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Influence of ecogeography and chromosomes on leaf stomatal variation and diversity in *Lilium amabile* Palibian

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Abstract. This study examines the impact of ecogeographical conditions and chromosomal variations on stomatal traits in *Lilium amabile* Palibin, a wild lily species native to East Asian countries, especially Korea. The stomatal traits, mainly density, size, and index, are appropriate indicators of plant adaptation to varying environmental conditions, and their variation often reflects the genetic and ecological dynamics of species across diverse habitats. In this study, leaf samples from 51 accessions of six different Korean habitats were analyzed to evaluate the impact of altitude, temperature, rainfall, and soil conditions on stomatal morphology. The cytogenetic analysis was also conducted to predict the role of chromosome numbers and types of B chromosomes. The findings revealed significant variation in stomatal traits across populations. The plants from high- altitude or drier regions exhibited smaller but more densely packed stomata, which suggest the adaptations to minimize water loss. Similarly, populations with higher chromosome numbers, particularly those carrying B chromosomes (25+2B), had significantly greater stomatal density but smaller stomata. This study shows that both ecogeography and chromosomal architecture have an effect on the morphological diversity of *L. amabile*. Understanding this relationship is crucial for evolutionary biology, conservation efforts, and breeding programs aimed at enhancing environmental resilience in lilies and related species.

Keywords: *Adaptive traits, B chromosome, chromosome number, ecogeography, and stomata density*

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1. Introduction

The lilies are herbaceous perennial flower crops belonging to the genus Lilium and the Liliaceae family, consisting of 110 species, which are further divided into seven sections that account for more than 10,000 officially registered cultivars with commercial values (McRae, 1998; Dhiman et al., 2018; Badarch et al., 2025). In East Asian nations, specifically China, Japan, and South Korea, varied climatic conditions facilitate the proliferation and survival of wild lily species. Notably, researchers have recorded around 15 endemic lily species in Korea alone (Lee, 1982; Wilson, 1925; Lucidos et al., 2013). *Lilium amabile* Palibin is a wild lily species that is only found in East Asia, especially Korea and parts of northeastern China. It is known for its striking phenotypic plasticity and ecological resilience (Kim & Kim, 2005). Furthermore, the distribution of *Lilium amabile* Palibian is considered from the south of Jeju Island of Korea to the east in Urleung Island, Korea, to the north in Liaoning province of China, to Manchuria of China to the west (Wilson, 1925; Chung, 1965; Synge, 1980; Haw, 1986; Badarch et al., 2025). One of the special features of this species is the variation in leaf stomatal characteristics, which are important for physiological processes, such as photosynthesis, transpiration, and gas exchange. Previous studies of L. *amabile* palibian were concerned with and confined to karyotype, B chromosome variation, and other cytological aspects, covering the diploid and B-chromosome aneuploidy as well as variations of trichromes, vegetative, and floral traits due to the ecogeography and chromosome number (Lee et al., 2019; Nguyen et al., 2019; Nguyen et al., 2021; Badarch et al., 2025).

The leaf stomatal traits, such as stomatal density, index, size, and distribution, are influenced by both intrinsic (genetic) factors and environmental pressures (Hetherington & Woodward, 2003). These characteristics are not only important for physiological performance under varying ecological conditions but also serve as taxonomic markers in systematics and phylogenetic studies (Hetherington & Woodward, 2003; Melotto et al., 2008). Eco-geographical factors such as altitude, temperature, relative humidity, and soil moisture content significantly shape stomatal morphology and distribution. In *L. amabile*, populations growing across distinct ecogeographic zones, including variations in altitude, temperature, humidity, and soil moisture, display divergent stomatal traits, indicating a strong ecogeographic influence on stomatal development and adaptive plasticity to microclimatic conditions (Lee et al., 2017).

Besides the environmental factors, chromosomal variation exerts a profound influence on stomatal traits. Cytogenetic investigations in the *Lilium* genus have documented extensive chromosomal polymorphisms, including polyploidy and structural chromosomal rearrangements (Lim et al., 2001). These karyotypic differences affect not only the size of the genome but also how cells divide and differentiate, which in turn changes the shape and number of stomata. In general, polyploidy, often associated with larger cell sizes, tends to result in reduced stomatal density but increased stomatal size, an adaptation that may be advantageous in high-altitude or drought-prone environments (Woodward et al., 2002).

It is essential to comprehend the synergistic impact of ecogeographical factors and chromosomal constitution on the stomatal characteristics of *L. amabile*, as this knowledge is vital for elucidating its evolutionary biology and for guiding conservation and breeding initiatives. The detailed information on these adaptive traits can assist in selecting resilient genotypes for horticultural improvement and climate change mitigation strategies. This study aims to identify the variation in leaf stomata distribution on *L. amabile* palibian due to geographical variations and chromosome number variations.

2. Materials and Methods

2.1. Plant material

This study investigated stomatal variation in *Lilium amabile* Palibin, a wild lily species distributed across six eco-geographical regions of South Korea. A total of 51 accessions were collected from Mt. Bobal (BB), Mt. Gongjak (GJ), Mt. Halla (HL), Mt. Hongcheon (HC), Mt. Mangdacam in Inje (IJ), and Mt. Kkothong in Yanggu (YG) (Table 1; Figure 1). The sampled accessions from each population were comprised distinct environmental gradients in term of altitude, rainfall, and temperature. The field-collected bulbs were transplanted into pots (5 cm diameter × 7 cm depth) filled with a 1:1 mixture of sterilized sand and peat moss to minimize soil-borne effects. The sampled accessions were cultivated under controlled greenhouse conditions at Kangwon National University (KNU), Chuncheon, with 14 h light (25°C) ,and 10 h dark (20°C) photoperiods to ensure uniform growth and reduce external variability (Hetherington & Woodward, 2003; Franks et al., 2009). The accession were sampled from the *L. amabile* populations those cytological studies including the availability or unavailability of B chromosome has been already investigated (Lee et al., 2019; Nguyen et al., 2021).

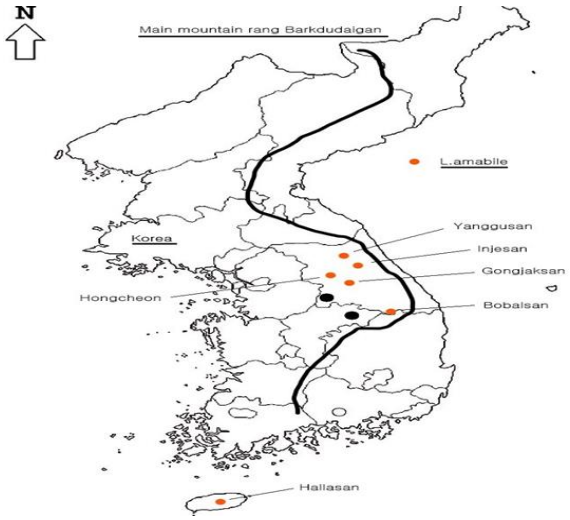

Figure 1. Location of sampled populations of *L. amabile* Palibian

Table 1. Geographical habitats, ecological and accession sampled information for the stomatal variation study in *L.ambile* Palibian

S.N.	Populations ID	Basic information of habitat						
		Location	Altitude	Soil texture	Mean annual rainfall (mm)	Mean temperature (°C)	Mean relative humidity (%)	No. of sample (accession) taken for study
1	BB	Mt. Bobal, Danyang-gun,Gangwon-Do	400	Loam	1900	9.1	73	15
2	GJ	Mt.Gongjak, Hongcheon-gun,Gangwon-Do	400-750	Sandy	1405	10.3	69.5	8
3	HL	Mt Halla, Jeju-Do	1450-1550	Drain humus	4670	6.55	80	6
4	HC	Mt. Hongcheon, Gangwon-Do	400-600	Sandy	1000	10.5	71	6
5	IJ	Mt.Mangdacam,Inge-gun,,Gangwon-Do	300-600	Sandy	1210	10.1	69.6	9
6	YG	Mt.Kkothong,Yanggu-gun, Gangwon-Do	400-600	Sandy	1000	10.5	71	7
Total								**51**

N.B. BB=Bobal,GJ=Gongjak,HL=Halla,HC=Hongcheon,IJ=Inje and YG=Yanggu;
*The climate characteristics of MD were retrieved from Wikipedia (https://en.wikipedia.org/wiki/Inje_County#Climate)

2.2. Observation of the variation in stomata density, length, and width

The investigation of variation of leaf stomatal density, length, and width for each accession were carried out taking one fully expanded, mature leaf from the midsection of the plant was selected to ensure consistent physiological age. Only the abaxial (lower) leaf surfaces were analyzed because preliminary microscopic inspection showed stomata were absent on abaxial surfaces, a common trait in *Lilium* species (Lawson & Blatt, 2014; Figure 2; Figure 3).

The epidermal imprints were obtained using the precise nail-polish method: a thin film was applied on the abaxial surface, allowed to dry, and gently peeled off with adhesive tape, which was then mounted on glass slides. Observations were made using a Nikon Eclipse E400 light microscope under 200 × magnifications.

For the calculation of mean stomatal density (number cm^{-2}), per leaf 10 random microscopic fields were analyzed (Figure 4; Table 2). In contrast, stomatal length and width (µm) were measured using an ocular micrometer and converted to an absolute scale using a stage micrometer calibration (Melotto et al., 2008; Franks et al., 2012; Badarch et al., 2025). Similarly, the variation in stomatal density caused by the number and types of B chromosomes was estimated, with details of each observation plotted in the diagram (Figure 5) and measurements presented alongside statistical analysis (Table 3).

Figure 2. The leaf surfaces (Abaxial) of *L. amabile* Palibian

2.3. Variability of stomata in terms of types and measurement

All stomata were identified as normocytic type, characterized by irregularly arranged subsidiary cells, consistent with previous reports for *Lilium* and other monocots (Franks & Beerling, 2009; Lee et al., 2017). No other stomatal morph types were

detected. Quantitative variability in stomatal traits i.e. density, length, and width among populations was assessed relative to their habitat characteristics, i.e.altitude, temperature, rainfall, and soil texture (Table 2).

For the evaluation of chromosomal influences, data from cytological confirmed accessions representing chromosome complements 2n=24, 24+1B, 24+2B, 24+2b, and 25+2b were used (Lee et al., 2019; Nguyen et al., 2019). Stomatal measurements for each cytotype were averaged across individuals to assess relationships between genome constitution and epidermal morphology (Woodward et al., 2002; Murray et al., 2020).

2.4. Statistical analysis

Analysis of the data regarding the stomata density based on the observation of numbers of stomata and their length and width across the populations of the different geoecological regions and based on the number of chromosomes and number of chromosomes and types of the B chromosomes present was performed using the standard statistical tools. In statistical analysis, descriptive statistics for the continuous variables were presented as mean and standard error of the mean. One-way ANOVA was used to compare group means. Following the ANOVA, Duncan's multiple comparison tests was performed to identify different variations in means. Pearson correlation coefficients were computed to determine linear relationships between the variables. In addition to correlation analysis, linear regression analysis was performed to predict dependent variables from independent variables (Table 2-3; Figure 4-5). The statistical significance level was considered to be 5% and 1%. The SPSS (Ver. 26) statistical program was used for all statistical computations.

3. Results and Discussion

3.1. Stomata types and distribution

In this investigation the microscopic observations confirmed that all *L. amabile* populations possess normocytic stomata restricted to the abaxial leaf surface (Figure 3). The absence of abaxial stomata aligns with xeromorphic adaptations that reduce transpiration water loss, commonly seen in montane *Lilium* species (Lawson & Blatt, 2014; Raissig et al., 2017).The findings of this experiment as uniformity in types of stomata among the studied populations demonstrates strong phylogenetic conservation, whereas inter-population differences in stomatal size and density imply ecological plasticity driven by local environmental conditions (Hetherington & Woodward, 2003; Franks et al., 2009).

Previous studies demonstrated similar types of findings in *Lilium lancifolium* and *L. tsingtauense*, in which variation in stomatal frequency were observed across altitude but there was no variation in stomata type (Lee et al., 2017). These results collectively highlight that stomatal architecture in *L. amabile* is genetically constrained, yet morphometric traits exhibit substantial ecological responsiveness.

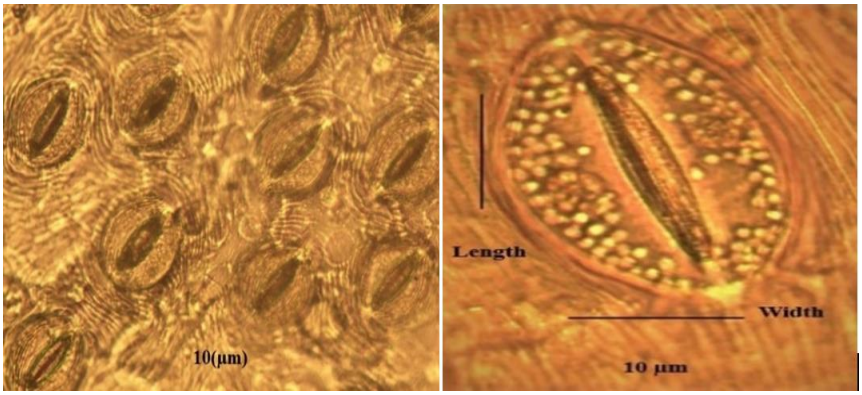

Figure 3. Stomata type of abaxial leaf surfaces of *L. amabile* Palibian

3.2. Variation in stomatal density, length, and width across eco-geographical regions

The estimated stomata density demonstrated significant variation among the six geographic populations under study (Table 2) which consisted the highest number of stomata (3528.5 stomata cm^{-2}) found in plants from Hongcheon, which

geographical region is characterized by moderate altitude and relatively low humidity, whereas the lowest number of stomata (2867.5 stomata cm^{-2}) was recorded in the populations of Inje, a region with higher humidity and lower evaporative demand. Conversely, the longest stomata were observed in high-altitude Halla San plants (18.5 µm), while the smallest occurred in Gongjak San (15.0 µm).

This type of findings of inverse relationship between stomatal size and density corroborates the well-established adaptive trade-off; plants in xeric or high-light environments often exhibit smaller but more numerous stomata to balance CO_2 uptake and water conservation (Woodward et al., 2002; Franks et al., 2009; de Boer et al., 2016). Furthermore, the higher stomatal density observed in mid-altitude populations may reflect evolutionary optimization for fluctuating vapor-pressure deficits typical of transitional mountain climates (Lawson & Blatt, 2014).

Additionally, the soil texture also appeared influential; sandy soils of Gongjak and Hongcheon populations showed higher stomatal frequencies than humus-rich Halla soils, indicating a possible linkage between edaphic stress and epidermal adaptation (Lee et al., 2017). In the previous studies similar findings were observed as eco-geographic plasticity has been observed in *Arabidopsis thaliana* (Dittberner et al., 2018) and *Oryza sativa* (Liu et al., 2015), supporting the hypothesis that local hydrothermal regimes fine-tune stomatal traits.

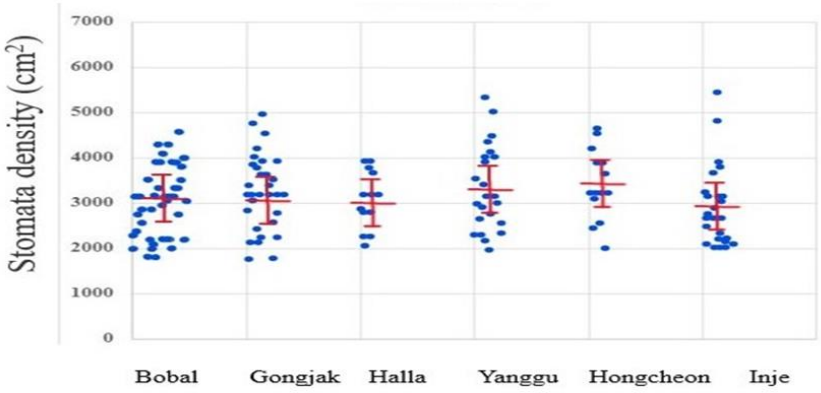

Figure 4. Variation of stomata density on the leaf surface (Abaxial)

3.3. Influence of chromosome number and B-chromosomes on stomatal traits

The estimated cytogenetic effect on stomatal density i.e. Number of stomata, length, and width, was presented in Table 3. It has revealed that populations with chromosome number 25+2b exhibited the highest stomatal density (5240.83 stomata cm^{-2}) while the smallest stomata was found in the plant populations with the chromosome number 24+2b (2762.92 stomata cm^{-2}) . While in contrast with this, those with 24 chromosomes without B elements showed lower densities (3096.77 stomata cm^{-2}) and larger guard cells (16.94 µm). These results resembles with earlier observations that increased chromosomal content or the presence of B chromosomes can alter cell size and developmental timing, thereby influencing epidermal cell differentiation (Nguyen et al., 2019; Lee et al., 2019).

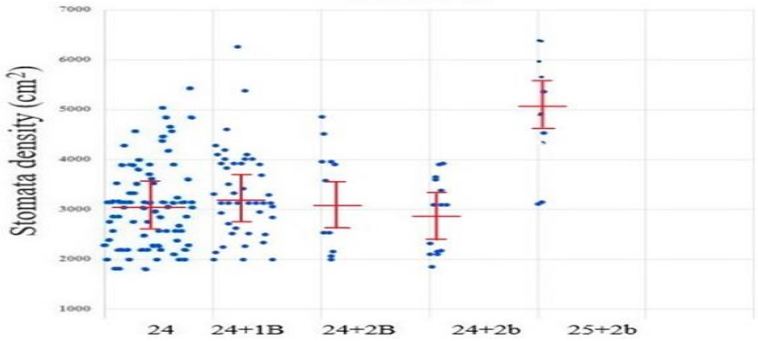

Figure 5. Variation of stomata density on the leaf surface (Abaxial) of different chromosome types

Moreover, smaller stomata associated with higher chromosome numbers may arise from enhanced gene dosage or epigenetic modulation linked to B-chromosome presence (Camacho, 2005).This sort of cytogenetic impact of epidermal features has been reported earlier in the studies of various crops viz. lilies, onions, and wheat, in which supernumerary chromosomes modify nuclear volume and stomatal initiation patterns (Franks et al., 2012; Murray et al., 2020).

Table 2. The stomatal density (number, length, and width) of the six different habitats

Plant	Stomatal density		
	Number	Length (µm)	Width (µm)
Bobal	3328.96±1224.92^{c}	15.41±0.67^{c}	10.33±0.78^{a}
Gongjak	3192.00±829.54^{cd}	15.00±0.86^{cd}	10.05±0.76^{ab}
Halla	3018.07±645.67^{cd}	18.55±2.01^{a}	9.87±0.64^{c}
Yanggu	3431.88±1133.89^{b}	16.62±1.04b^{c}	9.85±0.35^{c}
Hongcheon	3528.57±856.10^{a}	15.35±0.92^{cd}	10.07±0.47^{ab}
Inje	2867.59±862.96^{d}	17.33±1.63^{b}	9.75±0.50^{d}

*Values shown are means± SD. Small typed letters indicate significant differences in plant parameters according to the least significant difference post-hos analysis ($p \leq 0.05$) after one-way ANOVA.

In this way, the observed relationships revealed the model that both ecological stress and chromosomal architecture jointly regulate stomatal morphology- ecological gradients induce phenotypic plasticity, while chromosomal variations contribute to developmental constraints or facilitation (Woodward et al., 2002; Raissig et al., 2017). In *L. amabile*, the combined influence of high-altitude stress and B-chromosome polymorphism appears to enhance adaptive variability, thereby possibly improving water-use efficiency under fluctuating climates.

Table 3. The stomatal density (number, length, and width) having different chromosome number of six different habitats

Chromosome number	Stomata density		
	Number	Length (µm)	Width (µm)
24	3096.77±854.13^{c}	16.94±1.69^{a}	10.08±0.65^{b}
24+1B	3325.00±899.43^{b}	15.85±1.73^{b}	9.94±0.44^{c}
24+2B	3255.91±1010.84^{bc}	16.38±2.07^{ab}	10.25±1.26^{a}
24+2b	2762.92±717.19^{d}	15.25±0.50^{b}	10.25±1.26^{a}
25+2b	5240.83±2073.02^{a}	15.00±0.00^{c}	9.75±0.50^{d}

*Values shown are means± SD. Small typed letters indicate significant differences in plant parameters according to the least significant difference post-hos analysis ($p \leq 0.05$) after one-way ANOVA.

4. Conclusions

This study reveals both eco-geographical conditions and chromosomal variations significantly influence stomatal variation in *Lilium amabile* Palibin. The observed variation in stomatal density, length, and width were found among populations from different ecological regions, reflecting adaptations to altitude, temperature, humidity, and soil type. The accession or plants from higher or drier regions showed smaller but more numerous stomata, suggesting an efficient mechanism for water conservation. Chromosomal variations, particularly the presence of additional B chromosomes, were also found to affect stomatal morphology. Additionally the populations with extra chromosomes exhibited higher stomatal densities but smaller stomatal sizes, indicating a genetic influence on epidermal development. These findings highlight the combined role of ecological and genetic factors in shaping the adaptive characteristics of *L. amabile*. Understanding these relationships provides valuable insight for conservation strategies, breeding programs, and future studies on plant adaptation and resilience.

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Conflicts of interest. The authors mentioned that none of them have a conflict of interest when it comes to this article.

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References

Badarch, A., Rai, R. & Kim, J.-H. (2025). Estimation and analysis of the impact of habitats and chromosomes on trichome variation in *Lilium amabile* Palibian. Journal of Multidisciplinary Sciences, 7(1), 01-10.

Camacho, J.P.M. (2005). B chromosomes. In: T.R. Gregory (Ed.). The evolution of the genome (pp. 223–286). Elsevier.

Chung, T. (1965). Illustrated Encyclopedia of Fauna & Flora of Korea. Vol. 5. Tracheophyta. Genus Chrysosplenium. Ministry of Education. Seoul. pp. 497-483.

de Boer, H.J., Price, C.A., Wagner-Cremer, F., et al. (2016). Optimal allocation of leaf epidermal area between gas exchange and defense. New Phytologist, 210(3), 932-942.

Dhiman, M.R., Moudgil, S., Parkash, C., Kumar, R. & Kumar, S. (2018). Biodiversity in *Lilium*: A review. International Journal of Horticulture, p. 8(8), 83-97.

Dittberner, H., Korte, A., Mettler-Altmann, T., et al. (2018). Natural variation in stomata size contributes to the local adaptation of water-use efficiency in Arabidopsis thaliana. Molecular Ecology, 27(18), 4052-4065.

Franks, P.J., Drake, P.L. & Beerling, D.J. (2009). Plasticity in stomatal size and density: Implications for water-use efficiency. Plant, Cell & Environment, 32(10), 1508-1516.

Franks, P.J., Drake, P.L. & Froend, R.H. (2012). Anisohydric but isohydrodynamic: Seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance. Plant, Cell & Environment, 35(1), 141-153.

Haw, S.G. (1986). The lilies of China. B.T. Batsford Ltd., London.

Hetherington, A.M. & Woodward, F.I. (2003). The role of stomata in sensing and driving environmental change. Nature, 424(6951), 901-908.

Kim, D.K. & Kim, S.C. (2005). Genetic variation and population structure of the Korean endemic lily, *Lilium amabile* Palibin. Journal of Plant Biology, 48(1), 21-27.

Lawson, T. & Blatt, M.R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiology, 164(4), 1556-1570.

Lee, W.T. (1982). Lineamenta Florae Koreae. Academy of Sciences, DPR Korea.

Lee, J.H., Han, S.Y. & Choi, Y.J. (2017). Variation in leaf stomatal characteristics in populations of *Lilium amabile* in relation to ecological gradients. Korean Journal of Plant Research, 30(6), 573-581.

Lee, S.I., Nguyen, T.X., Kim, J.H. & Kim, N.S. (2019). Cytological variations and long terminal repeat (LTR) retrotransposon diversities among diploids and B-chromosome aneuploids in *Lilium amabile* Palibin. Genes & genomics, 41, 941-950.

Lim, K.B., Wennekes, J., De Jong, J., Jacobsen, E. & Van Tuyl, J.M. (2001). Karyotype analysis of *Lilium* species, hybrids and polyploids by chromosome banding and fluorescence in situ hybridization. Theoretical and Applied Genetics, 103(3), 390-400.

Liu, C., Xu, Y., Long, D., et al. (2015). Genetic variation of stomatal traits in rice germplasm. Plant Physiology and Biochemistry, 96, 81-88.

Lucidos, J.G. (2013). Bulb dormancy breaking and flowering physiology of Hanson lily (*Lilium hansonii*) [Master's thesis, Kyungpook National University, Daegu, Korea].

McRae, E.A. (1998). Lily Species. Lilies, Timber Press, Portland, Ore. USA, 319-330.

Melotto, M., Underwood, W., Koczan, J., Nomura, K. & He, S.Y. (2008). Plant stomata function in innate immunity against bacterial invasion. Cell, 126(5), 969-980.

Murray, B.G., Leitch, I.J. & Bennett, M.D. (2020). The genomic basis of polyploidy and genome size variation in plants. Annals of Botany, 125(1), 33-48.

Nguyen, T.X., Kim, B.R., Park, D.R., Kim, Y.K., Nguyen, V.Y., Na, J.K.,... & Kim, J.H. (2019). Karyotype and B chromosome variation in *Lilium amabile* Palibin. Genes & Genomics, 41, 647-655.

Nguyen, V.Y., Rai, R., Kim, J.H., Kim, J.Y. & Na, J.K. (2021). Ecogeographical variations of the vegetative and floral traits of *Lilium amabile* Palibian. Journal of Plant Biotechnology, 48(4), 236-245.

Raissig, M.T., Matos, J.L. & Bergmann, D.C. (2017). Abaxial–adaxial patterning in leaves: Symmetry, asymmetry, and polarity. Annual Review of Plant Biology, 68, 171-198.

Synge, P.M. (1980). Lilies, London: BT. Batsford, UK.

Wilson, E.H. (1925). The lilies of Eastern Asia. Dulau & Co. Ltd, London. pp 110.

Woodward, F.I., Lake, J.A. & Quick, W.P. (2002). Stomatal development and CO_2: Ecological consequences. New Phytologist, 153(3), 477-484.