Characteristics of Hybrids between Jakyungjong and Hwangsukjong in Korean Ginseng (*Panax ginseng* C. A. Meyer)

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Abstract

A large number of individual ginseng plants have been selected in the farmer's fields to develop new ginseng varieties with desirable traits since 1970s. Among them, Hwangsukjong with green stem and yellow berry was selected as a ginseng germplasm. The phenotype of Hwangsukjong is quite different from Jakyungjong that has violet stem and red berry and has been cultivated in most of ginseng fields. Therefore, Hwangsukjong was crossed with Jakyungjong to clarify the inheritance of stem color and then the characteristics of F_1 and F_2 hybrids were investigated.

F_1 hybrid plants were similar to Jakyungjong in most of aerial part characters and showed hybrid vigor in fresh weight of root and weight of 100 seeds. In F_2 generation, the stem color was segregated in a ratio of 3 violet to 1 green. From this result, it was elucidated that violet color was controlled by single dominant gene. In another experiment, DNA was extracted from parents (Jakyungjong and Hwangsukjong) and F_1 hybrid. For each primer evaluated, multiple band profile was produced comprising from one to five major bands plus a varying number of minor bands and amplified bands were detected among most primers. In case of UBC primer number 13, 17, 30, 31, and 43, band patterns of parents and F_1 hybrid were very similar, but the others were not. Especially, in #1, #4, and #33, specific band was produced in Hwangsukjong and F_1 hybrid while in #6, another specific band was produced in Jakyungjong and F_1 hybrid. Therefore, F_1 hybrid had all specific bands at these primers. So, these selective markers could be used for identification of characteristics of F_2 hybrids

Introduction

Korean ginseng (*Panax ginseng* C.A. Meyer) is the only one of the economical crop in our country. In the last decades, the products and consumption of ginseng have increased both in and

out of the country. There would be serious problems between supply and consumption of ginseng, if we do not plan to increase the production of good quality ginseng, because good cultivation area for production of good quality ginseng is decreased while the amount of ginseng consumption is increased. High quality ginseng is concerned by not only ginseng farmer but also consumer. Therefore, we have to increase the production per unit area by the development of new varieties which have good quality, high yield, and resistance to diseases. However, it is the fact that a single local variety, Jakyungjong, has only been cultivated across the country without the development of new varieties because breeding of this plant takes a long time. Fortunately, we have selected a lot of individual ginseng plants with desirable traits such as good quality, high yield, and distinctive traits since 1970s (Ahn *et al.*, 1985, 1987; Choi *et al.*, 1994, 1995, 1998, 2001; Chung *et al.*, 1992, 1993, 1995; Kwon *et al.*, 1991, 1994, 1998, 2000). Among them, a characteristic or promising lines were developed through comparative cultivation of several lines selected by pure line separation from local races in KT&G Central Research Institute (formerly Korea Ginseng & Tobacco Research Institute) (Choi *et al.*, 1998, 2001; Kwon *et al.*, 1991, 1994, 1998, 2000). Preliminary and advanced yield trials were performed over 8 years. The lines were then tested in regional yield and adaptation trials for 10 years. Finally, two lines, KG101 and KG102, were registered as cultivars in 1999 under the name of "Chunpoong" and "Yunpoong", respectively and seeds of certificated varieties have been releasing to ginseng farmers. We also developed Hwangsukjong as a ginseng germplasm. Hwangsukjong has yellow berry and green stem. The phenotype of Hwangsukjong is quite different from the local race, Jakyungjong. However, the informations on the genetical and molecular biological researches were very rare.

In the course of this experiment, Hwangsukjong, Korean ginseng variant with green stem and yellow berry, was crossed with Jakyungjong to develop new ginseng varieties and then the inheritance of stem color and the characteristics of Fand Fhybrids were investigated.

Materials and Methods

The Korean ginseng parents for the production of hybrids are Jakyungjong and Hwangsukjong. Jakyungjong is a local race with violet stem and red berry and Hwangsukjong is a green-stem variant with yellow berry. Characteristics of the aerial parts and roots were examined by using the three- or four-year old plants. In measurement, ten plants were used for each replication. And chi-square values were calculated to believe that in F_2 plants produced from the artificial cross

ginseng plants with violet stem and green stem would be in the ratio of 3 : 1.

DNA was extracted from young expanding ginseng leaves following the procedure of Wagner *et al.* (1987). Synthetic 10-mer oligonucleotide primers (UBC, Vancouver, Canada) were used, and total of 10 primers were screened for random amplified polymorphic DNA (RAPD) of ginseng DNAs (Jakyungjong, Hwangsukjong and F_1 hybrid). Polymerase chain reaction (PCR) was performed in 20 μl PreMixTM (Bioneer Corp., Korea) containing 50 ng DNA and 20 pg primer. Amplification was achieved in a DNA thermal cycler (Perkin Elmer Cetus, Fotodyne incorporated) programmed as follows: 45 cycles of 1 min at 96°C (denaturation), 1 min at 37°C (anneal), 2 min at 72°C (extension), followed by a final extension at 72°C for 15 min, and then held at 4°C. PCR products were analysed by electrophoresis (1hr at 50 V) in 1.4% agarose gel in TAE buffer. RAPD gels were scored for presence or absence of bands.

Results and Discussion

Two parents (Jakyungjong and Hwangsukjong) were crossed to clarify the characteristics of F_1 hybrid and the inheritance of stem color. And then the characters such as seed size, stem, leaf, and root were measured in parents, F_1, and F_2. Fig. 1 shows characteristics of seed size of parents and F_1 generation. Seed size may be weight, and the weight is determined by length, width, and thickness. These traits are the commonly used indicators for seed shape. The difference of seed size was found between parents and F_1 but not between each parent (Fig. 1). The F_1 was superior in length, width, and thickness of seeds. Stem diameter of Jakyungjong and F_1 plants was larger

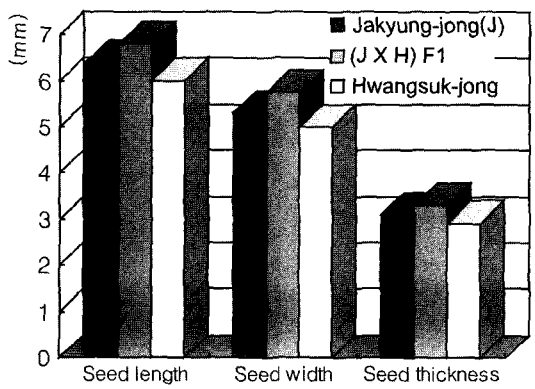

Fig. 1. Characteristics of ginseng seeds in parents and F_1 generation.

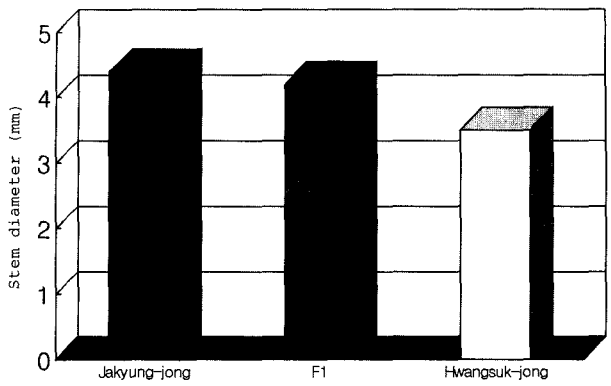

Fig. 2. Stem diameter of 3-year old ginseng plants in parents and F_1 generation.

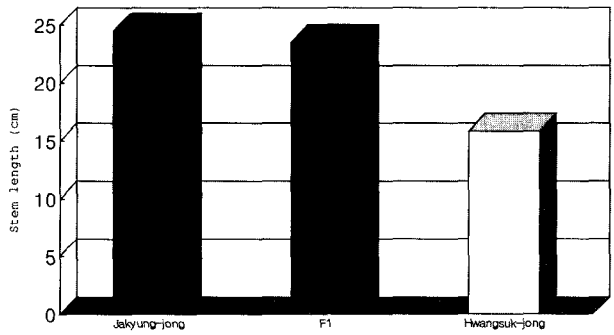

Fig. 3. Stem length of 3-year old ginseng plants in parents and F_1 generation.

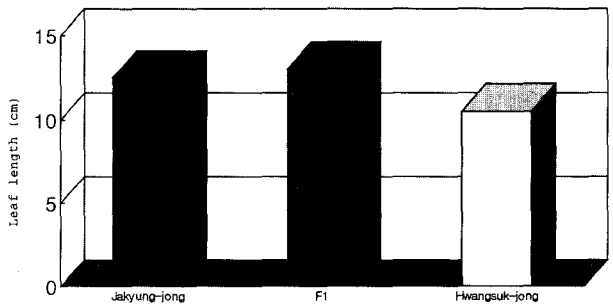

Fig. 4. Leaf length of 3-year old ginseng plants in parents and F_1 generation.

than that of Hwangsukjong (Fig. 2). Stem length, leaf length, and leaf width of Jakyungjong and F_1 plants were longer or wider than those of Hwangsukjong (Figs. 3-5). In the case of these characters, F_1 plants were similar to Jakyungjong. However, the number of leaflets of F_1 plants was

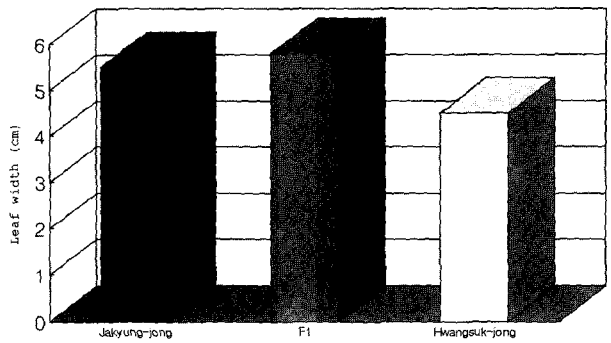

Fig. 5. Leaf width of 3-year old ginseng plants in parents and F_1 generation.

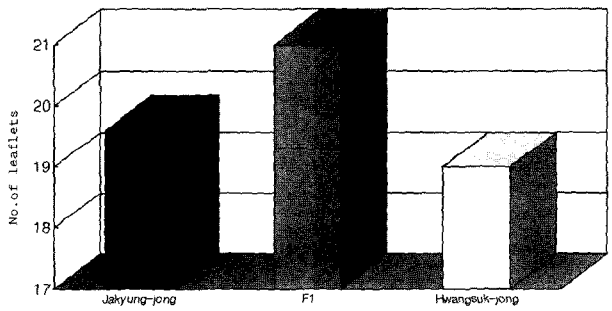

Fig. 6. Number of leaflets of 3-year old ginseng plants in parents and F_1 generation.

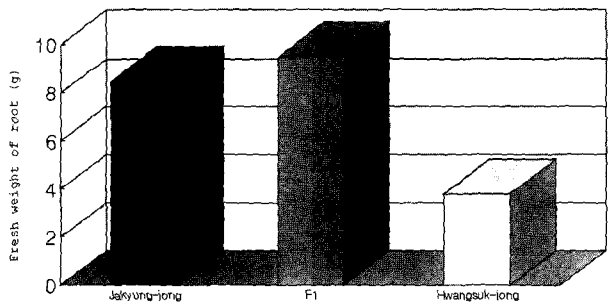

Fig. 7. Fresh weight of 3-year old ginseng root in parents and F_1 generation.

more than that of the parent races, Jakyungjong and Hwngsukjong (Fig. 6). Fresh weight of F_1 root was higher than that of Jakyungjong and Hwangsukjong (Figs. 7 and 8) and 100-seed weight of F_2 generation was higher than that of parents (Fig. 9). In this experiment, we found that these traits showed hybrid vigor. The F_1 hybrids may achieve exceptional vigor due to heterosis. In many cases, the heterosis contributes also to seedling vigor, so that the production ability of

Jakyungjong F_1 Hwangsukjong

Fig. 8. Whole plants of F_1 and parents.

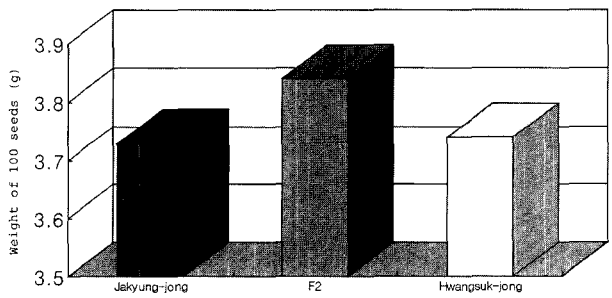

Fig. 9. Weight of 100 seeds harvested from 4-year old ginseng plants in parents and F_2 generation.

mature plants is much higher.

As shown in Table 1, the stem color of the F1 plants was violet. And then the stem color of F_2 plants was observed in order to clarify the inheritance of this character. Table 2 shows the number of plants with green- or violet-stem and chi-square test in F_2 generation. The chi-square test is

Table 1. Stem color of 4-year old ginseng plants in parents and F_1 generation

Cross combination	Stem color
Jakyung-jong (J)	Violet
Hwangsuk-jong (H)	Green
(J X H) F_1	Violet

Table 2. Chi-square test for the ginseng stem color in F_2 seedlings

Cross combination	No. of Plants observed	Observed frequency of stem color		Expected frequency of stem color		X^2	P
		Violet	Green	Violet	Green		
(J X H)F_2	1,044	787	257	783	261	0.0817	>0.75
(H X J)F_2	885	693	192	663.75	221.25	1.4211	>0.10

J : Jakyung-jong (violet-stem variant).
H : Hwangsuk-jong (yellow-berry variant).

a useful method for testing goodness of fit of Mendelian ratios. In the F_2 generation of a reciprocal crosses between two variants of Korean ginseng, Hwangsukjong and Jakyungjong, producing green stem with yellow berry and violet stem with red berry, respectively, the number of plants was obtained in the two phenotypic classes (Table 2). The test for deviations of the single factor ratio was made as in Table 2. In the counts of violet- and green-stem plants, we found x^2=0.082 and 1.421. As a matter of practical convenience, probability levels of 5% (0.05) and 1% (0.01) are commonly used in deciding whether to reject the null hypothesis. As seen from Table 2, these correspond to x^2 lower than 3.841 and x^2 lower than 6.635, respectively. In this experiment, it was found that the difference in the number of violet- and green-stem plants was not significant at the 5% level. Namely, the x^2 test was consistent with the hypothesis of a 3 to 1 ratio of violet- to green-stem ginseng plants. From this result, it was elucidated that violet color was controlled by single dominant gene.

Recently, DNA polymorphism amplified by arbitrary primers are being used usefully as genetic markers (Ahn *et al.*, 1996; Wang *et al.*, 1989; Welsh *et al.*, 1990; Williams *et al.*, 1990). For comparison among the variants of ginseng, DNA was extracted from parents (Jakyungjong and Hwangsukjong) and F_1 hybrid and RAPD analysis was applied. For each primer evaluated, multiple band profile was produced comprising from one to five major bands plus a varying number of minor bands and amplified bands were detected among most primers. In case of UBC primer number 13, 17, 30, 31, and 43, band patterns of parents and F_1 hybrid were very similar,

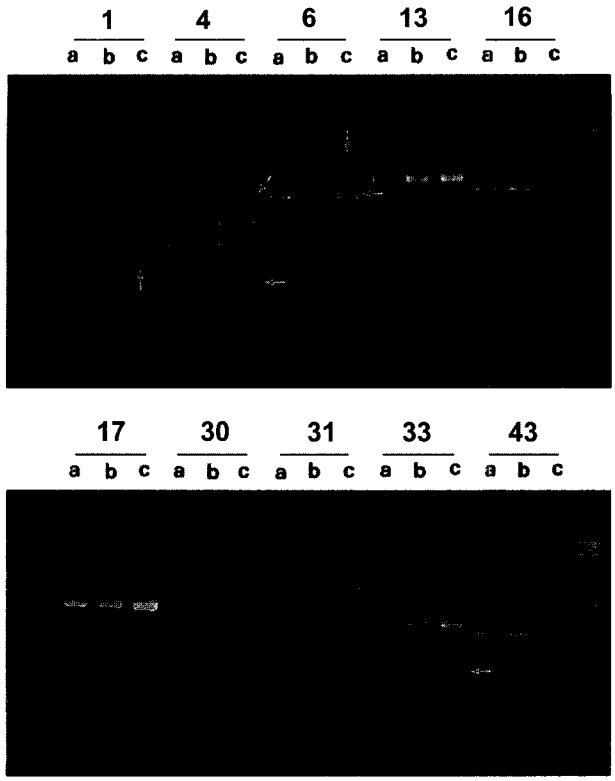

Fig. 10. Amplified bands of DNA with different 10-mer UBC primers in *Panax ginseng* C. A. Meyer variants, Jakyung-jong(a), Hwangsuk-jong(b) and F1-hybrid(c). Arrows are possible selective markers of ginseng hybrids.

1: CCTGGGCTTC 4: CCTGGGCTGG 6: CCTGGGCCTA 13: CCTGGGTGGA
16: GGTGGCGGGA 17: CCTGGGCCTC 30: CCGGCCTTAG 31: CCGGCCTTCC
33: CCGGCTGGAA 43: AAAACCGGGC

but the others were not (Fig. 10). Especially, in #1, #4, and #33, specific band was produced in Hwangsukjong and F_1 hybrid while in #6, another specific band was produced in Jakyungjong and F_1 hybrid (Fig. 10). Therefore, F_1 hybrid had all specific bands at these primers. So, these selective markers will be used for identification of characteristics of F_2 hybrids.

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