to test the value of the new autoploids preliminary through the determination of the agronomic characteristics and chemical ingredients. The traits of second and third generation polyploids were also determined. Our study would provide valuable materials for selection of plants with higher production potential of important diterpene compounds.

Materials and Methods

Plant materials and tissue culture

The seeds of AP used in the present study were purchased from market (Qiaoxu, Guigang, Guangxi, China) and authenticated by Prof. YU Bo-Yang, the corresponding author of this work. The AP seeds were kept underrunning tap water for 30 min, rinsed with distilled water, and then sterilized with 0.1% (W/V) solution of mercuric chloride (SCR, Sinopharm Chemical Reagent, Shanghai city, China) for 5 min, followed by five rinses in autoclaved distilled water. The disinfected seeds were inoculated on autoclaved tissue towel moistened with autoclaved tap water in Petri dishes disinfected seeds were inoculated on autoclaved tissue

Murashige and Skoog [18] medium (0.6% agar, 3% sugar) followed by five rinses in autoclaved distilled water. The

from N.S (Nanjing Chemical Reagent, Nanjing, Jiangsu, China) for 5 min, rinsed with distilled water, and then sterilized with 0.1% (W/V) solution of mercuric chloride (SCR, Sinopharm Chemical Reagent, Shanghai city, China) for 5 min, followed by five rinses in autoclaved distilled water. The disinfected seeds were inoculated on autoclaved tissue towel moistened with autoclaved tap water in Petri dishes parafilm.

Two way pieces served as the explants. After removal of the radicle, the cotyledonary nodes were inoculated in 200-mL glass jars (3 explants/jar) containing Murashige and Skoog \cite{18} medium (0.6% agar, 3% sugar) augmented with 0.2 mg L\(^{-1}\) of BAP (6-benzyl amino purine) and 0.1 mg L\(^{-1}\) of IBA (indolebutyric acid). The pH of the medium was adjusted to 5.8 prior to autoclave at 121 °C for 20 min. The reagents used for tissue culture were purchased from N.S (Nanjing Chemical Reagent, Nanjing, Jiangsu, China) and SCR (Shanghai city, China). All cultures were maintained at 25 ± 1 °C with 60% relative humidity under 2 000 lux with 16 h/8 h light/dark cycles.

Polyplolyd induction by colchicine

There were four kinds of treatments, respectively, for the internode, seedlings and seeds.

Test 1: The seven day-old axenic seedlings were treated with colchicine (Xi’an Shanchuan Biotechnology, Xi’an, Shanxi, China) solution for 24 h at 25 ± 1 °C. They were washed thrice with sterilized distilled water, cultured individually on MS medium for seven days, and then transferred to the proliferation medium for subculture.

Test 2: The single nodes were excised from tissue cultures and subsequently incubated in filter-sterilized colchicine solutions at 25 ± 1 °C, either for 2 or 12 h. The treated nodes were then cultured on proliferation medium, allowing the axillary buds to grow out and elongate.

Test 3: The seeds were immersed in filter-sterilized colchicine solution for 24 h at 55 ± 1 °C, and distilled water was used as a control. Soaked seeds were washed three times with sterilized distilled water and cultured on MS medium. The survival rate was determined after two months, and then the cotyledonary nodes were excised and inoculated into proliferation medium.

Test 4: The seeds were pre-soaked at 25 ± 1 °C for 12 h. For four treated groups, the seeds were soaked in colchicine solution at 4 ± 1 °C for several minutes, and then subjected to the fresh colchicine solution at 40 ± 1 °C for several minutes. And the control group was without any treatment. Finally, the seeds were washed with tap water for four h. All the seeds were planted in trays containing a 1 : 3 (vermiculite : peat, V/V) mixture substrate and then maintained in culture room (August 2012). The survival rate was determined after two months of culture.

Plant cultivation

For the first generation, when the seventh pair of true leaves had grown, 10 plants from each group were transplanted into 11-cm pots containing substrate (greenorchids, Foshan, Guangzhou, China) and then kept in culture room with natural light (25 ± 2 °C, September 2012 to February 2013) until analysis. The plants that remained in the trays were used in other experiments.

The second generation was planted in greenhouse with the fresh colchicine solution at 4 ± 1 °C. For four treated groups, the seed s were soaked in colchicine solution at 4 ± 1 °C for several minutes, and then subjected to the fresh colchicine solution at 40 ± 1 °C for several minutes. And the control group was without any treatment. Finally, the seeds were washed with tap water for four h. All the seeds were planted in trays containing a 1 : 3 (vermiculite : peat, V/V) mixture substrate and then maintained in culture room (August 2012). The survival rate was determined after two months of culture.

Flow cytometry analysis

For flow cytometry assays, 200 mg of fresh leaf from each test plant was cut with a scalpel and mixed with 2 mL of Otto’s buffer-1 (100 mmol·L\(^{-1}\) of citric acid and 0.5% Tween 20; Nanshi, Nanjing, China) \cite{19-20}. The homogenate was passed through a nylon filter (40 μm) and then centrifuged at 1 500 × g for 5 min at 4 °C. The supernatant was discarded, and the pellet was re-suspended with 1.5 mL of Otto’s buffer-1 and then centrifuged at 1 500 × g for 5 min at 4 °C. After the supernatant was discarded, the pellet was re-suspended with 1.5 mL of Otto’s buffer-1 and then centrifuged at 1 500 × g for 5 min at 4 °C. After the supernatant was discarded, the pellet was re-suspended with 2 mL of Otto’s buffer-2 (400 mmol·L\(^{-1}\) of NaH\(_2\)PO\(_4·12\)H\(_2\)O; Nanshi, Nanjing, Jiangsu, China), to which 20 μL of 1 mg·L\(^{-1}\) RNase (Aladdin, Shanghai, China) and 20 μL of 1 mg·L\(^{-1}\) propidium iodide (Aladdin,) were added, and the mixtures were incubated for 30 min at 4 °C, protected from light. For each sample, 20 000 nuclei were analyzed on a flow cytometer (FACSCalibur, BD, Franklin Lakes, NJ, USA). And the data were analyzed using ModFit LT (Verity Software, Topsham, ME, USA). The leaves from plants without treatment were used as control.
Analysis of morphologic characteristics

A set of morphologic traits was measured, including plant height, stem diameter (at the first node close to the bottom), stem tip morphology, length and width of true leaves, and canopy diameter. The pollen grain diameter (100 grains per plant, 10 × lens) and the stomatal density of the lower epidermis (10 visual fields per plant, 40 × lens) were also observed (Leica DM2500, Leica, Bensheim, Germany). Ten plants from each test line were used, and the untreated normal plants were used for comparison. A ruler was used for the measurement of leaf width, length, plant height, and canopy. Vernier caliper was used for the stem diameter. Analytical Balance (AB135-S, Mettler Toledo, Zurich, Switzerland) was used for measurement of seed weight.

Determination of andrographolide content

We tested the andrographolide contents and compared the chemical compositions of the leaves of the experimental plants by using HPLC according to the Hong Kong Chinese Materia Medica Standards [21]. The collected plants were dried at 60 °C in a hot air oven for 12 h, and then used for extraction. For the sample preparation, the powdered sample was weighed (0.2 g) and placed in a 50-mL centrifuge tube, to which 20 mL of methanol (AR grade, Hanbon, Huai’an, Jiangsu, China) was then added. The mixture was sonicated for 30 min and centrifuged at about 3 000 × g for 5 min, and the supernatant was transferred to a 50-mL volumetric flask. The extraction procedure was repeated once and the supernatants were combined and then made up to the mark with methanol. At last, the resultant solution was filtered through a 0.45-μm PTFE filter directly into a vial for determination.

The liquid chromatography system was equipped with a DAD (Waters 600 controller, 996 detective, Waters, Milford, MA, USA) and the detections were performed at 215 nm for the fingerprint and 225 nm for andrographolide; the a column (4.6 mm × 250 mm, C18, 12 nm, 5 μm, Hanbon, Huai’an, Jiangsu, China) was used. The temperature was maintained at 25 °C, with injection volume of 10 μL. The mobile phase was consisted of (A) Elix water (Millipore, Bedford, MA, USA) and (B) acetonitrile (HPLC grade, Tedia, Fairfield, OH, USA), and the flow rate was set at 1 mL·min⁻¹. The gradient program was optimized as follows: 0–5 min, 22%–25% B; 5–30 min, 25%–30% B; and 30–60 min, 30%–45% B. The standards of andrographolide (lot number 110797–201108) and dehydroandrographolide (lot number 110854–201007) were purchased from National Institutes for Food and Drug Control (Beijing, China).

For polyploids in the first and second generations, the stems were not harvested in order to harvest more seeds, and therefore the total andrographolide concentration was calculated for the leaves only.

Statistical analysis

The Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA, USA) was used for data processing and analysis. The values were presented as means ± SD for the samples in each group. Quantitative data between two variable groups were compared by using one-way ANOVA. P < 0.05 was considered statistically significant.

Results

Survival rate after colchicine treatment

Treated nodes (from tissue culture) and seedlings were withered in a week after subculture, while untreated nodes continued to grow and proliferate (Table 1, Fig. 1A). The subsequent overall germination levels of the colchicine-treated seeds cultured in the MS medium were higher,

<table>
<thead>
<tr>
<th>Explant</th>
<th>No. of explant</th>
<th>Colchicine concentration/%</th>
<th>Temperature and time</th>
<th>Survival or germination rate</th>
<th>Induction rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling</td>
<td>20</td>
<td>0</td>
<td>25 °C 24 h</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.1</td>
<td>25 °C 24 h</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.2</td>
<td>25 °C 24 h</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Node</td>
<td>30/30</td>
<td>0</td>
<td>25 °C 2 h/12 h</td>
<td>82/80</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30/30</td>
<td>0.1</td>
<td>25 °C 2 h/12 h</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>30/30</td>
<td>0.2</td>
<td>25 °C 2 h/12 h</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Seed</td>
<td>30</td>
<td>0</td>
<td>55 °C 4 h</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.05</td>
<td>55 °C 4 h</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.1</td>
<td>55 °C 4 h</td>
<td>50</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.15</td>
<td>55 °C 4 h</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.2</td>
<td>55 °C 4 h</td>
<td>65</td>
<td>–</td>
</tr>
</tbody>
</table>

Statistical analysis

The Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA, USA) was used for data processing and analysis. The values were presented as means ± SD for the samples in each group. Quantitative data between two variable groups were compared by using one-way ANOVA. P < 0.05 was considered statistically significant.

Results

Survival rate after colchicine treatment

Treated nodes (from tissue culture) and seedlings were withered in a week after subculture, while untreated nodes continued to grow and proliferate (Table 1, Fig. 1A). The subsequent overall germination levels of the colchicine-treated seeds cultured in the MS medium were higher,
compared to water-treated controls, especially at the higher levels of colchicine (Table 1), and the shape of these seedlings was changed (Fig. 1A). However, they were also withered on the proliferation medium, and the control seedlings continued to grow and proliferate. Colchicine treated seeds of AP sowed in the substrate (vermiculite: peat), had higher survival rate (Table 1). A 0.1% concentration of colchicine resulted in 40% of polyploids when seeds were treated for 60 min, respectively, indicating that a prolonged exposure did result in higher numbers of tetraploids.

**Characteristics of polyploids**

Among the individuals in each treatment group, some special mutations occurred (Figs. 1B and 1C). Later florescence, curly leaves, special burls that could shoot up into four first-level branches and a withered shoot apex were used to distinguish these special plants from others. Flow cytometry analysis was used to identify the ploidy of these special plants (Table 2; 1-7: the No. 7 of group 1). Their leaves were used for flow cytometry analysis. Fig. 2 shows the typical histograms of the RFI of nuclei isolated from young leaves of special and normal plants. The peak RFI for the normal plants was set at around channel 25 (Fig. 2A), whereas the peaks for all of the special ones were concentrated at around channel 50 (Fig. 2B). We could conclude that these special ones were tetraploids, according to the results of the FCM.

**Agronomic traits of 1st generation**

Colchicine treatment led to crimping and wrinkling of leaf tips, and the leaf index of the polyploids (Table 2) decreased significantly compared with normal plants. Leaf index varied from 2.26 to 2.95 between the polyploids, while the normal plants had a higher leaf index. The mean stomatal density of the polyploids was decreased significantly than the

![Fig. 1 Effects of colchicine treatment on *Andrographis paniculata*. (A), untreated nodes/seedlings in the proliferation medium (left), treated nodes/seedlings in the proliferation medium (middle), treated seeds germinated in the MS medium (right); (B), the 3rd pairs true leaves of first generation, normal (left), and tetraploid (right), bar = 1 cm; (C), plant of diploid (left) and tetraploid (right), bar = 10 cm; (D), stomata in diploid (left) and tetraploid (right), bar = 10 μm](image-url)
normal (Fig. 1D), their average pollen size was significantly larger, and the growth cycle of polyploids was longer than the normal plants (Table 2).

There were four chromatographic peaks as required by the standard [20] (Fig. 3). Peaks 1 (andrographolide) and 4 (dehydroandrographolide) were the qualitative and quantitative components in the standard. Peaks 2 and 3 were the qualitative components in the standard, but the quantitative determination did not need. And the resolution of the four peaks conformed to the requirement. As shown in Fig. 4, the andrographolide concentration of the polyploids were higher than the normal, ranged from 2.43% to 6.55% in flowering stage, and the mean concentration (3.73%) was significantly higher than that of the normal plants (2.28%). In fruiting stage, the andrographolide concentration of the leaves was decreased, except 1-7 and 4-7, and the mean concentration of polyploids (3.12%) was also significantly higher than that of the normal plants (1.93%).

The seeds of all the polyploids germinated later and the seedlings grew slowly (Table 2). Serial 3-7 grew faster and had more seeds than others, so we chose it for further study.

Morphologic traits of 2nd and 3rd generations (serial 3-7)

Similarly to the 1st generation, the 2nd and 3rd generations of serial 3-7 also had significantly broader leaves, and a lengthened growth period (Table 3). In contrast to the previous generation, however, the stems (Table 3) of the polyploids were thicker (13%, 2nd; 41%, 3rd) than that of normal plants.

The stomatal density of polyploids (Table 3) was also decreased. More importantly, the seeds of serial 3-7 (2nd) were significantly heavier than that of the normal plants which often had many empty fruits and immature seeds (Table 3). In the flowering phase, the leaf yield per plant and total yield per plant of 3rd generation serial 3-7 were significantly higher than that of the normal plants (Table 3).

Chemical components of 2nd and 3rd generations (serial 3-7)

There were also four chromatographic peaks as required by the standard [21] (graphs not shown). In reproductive phase (Fig. 5), the leaves of 2nd serial 3-7 had significantly higher
andrographolide content than normal plants. And the content of serial 3-7 was decreased in growth stage, while the content in normal plants was not changed from seedlings to early flowering stage.

The mean andrographolide concentrations in leaves of 3rd generation serial 3-7 showed slower accumulation from seedlings to blooming stage, while the andrographolide concentration of normal plants accumulated quickly, in early flowering stage, it could reach the maximum (Fig. 6). The leaves from serial 3-7 had significant higher concentration of andrographolide in blooming stage (4.57%) than the normal ones (3.57%). Similarly, the mean andrographolide concentration in stems of 3rd generation serial 3-7 (1.46%) was significantly higher than normal plants (1.09%) in early flowering stage (Fig. 7). And the andrographolide yield per plant of 3rd generation serial 3-7 was also significantly higher than the normal plants.

The andrographolide accumulation trends of the normal groups’ leaves in 2nd and 3rd generations were different from growth stage to early flowering stage (Figs. 5 and 6).

![Fig. 4](image)

**Fig. 4** The andrographolide concentration of 1st generation leaves in flowering and fruiting stages. Normal = mean concentration of normal plants, mean ± SD, n = 5; Average = mean concentration of polyploids (1-7, 2-1, 2-10, 3-4, 3-7, 4-7 and 4-10), mean ± SD, n = 7. The asterisks * indicate significant differences between average of polyploids and normal plants at *P* < 0.05

![Fig. 5](image)

**Fig. 5** The andrographolide concentration of 2nd generation leaves (Normal and Serial 3-7). Mean ± SD, n = 5. The asterisks * indicate significant differences between serial 3-7 and normal plants at *P* < 0.05

**Table 3** Morphologic characteristics of 2nd and 3rd generations

<table>
<thead>
<tr>
<th>Serials/Traits</th>
<th>Normal (2nd)</th>
<th>3-7 (2nd)</th>
<th>Normal (3rd)</th>
<th>3-7 (3rd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf index</td>
<td>3.70 ± 0.02</td>
<td>3.33 ± 0.01*</td>
<td>4.17 ± 0.01</td>
<td>3.45 ± 0.02*</td>
</tr>
<tr>
<td>Stem diameter/mm</td>
<td>5.65 ± 1.12</td>
<td>6.38 ± 0.63</td>
<td>4.46 ± 0.76</td>
<td>6.31 ± 1.94*</td>
</tr>
<tr>
<td>Plant height/cm</td>
<td>73.0 ± 6.0</td>
<td>66.2 ± 7.8</td>
<td>47.3 ± 3.9</td>
<td>61.4 ± 5.2*</td>
</tr>
<tr>
<td>Stomatal density</td>
<td>28 ± 2</td>
<td>24 ± 3*</td>
<td>30 ± 1</td>
<td>27 ± 2*</td>
</tr>
<tr>
<td>Seeds weight (10 grains)/mg</td>
<td>14.01 ± 0.76</td>
<td>18.42 ± 0.65*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Leaf yield per plant (dry weight)/g</td>
<td>–</td>
<td>–</td>
<td>1.37 ± 0.99</td>
<td>3.43 ± 1.34</td>
</tr>
<tr>
<td>Yield per plant (dry weight)/g</td>
<td>–</td>
<td>–</td>
<td>5.47 ± 4.08</td>
<td>12.76 ± 6.12</td>
</tr>
<tr>
<td>Andrographolide yield per plant/mg</td>
<td>–</td>
<td>–</td>
<td>59.13 ± 46.09</td>
<td>179.98 ± 65.84</td>
</tr>
<tr>
<td>Average growth cycle/d</td>
<td>200</td>
<td>245</td>
<td>190</td>
<td>220</td>
</tr>
</tbody>
</table>

Mean ± SD (leaf index, stem diameter, plant height and stoma, n = 10; seeds weight, n = 50; yield of leaf, plant and andrographolide, n = 5); *Significantly different from normal plants, *P* < 0.05

**Discussion**

Many plants, including herbs, have been induced to become polyploids to increase their yield and improve their quality [2, 22]. The induction result showed that under the same colchicine concentration, the extension of the processing time could increase the induction rate. In our research the treatment of colchicine changed the morphologic traits of AP, and the seed germination rates, organ sizes and andrographolide content differed among the polyploids, indicating that different treatments have different effects on the plant traits, which could produce different phenotypes or genotypes. The colchicine might be toxic for seedlings and nodes (tissue culture), and the proliferation medium might be unsuited for the treated nodes. While the resistance and adaptability on stress of the seeds and embryos were higher, so polyploidy induction through seeds had yielded success, and the in vitro induction also laid the foundation for future work.
Fig. 6  The andrographolide concentration of 3rd generation leaves (Normal and Serial 3-7). Mean ± SD, n = 5. The asterisks * indicate significant differences between serial 3-7 and normal plants at P < 0.05

Fig. 7  The andrographolide concentration of 3rd generation stems (Normal and Serial 3-7). Mean ± SD, n = 5. The asterisks * indicate significant differences between serial 3-7 and normal plants at P < 0.05

Polyploids are often stronger, and thus its economic value is increased [16, 23-25]. And the decrease in stomatal density and the increase in pollen size observed in the plants are identified as characteristic polyploid markers [26]. Compared with diploids, AP polyploids showed obvious changes in their morphologic traits, such as larger leaves, special burls, and decreased stomatal density. All the polyploids of the three generations had larger leaves, the stems of 2nd and 3rd generation plants (serial 3-7) were thicker, and in the third generation, polyploids (serial 3-7) had significantly higher leaf yield and total yield per plant.

Some reports have shown that self-pollinated plants have more bad seeds or higher odds of infertility [27-28]. Withered and immature seeds often appeared in normal AP; however, in our experiments the seed quality of polyploid AP was improved, especially in the second generation (serial 3-7), and those seeds germinated normally, which could be considered as a good omen. And it was very useful for the subsequent cultivation work.

With the increasing organ size and chromosome number, the nutritional components and secondary metabolites of plants may change to a large degree [4, 29]. Similarly to other herbs, AP polyploids produced more andrographolide. The average leaf andrographolide concentration of 1st generation polyploids was increased by 72%, significantly higher than that of the normal plants. As the same to the 1st generation, the leaf andrographolide contents of 2nd and 3rd generation polyploids (serial 3-7) were also significantly higher. And the 3rd generation polyploids (serial 3-7) had significantly higher andrographolide yield per plant in flowering stage.

Considering that the leaves are the main medicinal part and andrographolide is the main medicinal ingredient of AP [30-31], larger leaves and increased andrographolide concentration would help improve the quality and raise the economic value of polyploids.

The normal groups in 2nd and 3rd generations had different trends from growth stage to early flowering stage. These plants were planted in two years and they were two generations, and the temperatures, light, soil and rainfall had some changes, which might lead to changes in growth cycles length, and affect the trends. Therefore, the present study focused on the plants planted in the same year, to avoid interference by the environment factors.

Polyploidy is a major mechanism of chromosome evolution and speciation in flowering plants, and some plants are comprised of many cytotypes that represent autopolyploids of the basic diploid cytotype [32-35]. Although somatic chromosome doubling produces only additional copies of existing genes and chromosomes, many genome alterations occur after mitotic polyploidization [36]. In this sense, the appearance of novel phenotypes in new polyploid plants probably involves changes in gene expression. In our study, the AP polyploids phenotypes varied because they had different agronomic traits and andrographolide concentration, and the accumulation and distribution of andrographolide in polyploid plants were different from that of the normal plants. The production of synthetic autopolyploid lines could introduce useful materials for expanding the limited AP cultivars, although it is not known whether such autopolyploids have different genotypes, which needs to be further studied.

Conclusion

In the present study, we induced A. paniculata autopolyploids with different morphologic traits successfully for the first time. Key morphologic variations observed in the polyploids included larger leaves, lower stomata density, increased pollen diameter and later maturity. The average andrographolide content of the leaves of AP polyploids was significantly higher than that of the normal plants. In addition, the 2nd and 3rd generation polyploids retained a lot of good traits, such as large leaves with high andrographolide content and plump fruits and seeds. The stable 2nd and 3rd generations
indicated good prospects for the application of polyploids. Thus, regarding self-pollinated herbs, such as AP, polyploid induction by using colchicine is a practical choice to provide materials for breeding cultivars and quality improvement.

Acknowledgements

The authors thank Professor GAO Shan-Lin and the Medicinal Botanical Garden of China Pharmaceutical University for their excellent technical assistance.

References


