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https://doi.org/10.5109/1799304

出版情報：九州大学大学院農学研究院紀要. 62 (1), pp.69-74, 2017-02-24. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **62** (1), 69–74 (2017)

The Classification of the Morphological Characteristics of Aerial Vegetative Tissues in a Large Germplasm Collection of Korean Ginseng (*panax sp*.)

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(*Received October 28, 2016 and accepted November 4, 2016*)

This study was conducted to clarify the morphological characteristics related to the qualitative characters of aerial vegetative tissues in a large collection of ginseng germplasms. A total of 380 germplasm lines collected from different regional sites of Korea were morphologically characterized in detail. We also characterized additional 18 lines that were generated by the mutagen MNU (N–methyl–N–nitrosourea) treatment to 'Gopoong' and 'Gumpoong' varieties. The color and shape of stem and leaflet of ginseng germplasms were divided into specific sub–categories. The stem color was divided into 5 classes such as light green, green, light purple, purple, dark purple colors. Anthocyanin expression was also determined based on the extent and distribution in stem and petiole. Four categories were observed for the shape of leaflets. We also characterized the appearance of cross–sectioned leaves and found 3 classes such as concave, plane and convex type. The leaflet color at senescence was divided into 5 classes such as yellow, brown, reddish brown, red, dark red. Our thorough investigation broadens genetic resources for ginseng breeding and provides useful information of morphological diversities in ginseng germplasms in South Korea. Morphological distinction established in this study will be applied in different germplasm collections.

Key words: Ginseng, germplasms, morphological traits, anthocyanin, breeding

INTRODUCTION

Ginseng (*Panax ginseng* C.A. Meyer) was originated in Korean and China and has been used for a medicinal plant for more than 4,000 years. Ginseng is cultivated in all regions of the Korean Peninsula (34–43°), three Provinces of Northeast China (43–47°), and Primorye (42.5–48°) of Russia (Kim, 2012). The natural environmental conditions for the growth of ginseng is quite limited in terms of the longitude and latitude. Such limited availability of area for spontaneous growth of ginseng suggests that the cultivation area for ginseng is narrow.

Ginseng is the perennial plant that belongs to Umbelliflorae *Araliaceae Panax*, emerging in mid–April and flowering in early May (Kwon *et al*., 2001). The flower is the perfect flower with 5 petals and sepals, 5 stamens and 1 pistil, and 2 stigmas. Although it reproduces mainly via self–fertilization, pollinator such as wind and insects are also important player for pollination.

The seeds of ginseng have been domesticated and maintained by local farmers since cultivated. Domestication has focused on the development of ginseng with vigorous growth characteristics, particularly root growth. All species that have been nurtured were bred through the pure line selection from the land race. The breeding through the cross or mutation breeding still remains at infant stage. The ginseng inflorescence also occurs in 2–year old ginseng when the ginseng seedling with excellent growth traits is transplanted to the main field (Chung *et al*., 1989). However, inflorescence and fruiting of ginseng generally occur in 3–year old ginseng. So, it would take at least 30 years until new varieties are developed by cross breeding (Cha *et al*., 2003). In addition, there is limited number of germplasms that show genetically fixed superior traits and genetic diversity. Thus, generation of new ginseng cultivars through cross–breeding is a major obstacle in ginseng breeding program.

Recent characterization of ginseng germplasms focused on quantitative and qualitative characters of 27 items based on International Union for the Protection of New Varieties of Plants (UPOV) (KSVS, 2015). Although molecular markers have been used to classify the varieties of ginseng, there are few reports based on the classification by quantitative and qualitative characters for available germplasms (Rhim *et al*., 2010; Wang *et al*., 2010; Bang *et al*., 2011). Because ginseng farmers require novelty, distinctness, uniformity and stability for a new ginseng variety, it is necessary to establish more detailed and clear criteria to ensure distinctness based on morphological characteristics of germplasms. The direction for the ginseng breeding is currently based on

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superior root shape, tolerance to biotic/abiotic stresses, and high ginsenoside content. In order to develop facile strategy in ginseng breeding program, comprehensive morphological analysis of ginseng germplasms is required, which will eventually provide invaluable initial resources.

The objective of this study was to establish the methodology to clarify qualitative morphological distinction of germplasms collected in Korea and abroad.

MATERIALS AND METHOD

Germplasm collection

Three hundred eighty one germplasms were collected from different geographical regions in South Korea during 2005 to 2011 (Table 1 and Fig. 1). In addition, eighteen lines induced by the mutagen MNU (N–methyl N–nitrosourea) treatment to 'Gopoong' and 'Gumpoong' varieties were also included. A total of 398 lines were used in this study.

Fig. 1. Collection sites of ginseng germplasms in South Korea.

Growth conditions

Germplasm seeds were stratified in wet sand at 15–20°C for 3 months, followed by dormancy breaking period for 100 days at 2–4°C. After seed stratification, seeds were germinated in seed bed and ginseng seedling was transplanted to the main field on March with the planting density of 7 plant/10 rows/1.6 m^2. The shading facility was installed before germination by using the 4–layered shade net consisting of 3 blue layers and 1 black layer, and then was covered with the black double–woven shade net to prevent high–temperature stress. Soil consisted of sandy loam with pH (1:5) 5.8, EC 0.6 dS/m, 45 mg/kg nitrate nitrogen, 21 g/kg organic substance, and 123 mg/kg phosphoric acid. Other cultivation management, such as pest control, weed removal, etc., was conducted in accordance with GAP (Good Agricultural Practice) standard cultivation guidelines of ginseng (RDA, 2009).

Morphological characterization of aerial vegetative tissues of germplasms

Several morphological characteristics (stem color, distribution of anthocyanin on stem and petiole, leaf shape, leaf stipule, leaf surface blistering, and color at senescence) were visually assessed from mid–June to late–June for four–year–old individual plants. At least 20 individual plants of each germplasm were investigated for each characteristic.

RESULTS AND DISCUSSION

In order to provide useful genetic resources in the field of ginseng breeding program, a large number of germplasms (398 lines) were collected and its vegetative tissues were investigated (Table 1 and Fig. 1). Since most

Table 1. Geographical location of germplasm seed collection used in this study.

No.	Geographical region	Latitude	Longitude	No. of collected lines
1	Yeoju	37°12′ 6.73″N	127°32′ 42.74″E	115
2	Suwon	37°17′ 9.61″N	126°56′ 17.56″E	91
3	Daejeon	36°23′ 21.17″N	127°20′ 50.38″E	49
4	Eumseong	36°55′ 25.00″N	127°45′ 27.42″E	36
5	Pocheon	38°5′ 20.67″N	127°16′ 31.68″E	34
6	Hongcheon	37°40′ 40.97″N	127°56′ 6.24″E	25
7	Haenam	34°38′ 40.93″N	126°26′ 52.53″E	15
8	Yangju	37°49′ 28.57″N	126°59′ 1.25″E	4
9	Gimpo	37°43′ 1.57″N	126°33′ 9.05″E	11
10	Mutant	–	–	18
Total				398

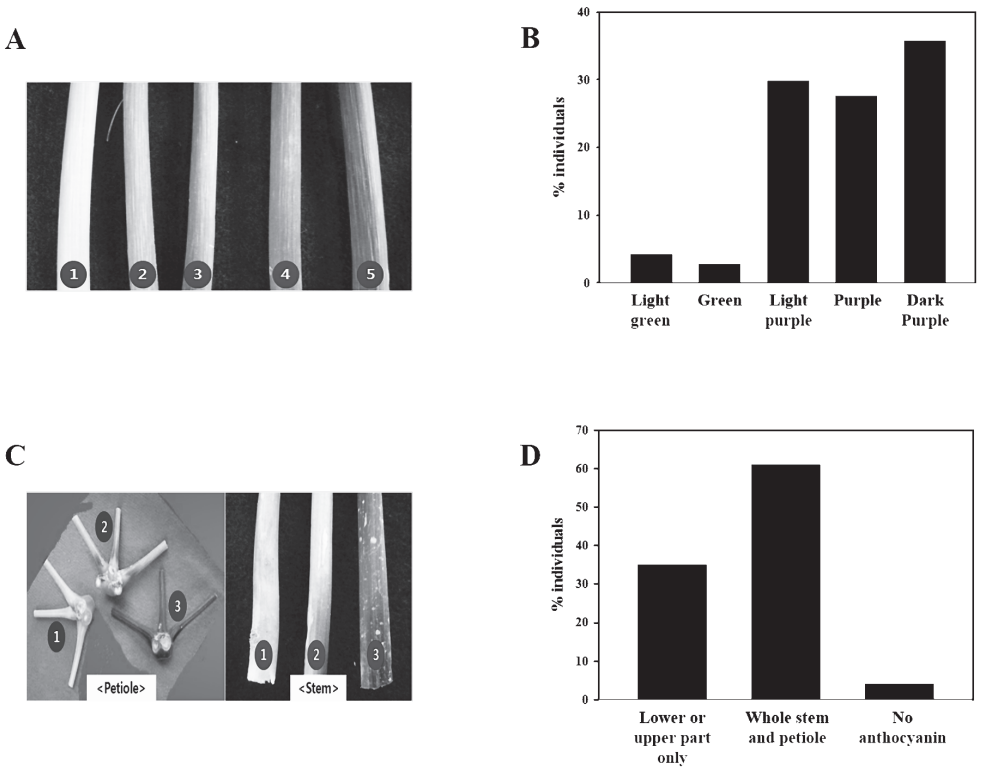

Fig. 2. Characterization of stem color of germplasms.
(A) Five categories for stem color classification. 1: Light green, 2: Green, 3: Light purple, 4: Purple, 5: Dark purple. (B) Percentage of each category of stem color among germplasms. (C) Three categories for the anthocyanin accumulation in petiole (Left panel) and stem (Right panel). 1: No anthocyanin, 2: Anthocyanin accumulated in only upper part or lower part, 3: Anthocyanin in both stem and petiole. (D) Percentage of each category of the anthocyanin accumulation among germplasms

of germplasms exhibited unique morphological differences in aerial parts from 4–year–old ginseng, we performed our assessment at this period.

Stem

First observable morphological difference among germplasms upon bolting was stem color. Although UPOV classifies only two categories of stem color of ginseng (light green and purple color), we expanded this analysis by subdividing the stem color into five categories, light green, green, light purple, purple, and dark purple (Fig. 2A). Three hundred seventy lines showed purple color. Based on the purple intensity, 142 (35.7%), 109 (27.4%), or 119 (29.8%) lines were categorized as dark purple, purple, or light purple, respectively (Fig. 2B). Seventeen lines (4.3%) showed light green color while only 11 lines (2.8%) exhibited green color (Fig. 2B). We categorized that accounting for 35.7%. The lines with purple and light purple color numbered 109, accounting for 27.5% and 29.8%, respectively. The lines with light green color and green color numbered 17, accounting for 4.3% and 2.7%, respectively. Thus, approximately 93% of germplasms appears to accumulate large quantity of anthocyanin on stem, albeit different extent. Previous study indicates that Korean ginseng cultivars have two categories of stem color, purple (violet variant) and green (green variant) (Chung *et al*., 1992). Yunpoong, Gopoong, Sunpoong, Sunun, Sunwon, and Sunhyang belong to violet variant. Most landraces being cultivated by farmers in South Korean show violet variant (Kwon *et al*., 1998; Kwon *et al*., 2000; Kwon *et al*., 2003; Lee *et al*., 2008). Gumpoong is the variety belonging to green stem variant. The functional, physiological roles of stem anthocyanin during ginseng growth and development remain to be determined.

Since we also observed the tissue–specific difference of anthocyanin accumulation and distribution. As shown in Fig. 2C, three categories can be identified. The line can be classified based on the distribution of anthocyanin coloration in stem as shown in Fig. 2C. The stem can be classified into the lines that do not manifest any anthocyanin pigment, the lines that show purple color only in base part, the lines that show light purple color in the part of stem, and the line that has purple color in whole stem. The germplasms with purple color in both stem and petiole accounted for 61% while 23.3% showed purple color in base stem and petiole (Fig. 2D). Ginseng is grown in the shady environment and the leaves of 4–year–old ginseng increase the shading. Because the extent of anthocyanin accumulation is largely affected by sunlight exposure, research needs to be conducted to reveal the correlation between light intensity and anthocyanin accumulation in different aerial parts of ginseng. It is also recommended that the extent of anthocyanin

accumulation in petiole, which is considered to be one of the very important qualitative factor in germplasm classification, should be included in the list of morphological characterization in the 'Variety Registration Characteristics Table'

Leaflet

The type and position of ginseng leaflet are depicted in Fig. 3A. Ginseng typically has the palmately compound leaf and usually consist of five leaflets; 1 central leaflet, 2 first lateral leaflets, and 2 second lateral leaflets. It was found that the central leaf and the first lateral leaf showed regular shapes. Four representative leaf shapes were observed among germplasms, long elliptic, elliptic, slender, and spatulate (Fig. 3B). We determined the leaf shape based on leaf index (the ratio of leaf width and leaf length). Under these criteria, the long elliptic type is the leaflet with the ratio of leaflet width/leaflet length equal to or exceeding 1/3, and the elliptic type is the leaflet with the ratio of leaflet width/leaflet length exceeding 1/2. The large proportion (287 lines, 72%) of germplasms was classified as elliptic shape of leaflet (Fig. 3C). The slender type and long elliptic type account for 15.9% and 11.7%, respectively. Only two lines had spatulate leaflets. Leaf shape is a representative morphological marker to distinguish between American ginseng *(Panax qinoquefolium* L.) and Korean ginseng. American ginseng contains the spatulate type leaf. Considering the fact that different size and shape of leaflets are occasionally observed in same varieties or lines, it is generally believed that more than one gene determines this trait. Choi *et al*. (1980) reported that the number and shape of palmately compound leaves were governed by the gene.

Next, we investigated the shape of cross section of leaflet. When the leaflet of ginseng was laterally cut, the shape of cross section was classified into three categories, concave, plane, and convex type (Fig. 4A). Interestingly, this trait appeared to be genetically controlled by a single gene because no variation was observed regardless of different growth conditions (temperature and humidity). Among germplasm, 300 lines (75.4%) were classified to the plane type (Fig. 4B). The convex and concave types account for 12.9% and 11.7%, respectively (Fig. 4B).

The degree of wrinkles on the leaf surface differs among germplasm lines (Fig. 4C). The leaves of 94 lines (23.6%) were strongly wrinkled whereas 220 lines (55.3%) were intermediate. Also, 84 lines (22.1%) were weakly wrinkled (Fig. 4D).

Five leaves are typically observed in 4–year–old ginseng (Fig. 3A). During the course of investigation, we notice some lines bear additional leaves under the secondary leaflet (Fig. 5A). Based on the number of additional leaf (stipule), three categories were classified. No stipule occurrence was observed in 263 lines (66.1%). However, 37 lines (9.3%) produced more than two stipules and 98 lines (24.6%) had one stipule (Fig. 5B).

We also found that germplasms displayed different leaf color at senescence. Five categories were evident; yellow, brown, reddish brown, red, and dark red (Fig. 5C). The brown color and red color were observed in 210 lines (52.8%) and 118 lines (29.7%), respectively. The light reddish brown and dark red color comprised 8.2% and 5.7%, respectively (Fig. 5D). The lines that showed the yellow color without any red color accounted for

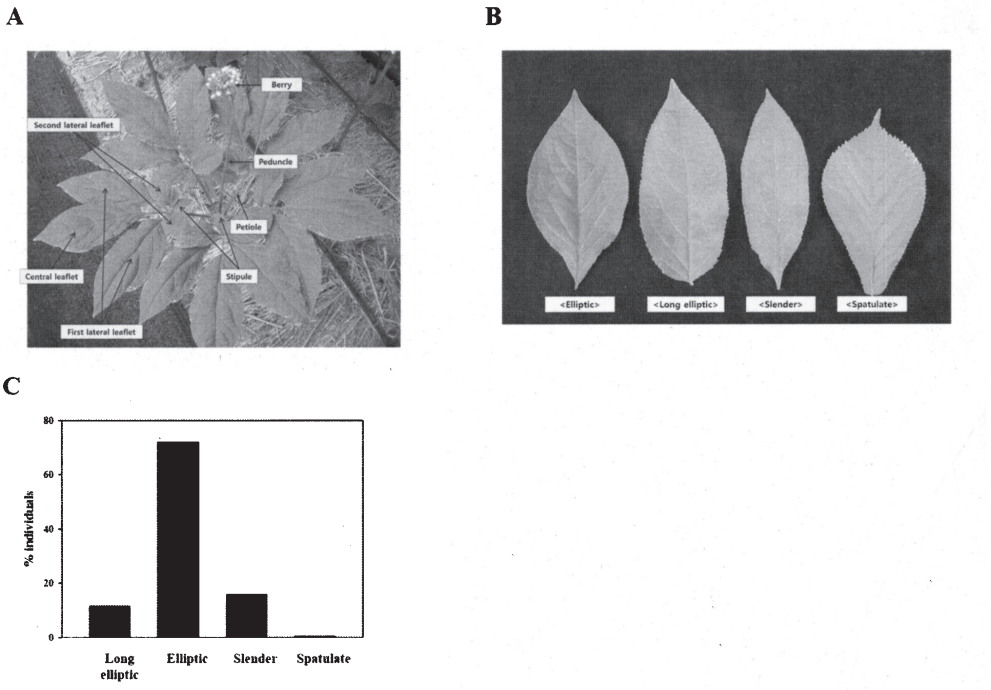

Fig. 3. Structure of aerial parts of ginseng and leaf shape.
(A) Schematic representation of the structure of aerial parts of 4–year–old ginseng (B) Four categories for the leaf shape. (C) Percentage of each category of the leaf shape among germplasms.

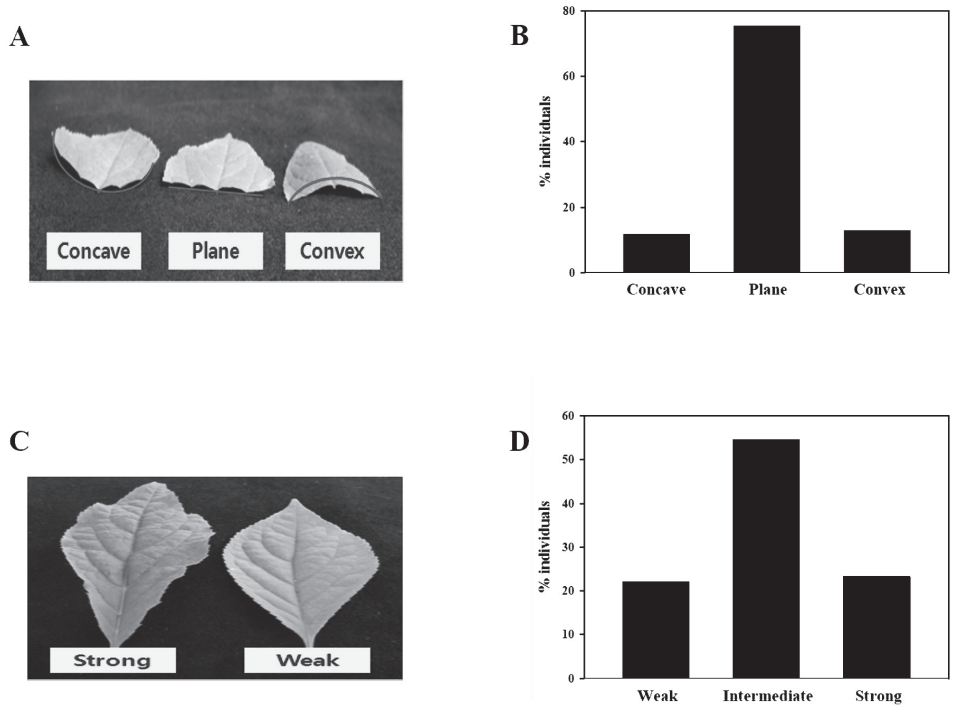

Fig. 4. Visual assessment of cross-section and the extent of surface wrinkle of germplasm leaves. (A) Three categories of appearance of cross-section leaf. (B) Percentage of each category of appearance of cross-section leaf. (C) Presence of wrinkle on the leaf surface. Categorized as weak, intermediate, and strong. (D) Percentage of each category of the extent of wrinkles on leaf surface among germplasms.

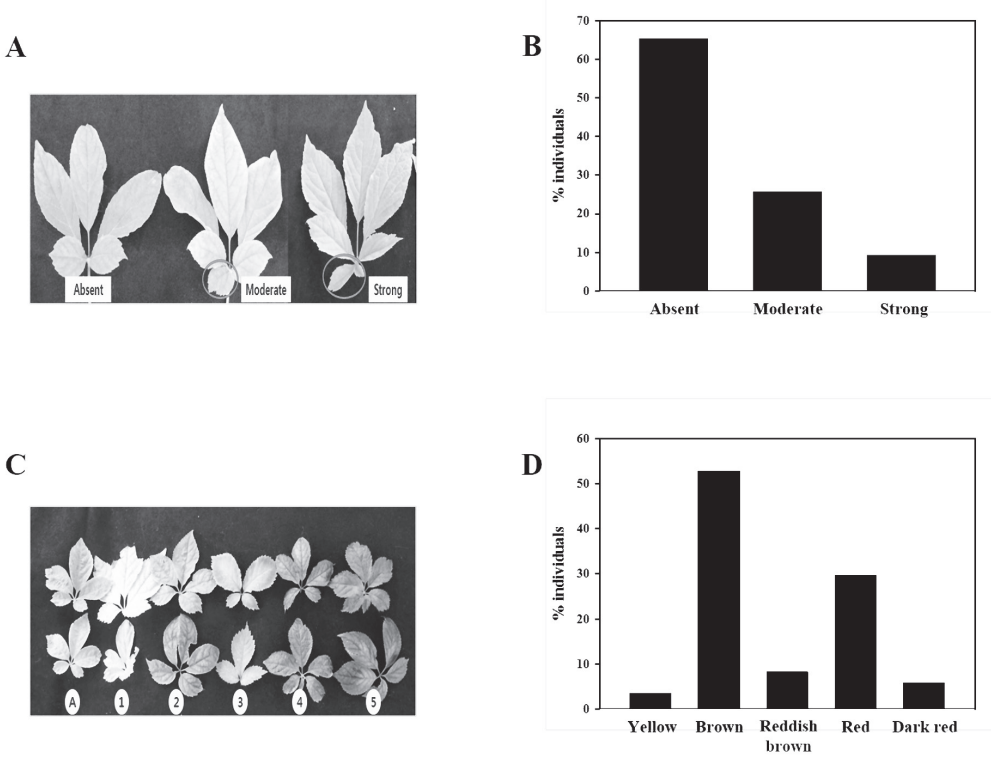

Fig. 5. Morphological characteristics of the presence of additional leaves and leaf color at senescence. (A) Three categories of the presence or absence of stipules. (B) Percentage of each category of the presence or absence of stipules. (C) Five categories of leaf color at senescence. A: control young leaf, 1: Yellow, 2: Brown, 3: Reddish brown, 4: Red, 5: Dark red. (D) Percentage of each category of the leaf color at senescence among germplasms.

3.6%. Although different growth conditions including the type of shade net and shade sheet, soil environments, and climate affect the leaf color at senescence, we found that the violet stem lines always contained reddish leaves at senescence, indicating the presence of genetic program controlling this trait.

CONCLUSIONS

In this study, we characterized aerial vegetative tissues (stem and leaf) in a large collection of ginseng germplasms mainly collected from different geographical regions of South Korea. Development of new varieties with better agronomical traits requires outcrossing–based breeding strategies. One of the main bottlenecks in such effort in ginseng breeding is the lack of intermediate or mating parents with desired traits. As a first step toward generating useful germplasm resource, a thorough/systematic morphological investigation was performed. We found that current classification methodologies are not suitable to cover all the characteristics we tested. We showed that stem color can be classified at least five categories (Fig. 2A) and further subdivided based on the color distribution in the petioles. In relation to the criteria for distinguishing the leaflet shape, Choi *et al*. (1982) used 7 categories while Ahn *et al*. (1984) classified into 3 shapes. Chung (2007) classified the leaflets into 2 shapes. In this study, the shapes of leaflets were classified, depending on the ratio of leaflet width/leaflet length. The leaflet shapes of ginseng were typically classified on the basis of visual assessment. Therefore, it would be necessary to adopt systematic methodology for classification of leaf shape. We also found that some germplasm lines produced additional leaves (stipule). The formation of multiple leaflets may lead to the enhanced capability of photosynthesis. It remains to be determined whether lines with more leaves produced the increased yield of seed or root weight in the future.

ACKNOWLEDGMENTS

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01018701)" Rural Development Administration, Republic of Korea.

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