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Original Research Article

Quality Change in Plug Seedlings of Three Indigenous Medicinal Plants after Short-term Cold Storage

Hye Jin Oh, Ji Eun Park, Yoo Gyeong Park and Byoung Ryong Jeong[1,2]*

Department of Horticulture, Division of Applied Life Science (BK21 Plus), Graduate School of Gyeongsang National University, Jinju 660-701, Korea

[1]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Korea

[2]Research Institute and Life Science, Gyeongsang National University, Jinju 660-701, Korea

Abstract - To test the quality change of seedlings of three domestic medicinal plants raised in plug trays, a short term storage experiment was conducted. Seedlings were kept in growth chambers for two weeks at 4 or 8℃ temperature combined with 0 or 5 μmol·m^{-2}·s^{-1} PPFD. Quality of glasshouse-raised seedlings was assessed after two weeks of cold storage in the growth chamber and one week of acclimation in the greenhouse. After two weeks of storage in the growth chamber of *Perilla frutescens* var. acuta Kudo, plant height was the greatest in the treatment 8℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD. Internode length of *P. frutescens* var. *acuta* Kudo was the greatest in the treatment of 4℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD. After one week of acclimatization in a glasshouse, the growth and development, such as plant height, internode length and leaf size, were greater in the 8℃ combined with 5 μmol·m^{-2}·s^{-1} PPFD than in the other treatments. After two weeks of storage in the growth chamber of *Sophora tonkinensis*, plant height increased more in the treatment of 4℃ than 8℃. After one week of acclimatization in a glasshouse, number of leaves did not change in the treatment of 4℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD, but it increased in the other treatments. Leaf width increased more under the dark than light condition. Leaf length did not observably change in any treatments. After two weeks of storage in the growth chamber, plant height of *Angelica gigas* Nakai was the greatest in the treatment of 8℃. Number of leaves was the greatest in the treatment of 8℃. Leaf growth was greater under dark than light condition. These results suggested that optimal storage environment was 8℃ combined with 5 μmol·m^{-2}·s^{-1} PPFD for *P. frutescens* var. *acuta* Kudo, and 4℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD for *S. tonkinensis* and *A. gigas* Nakai. Hence, proper combination of temperature and PPFD were necessary for better storage, and acclimatization and growth, thereafter, of the plug seedlings of theses plant species.

Key words - *Perilla frutescens* var. *acuta* Kudo, *Sophora tonkinensis*, *Angelica gigas Nakai*

Introduction

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. The World Health Organization (WHO) has emphasized the importance of the traditional indigenous medicines, since a large majority of rural people in the developing countries still use these medicines as the first defense in health care (Goleniowski *et al*., 2006). The world medicinal herb sector is called as an augmentative and alternative pharmaceutical industry, which grew from 60 billion US dollars in 2002 to 212 billion US dollars in 2007, and is expected to grow to 5 trillion US dollars in 2050. Total production and output of medicinal plants are steadily increasing every year. However, when the weather condition is not suitable for medicinal plants, it will cause difficulty in their cultivation and change the contents of medicinally-active compounds. Also currently there is not enough supply of medicinal herbs to meet the increasing demand in Korea.

The *P. frutescens* have been used as an important traditional herbal medicine for treating various disease including depression, anxiety, tumor, cough, antioxidant, allergy, intoxication, and some intestinal disorders (Ha *et al.*, 2012; Makino *et al*., 2003; Yang *et al.*, 2012) in East Asian countries such as Korea, China, and Japan. *Sophora* is a genus of the Fabaceae family, contains about 52 species, nineteen varieties, and seven forms that are widely distributed in Asia, Oceania, and the

*Corresponding author. E-mail : brjeong@gmail.com

Pacific islands (Krishna *et al*., 2012). Its stems and roots are commonly used as a traditional drug to treat acute pharyngolaryngeal infections and sore throats (Krishna *et al*., 2012). The root of *Angelica gigas* Nakai (Umbelliferae), popularly known as Korean "Dang Gui", has been used to treat female afflictions and anemia in traditional oriental herbal medicine since ancient times in Korea (Konoshima *et al*., 1968; Son *et al*., 2010).

Plug transplants are seedlings or small propagation plants raised in uniform individual cells called plugs, which are filled with a cohesive medium, and to be transplanted to other growing systems. In plug system usually seeds are sown to plug trays by an automated seeder, and with a few exceptions, only one plant per cell is raised (Jeong, 1998, 2000). Transplanting is often delayed by lack of time or equipment, or by labor problems or weather (Jeong, 1998; Sato *et al*., 2004). This can result in overgrowth of plants in plug trays and loss of seedling quality (Kaczperski and Armitage, 1992).

Seedling storage is needed for coordinating the supply of seedlings with variable demands (Sato *et al*., 2004). It's important to realize that plant storage is an operational necessity, not physiological requirement (Landis, 2000). Plug producers would benefit from a system of storing plugs for short periods to increase total output during the marketing period (Kaczperski and Armitage, 1992). To ensure that there is an adequate supply of seedlings, it is sometimes necessary to store them for periods of time, for example, during shipping, while waiting for optimal weather for transplanting, or because of labor shortages for planting (Jiang *et al*., 2012). Although low-temperature storage in darkness is widely used to preserve seedling vigor (Kaczperski *et al*., 1996; Kubota *et al*., 2002), temperature stress and lack of light can decrease their quality (Jiang *et al*., 2012). Kubota and Kozai (1995) suggest that proper combinations of temperature and PPFD would be necessary for better storage. Effects of photosynthetic photon flux density (PPFD) and air temperature were also investigated for a short-term experiment (Dorion *et al*., 1991). On the other hand, the visual quality of the seedlings stored in light was almost the same regardless of the combinations of PPF and photoperiod (Kubota *et al*., 2002). Cold storage temperatures can partially satisfy the chilling requirement of dormant stock and refrigerated storage showed to improve plant quality (Ritchie, 1989). Cold storage is a common treatment that is used in order to protect seedlings during the lifting-planting period (Genç and Yahyaoğlu, 2007).

This study aims to assess changes in quality of medicinal plant seedlings when stored at low temperatures for a short period of time.

Materials and Methods

Seeds of *Perilla frutescens* var. *acuta* Kudo, *Sophora tonkinensis,* and *Angelica gigas* Nakai were sown in 200-cell plug trays, containing a commercial medium (Tosilee medium, Shinan Grow Co., Korea), on Sept. 16, Sept. 7, and Sept. 21, 2012, respectively. Starting from 22, 31, 18 days after sowing, respectively, seedlings of *Perilla frutescens* var. *acuta* Kudo, *Sophora tonkinensis,* and *Angelica gigas* Nakai were fed with a nutrient solution [containing in $mg \cdot L^{-1}$ 436.6 $Ca(NO_3)_2 \cdot 4H_2O$, 232.3 KNO_3, 272.0 KH_2PO_4, 209.1 $MgSO_4 \cdot 7H_2O$, 80.0 NH_4NO_3, 15.0 Fe-EDTA, 17.4 K_2SO_4, 1.4 H_3BO_3, 0.2 $CuSO_4 \cdot 5H_2O$, 2.1 $MnSO_4 \cdot 4H_2O$, 0.12 $NaMoO_4 \cdot 2H_2O$, and 0.8 $ZnSO_4 \cdot 7H_2O$] once a day. Seedlings were held under the condition of 4 or 8℃ ± 0.2℃ temperature combined with 0 or 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD provided by the white fluorescent lamp (F48T12/OW/VHO, Philips, USA) in growth chambers (KGC-175VH, Koencon Co., Hanam, Korea) for two weeks. Relative humidity (RH) in the chambers was kept at 70%. Then seedlings were moved to a glasshouse and acclimatized for one week under the condition of 70% RH and a 18-25℃ temperature set point.

Growth parameters such as plant height, internode length, leaf length and width, and number of leaves were measured prior to, after storage, and after acclimatization in the glasshouse. Internode length was measured at the closest node to the top with the full-grown leaf.

The experiment had 10 plants per replication and three replicates laid out in a completely randomized design. Data collected were analyzed for statistical significance with the SAS (Statistical Analysis System, V. 9.1, Cary, NC, USA) program. The experimental results were subjected to an analysis of variance (ANOVA) and Duncan's multiple range tests at 5%. Graphing was performed with the Sigma Plot 10.0 (Systat Software, Inc., San Jose, CA, USA).

Results and Discussion

After two weeks of storage in the growth chamber, *P. frutescens* var. *acuta* Kudo seedlings were festered at leaf tips and internode was not much affected by the low temperature. Plant height in all treatments increased after two weeks of storage in growth chambers. Especially, the treatment of 8℃ combined with 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD showed increase in plant height which implies less efficacy of short-term cold storage than other treatments (Fig. 1). However, plant height in the 8℃ combined with 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD increased after one week of acclimatization in a glasshouse. Internode length increased more in the 4℃ than 8℃ treatment after two weeks of storage, especially in the 4℃ combined with 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. In all treatments it increased more after one week of acclimatization in a glasshouse than after two weeks of storage in growth chambers. Internode length did not significantly change overall after two weeks of storage (Fig. 1). However, plant height, internode length, and leaf growth in all treatments increased after one week of acclimatization in a glasshouse, especially under a high light condition. These results indicate overall the cold storage was effective in holding growth of the seedlings, and seedling growth resumed during the acclimatization period. After one week of acclimatization in a glasshouse, leaf tips of the *P. frutescens* var. *acuta* Kudo were necrotic and stem was slightly bent, but growth resumed slowly, presumably due to shock caused by changes in the environment. However, internode length and plant height at 8℃ combined with 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD were affected. Leaf length was greatly increased in the 4℃ combined with 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD after two weeks of cold storage (Fig. 2). After one week of acclimatization in a glasshouse, it was not significantly different among treatments. Leaf width showed a similar trend as the leaf length. Leaf width was greatly increased in the 4℃ combined with 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD after two weeks of storage, showing that a short-term cold storage affected leaf size of the seedlings (Fig. 2). However, the treatment of 4℃ combined with 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD increased leaf width after one week of acclimatization in a glasshouse. Leaf size increased in the 4℃ combined with 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD after two weeks of storage and this combination should be avoided if it is intended to stop the leaf growth (Fig. 2).

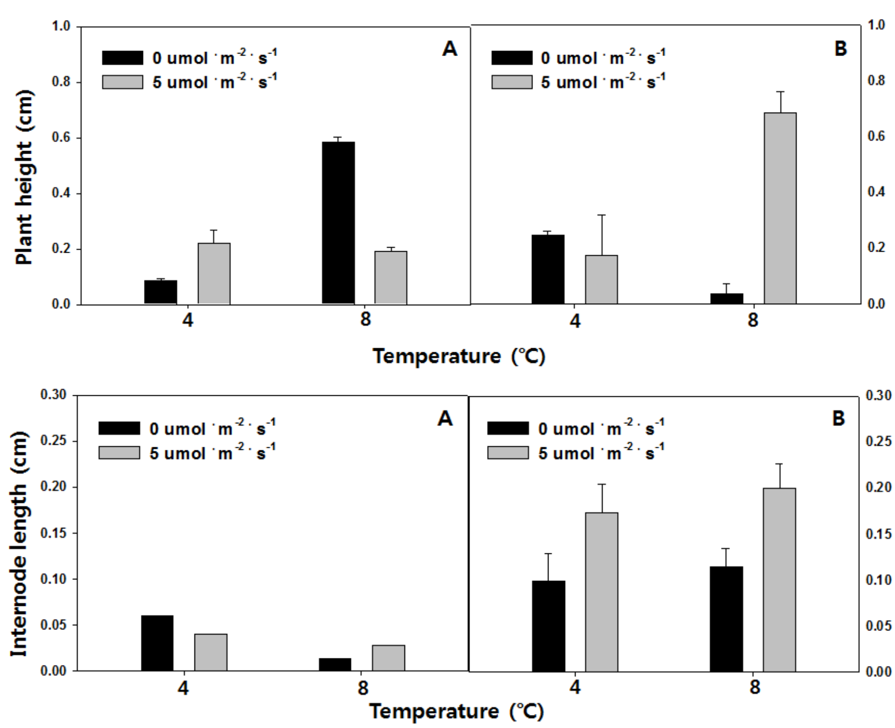

Fig. 1. Changes in plant height and internode length after two weeks of cold storage and one week of acclimatization of *P. frutescens* var. *acuta*: A, after two weeks of storage in growth chambers; and B, after one week of acclimatization in a glasshouse.

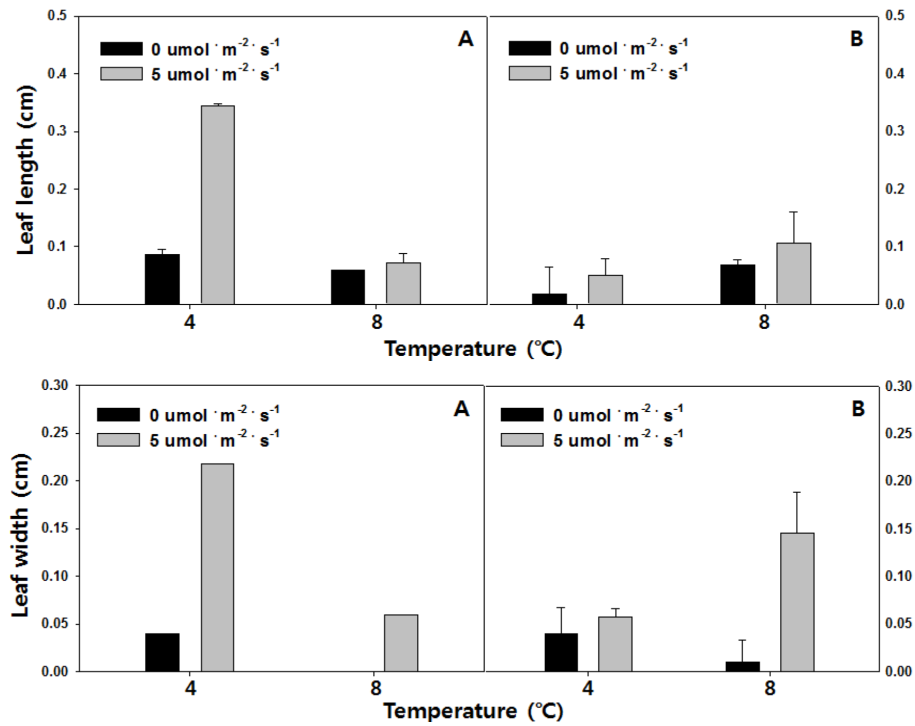

Fig. 2. Changes in leaf length and width after two weeks of cold storage and one week of acclimatization of *P. frutescens* var. *acuta*: A, after two weeks of storage in growth chambers; and B, after one week of acclimatization in a glasshouse.

Leaves of *S. tonkinensis* were shrunken at after two weeks of storage in the growth chamber, and they resume normal growth at after one week of acclimatization in a glasshouse. Plant height of *S. tonkinensis* increased more in the 4℃ combined with 0 or 5 μmol·m^{-2}·s^{-1} PPFD than in the 8℃ combined with 0 or 5 μmol·m^{-2}·s^{-1} PPFD after two weeks of storage in growth chambers (Fig. 3). As compared to the greenhouse cultivation, it showed not much change beside shrinkage in the leaf as a whole. This means that to slow the growth rate of plant height, it is better to store at 8℃ than 4℃. However, after one week of acclimatization in a glasshouse, it increased more in the 8℃ than 4℃ treatment. Therefore, for withholding plant height the seedlings should be stored at 8℃ rather than 4℃. Number of leaves in all treatments increased after two weeks of storage in growth chambers, especially in the 4℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD (Fig. 3). Number of leaves did not change in the 4℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD. However, it increased after one week of acclimatization in a glasshouse. Leaf length increased after two weeks of storage in the 8℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD, but not in other treatments. However, after one week of acclimatization in a glasshouse there were no observable changes in all treatments. Leaf width after two weeks of storage at 4℃ in growth chambers increased at an average of 0.1 cm more than that at 8℃ (Fig. 4). Thus, short-term cold storage was not effective for growth inhibition of leaf width. Leaf width increased more for those stored under a dark condition than a light condition after one week of acclimatization in a glasshouse (Fig. 4). Similar results were reported that plant quality was improved with the addition of light during storage and as storage temperature increased (Heins *et al*., 1994).

Leaves of *A. gigas* Nakai were drooped to the side at after two weeks of storage in the growth chamber, and they resume normal growth after one week of acclimatization in a glasshouse. Plant height of *A. gigas* Nakai was the greatest in the 8℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD both after two weeks of storage in growth chambers and after one week of acclimatization in a glasshouse (Fig. 5). Number of leaves was the greatest at 8℃ combined with 0 or 5 μmol·m^{-2}·s^{-1} PPFD after two weeks of storage in growth chambers, implying less effectiveness of this treatment than other treatments (Fig. 5). After one week

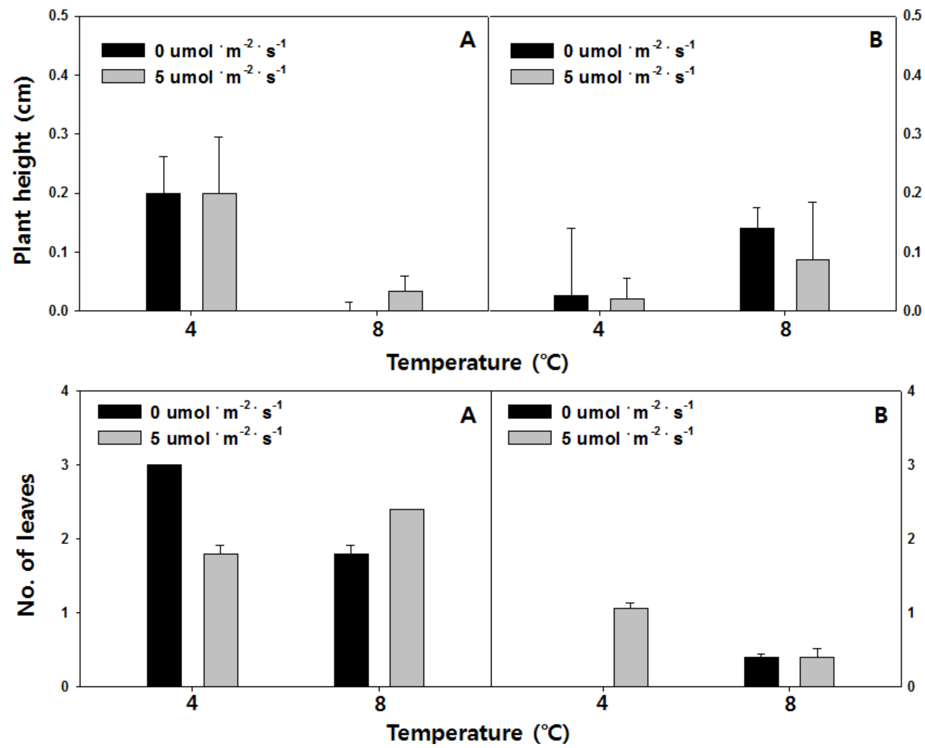

Fig. 3. Changes in plant height and no. of leaves after two weeks of cold storage and one week of acclimatization of *S. tonkinensis*: A, after two weeks of storage in growth chambers; and B, after one week of acclimatization in a glasshouse.

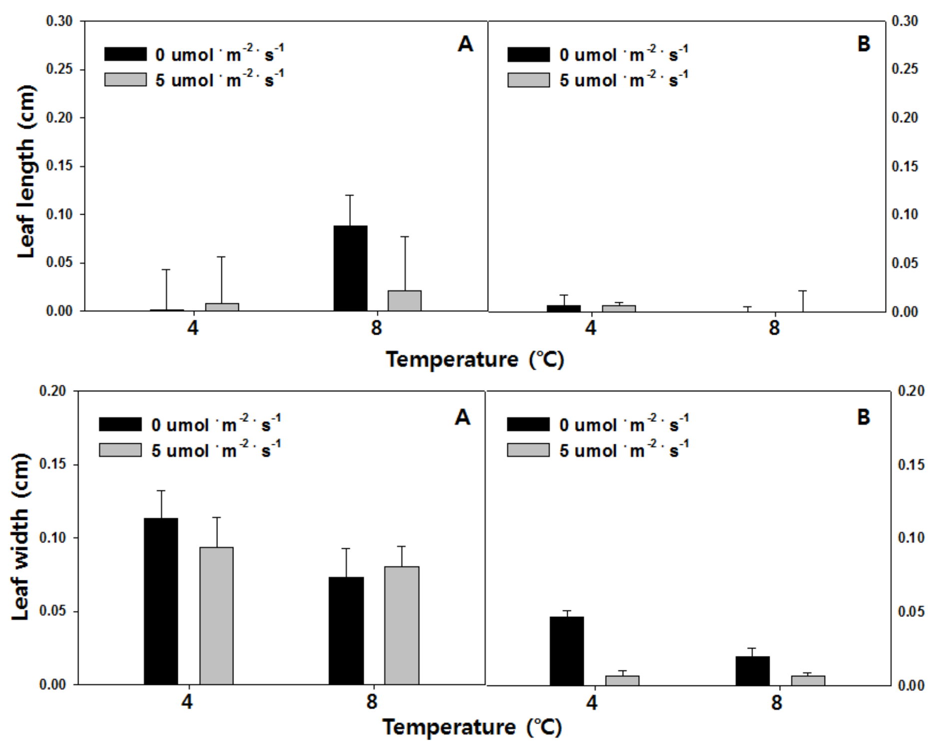

Fig. 4. Changes in leaf length and width after two weeks of cold storage and one week of acclimatization of *S. tonkinensis*: A, after two weeks of storage in growth chambers; and B, after one week of acclimatization in a glasshouse.

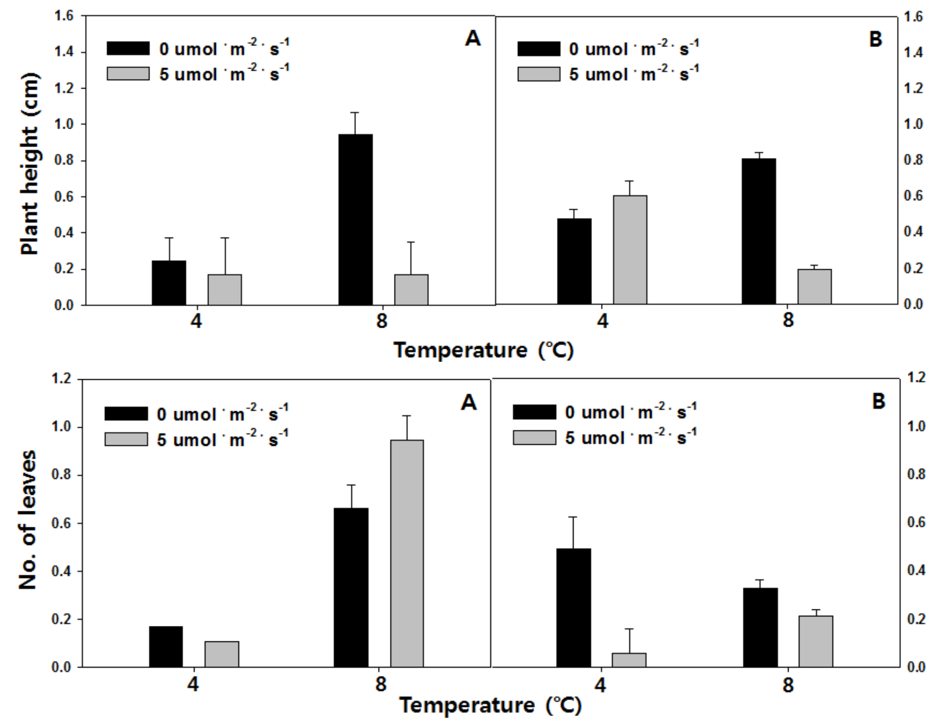

Fig. 5. Changes in plant height and no. of leaves after two weeks of cold storage and one week of acclimatization of *A. gigas* Nakai: A, after two weeks of storage in growth chambers; and B, after one week of acclimatization in a glasshouse.

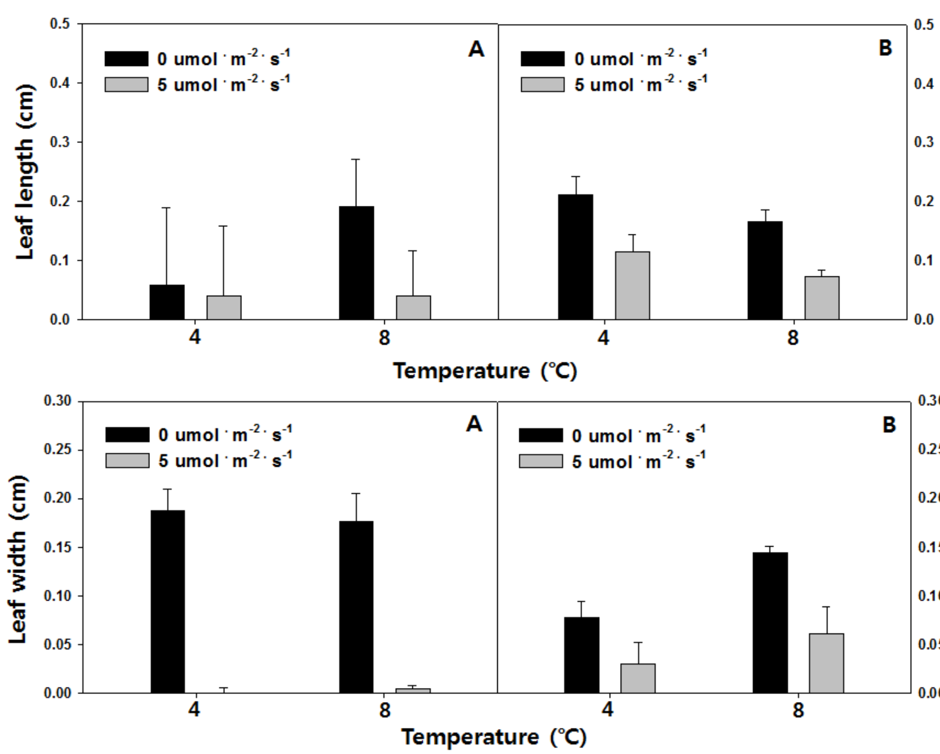

Fig. 6. Changes in leaf length and width after two weeks of cold storage and one week of acclimatization of *A. gigas* Nakai: A, after two weeks of storage in growth chambers; and B, after one week of acclimatization in a glasshouse.

of acclimatization in a glasshouse, no. of leaves was the greatest in the 4℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD, and it was greater under a dark than light condition. Leaf growth was greater under a dark than light condition in both 4 and 8℃ (Fig. 6). Low temperature storage in darkness is commonly used to preserve quality in many seedlings (Kaczperski and Armitage, 1992; Kaczperski *et al*., 1996; Leskovar and Cantliffe, 1991; Risse *et al*., 1985) and is used as a holding method for numerous bedding plant species (Lange *et al*., 1991).

Seedling growth can be suspended by control of light and temperature levels in the growth environment (Heins *et al*., 1994; Kubota *et al*., 2002). The stem elongation during seedling storage is a consequence of the environmental conditions in which the growth of seedlings was not completely suppressed (Heins *et al*., 1994). Storing plug seedlings is also useful for growers, since holding the seedlings for a few weeks before transplanting allows flexible crop scheduling and labor management (Kubota *et al*., 2002). Producers of medicinal plant seedlings would benefit from a system of storing plugs for short periods of time to increase total output during the marketing period as Kaczperski and Armitage (1992) pointed out. The success of storage depends on the physiological status of seedlings on the date of lifting and storage conditions. Low temperature storage under light showed to significantly increase seedling quality (Heins *et al*., 1994; Kubota *et al*., 2002). Seedlings should also be physiologically ready for cold storage conditions (Deligöz, 2013). There have been numerous studies (Burr and Tinus, 1988; Ericsson *et al*., 1984; Généré *et al*. 2004) on changes in seedling quality during cold storage, as well as on survival and growth after planting.

These results suggested that optimal storage environment were 8℃ combined with 5 μmol·m^{-2}·s^{-1} PPFD for *P. frutescens* var. *acuta* Kudo and 4℃ combined with 0 μmol·m^{-2}·s^{-1} PPFD for *S. tonkinensis* and *A. gigas* Nakai. Hence, proper combinations of temperature and PPFD were necessary for better storage, and acclimatization and growth, thereafter, of the plug seedlings of these plant species.

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