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Estimation and analysis of the impact of habitats and chromosomes on trichome variation in *Lilium amabile* Palibian

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Abstract. The leaf trichome, a unique and distinctive feature of *Lilium amabile Palibian*, sets this species apart from the 15 endemic lilies grown in Korea. This experiment examined the leaf trichome type and its variation in density, length, and width by geographical region (habitat). It focuses on investigation how chromosome/b chromosome and trichome variation parameters (density, length, and width) are related in *L. amabile Palibian*. Altogether, 51 leaf samples were examined, each representing every accession from six geographical regions in Korea. The mature intact leaves of each population was preserved in an acetic/ethanol (1:3) solution for up to 14 days. Subsequently, leaf trichome observation was conducted using light microscopy, a reliable and widely accepted method in the field of such types of research. Trichomes, found on both sides of the leaf (adaxial and abaxial), varied in density, length, and width and were categorized into four groups i.e. conical, cylindrical, glandular, and hooked. The conical type of trichome was commonly recorded in every population and sample. The results suggested that the primary ecological factors of habitat could influence the shape, size, and density of leaf trichomes. The findings confirmed that ecology, elevation, sunlight, and chromosomal changes affect trichome morphology and density. It was also observed that chromosome/B chromosome changes do not significantly affect trichome type and density.

Keywords: *Abaxial surface, accession, adaxial surface, ecological factors, and geographical region*

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1. Introduction

Lilies are perennial herbaceous floricultural crops that are used for multiple purposes, viz., as cut flowers, pot flower purposes, landscape gardening purposes, medicinal purposes, as food and cosmetic purposes, and for various ethnic ritual purposes (Dhiman et al., 2018; Nguyen et al., 2021). The lily comes under the genus Lilium and the family Liliaceae, which exist globally and comprise 110 species, 7 sections, and more than 10,000 formally registered commercial cultivars (McRae, 1998; Matthews, 2007; Bakhshaie et al., 2016; Dhiman et al., 2018) is a testament to the adaptability and evolutionary success of this plant family. The main distribution areas of lilies are 60 species in Southeast Asia and East Asia, 25 in Europe, and 14 in North America. Most of the species of lilies are native to the temperate northern hemisphere, though their range extends into the northern subtropics (Dhiman et al., 2018).

Korea is considered a significant center of wild lilies in East Asia, as it harbors diverse climatic conditions suitable for Lilium's growth and existence (Lucidos et al., 2013). About 15 endemic lily species are growing in Korea (Wilson, 1925; Lee, 1982). In the previous five various species of Lilium have been studied so far by our floricultural breeding laboratory, viz., *Lilium tigrium* (Nguyen et al., 2016), *Lilium leichtlinii* var. maximowiczii (Nguyen et al., 2015; Rai et al., 2021), *Lilium brownie* var. colchesteri (Rai et al., 2022), *Lilium longiflorum* Thunb. (Rai & Kim, 2020a; Rai & Kim, 2021), and *Lilium formolongi* Hort. (Rai et al., 2019; Rai et al., 2020; Rai & Kim, 2020b), and *L. amabile* is one species in which recent research is focusing on exploring the different aspects of this species. *L. amabile* is distributed from the south in Jeju Island, Korea, to the east in Urleung Island, Korea (Wilson, 1925; Synge, 1980), to the north in Liaoning Province, China (Haw, 1986), and to the west in Manchuria, China (Chung, 1965).

Lilium amabile contains a large number of black spots with orange-red flowers. Its bulbs are edible as a tonic bulb, but it is primarily used as an ornamental plant in Korea (Lee, 1982; Song et al., 1989). Nguyen et al. (2021) studied the variations of vegetative and floral traits of *L. amabile* due to the ecology of the particular geography. Furthermore, recent genetic studies confined karyotype, B chromosome variation, cytological variation, and long terminal repeat retrotransposon diversities among diploid and B-chromosome aneuploidy (Lee et al., 2019; Nguyen et al., 2019).

There are few studies on the type, distribution, and density of leaf trichomes in *L. amabile*. This study, however, is a novel exploration into the type, distribution, and density of trichomes in *L. amabile* and their relationship with the B chromosome. The morphology and density of leaf trichomes vary considerably between plant species and may differ from one population to another. The trichome type can vary between more than one cell, and the trichome can be straight, spiral, hooked, branched, unbranched, columnar, or non-glandular (Werker et al., 1985). Trichomes are specialized epidermal cells that generally reduce transpiration and act as a deterrent to herbivory. The present study investigated the trichome type, region variation, and relationship between the B chromosome trichome densities of *L. amabile* leaf trichomes. This study determines the effect of *L. amabile* ecological factors and the relationship of B chromosomes.

2. Materials and Methods

2.1. Plant material

In this investigation, the type and frequency of glandular and non-glandular trichomes were examined on both leaf sides of six populations of *L. amabile* Palibian. I have collected 51 *L. amabile* accessions from six geographical regions in Korea (Table 1, Figure 1). The findings from this investigation are significant. For sample collection, the bulbs were taken from each plant and grown in pots (5 in diameter × 7 in-depth) containing sand and peat moss (1:1). The plants were then grown with ten h dark (20°C) and 14 h light (25°C) cycles in the greenhouse that was located in the University Farm of Kangwon National University, Chuncheon.

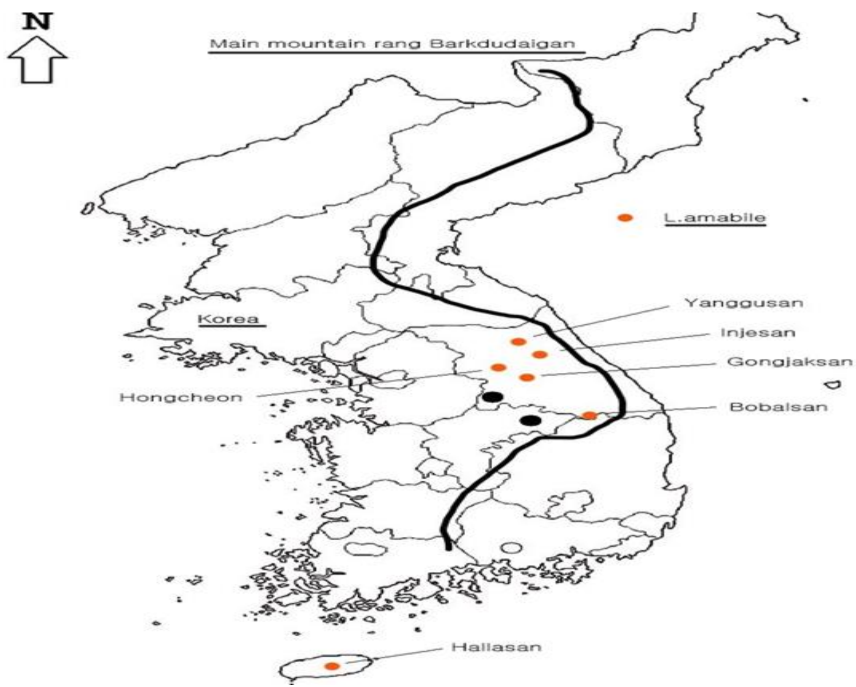

Figure 1. Map of *L.amabile* Palibian populations location sites

2.2. Trichome observation (Variation in trichome density, length, and width)

One mature leaf was selected from the middle section of each population of each stem. The very young leaves, which were the active trichomes used, were meticulously chosen and collected in late June. These leaves were then studied by light microscopy only. For this, each sample's fresh adult plant leaves were saved in an acetic/ethanol (1:3) solution for 14 days and rinsed in water. Following clearing, leaves were dehydrated in ethanol and stained in 1% carmine (in 99% water) for 15 min; each leaf was divided into four parts and mounted onto microscope slides. Suitable thin slices were placed on glass lamina in the final stage, observed, and photographed using a light microscope (Nikon E400).

Table 1. Habitat location, population denotation, and other basic characteristics feature in *L. ambile* Palibian

Population	Habitat locality	Time of flowering	Altitude (m)	No. of plants studied	Soil texture
BB	Mt. Bobal, Danyang-gun	20-Jun	400	15	Loam
GJ	Mt.Gongjak, Hongcheon-gun	22-Jun	400-750	8	Sandy
HL	Mt Halla, Jeju	11-Jul	1450-1550	6	Drain humus
HC	Mt. Hongcheon, Gangwon	16-Jun	400-600	6	Sandy
IJ	Inge-gun	22-Jul	300-600	9	Sandy
YG	Yanggu-gun, Gangwon	18-Jul	400-600	7	Sandy
Total				51	

The sample was collected from six locations with similar elevations except for Halla. As for the soil, Gonjak, Yanggu, Inje, and Hongcheon had sandy soils, Bobal had loam, and Halla had drain humus soils.

2.3. Trichome variability (type and measurement)

The density of hairs on the leaf's surface varied in each population, and the plants in all populations had more dense hairs on the upper side than on the lower side of the leaves. This variation in trichome density and size is significant as it can indicate different adaptive strategies in response to environmental conditions. The hairs on the lower surface of the leaves are located in the veins along the non-rectangular surface. The size of trichomes, a crucial aspect of our study, was meticulously measured using a Nikon E400 microscope. The measurements were taken on a 20 × 10 eye piece and objective, ensuring precision, and then converted to (cm²) square centimeters, providing accurate and reliable data for our analysis. This study revealed a fascinating diversity in plant structures. Four different types of hairs were observed in all plants in the studied populations, with this variety displayed on both surfaces of the leaves.

2 (A). The adaxial surface of the leaves **2(B).** The abaxial surface of the leaves

Figure 2. *L. amabile* leaf surface (A) Adaxial and (B) Abaxial

2.4. Trichome density, length, width variability, and relationship with chromosome number and B chromosome

The trichome density, length, and width variation were observed as described in above 2.2 with the sample drawn as shown in Table 1. The trichome variability in terms of trichome density and its types were measured as described in above 2.3. The chromosome number and B chromosome number of the accessions of each population were confirmed in the previous experiment

(Lee et al., 2019; Nguyen et al., 2019) and were taken for references to measure the relationship with trichome density, length, and width.

Leaf samples were taken from plant accessions provided by the Floricultural Breeding Lab, Department of Horticulture, Kangwon National University (KNU). For this experiment, *L. amabile* populations from different geographical sites whose chromosomes have already been confirmed, ensuring the reliability of our findings.

2.5. Statistical analysis

The raw data about leaf trichome density, length, and width of different habitats, i.e., geographical populations (Table 2), and comprised different chromosome numbers (Table 3) were obtained through the light microscope (Nikon E400) and prepared using MS Excel 2016, and further analysis of variability parameters was carried out using the TNAUSTAT statistical packages. The ANOVA analysis was carried out according to the least significant difference post hoc analysis ($P \leq 0.05$) after one-way ANOVA. Likewise, the DMRT (Duncan's multiple range test) was estimated at the 5% significance level for separating the estimated means of all the treatments following the DMRT comparison tools under the general designs head of the TNAUSTAT statistical package.

3. Results and Discussion

3.1. Trichome type analysis

3.1.1. Trichome type

Plant trichrome is natural hairs or hair-like structures generally produced on plant surfaces, especially on leaves, stems, and fruits (Xiao et al., 2017; Shahzad et al., 2020; Watts & Kariyat, 2021a; Gostin, 2023). Trichomes are specialized epidermal cells on plant surfaces that provide a hairy texture and act as a defense mechanism against insects (Jayanthi et al., 2018). In another sense, it is considered as the unicellular or multicellular epidermal appendages in plants (Wang et al., 2021; Watts & Kariyat, 2021a; Gostin, 2023). Plant trichomes can be classified as glandular and non-glandular trichrome (Jayanthi et al., 2018; Shahzad et al., 2020; Zhang et al., 2020; Asikin et al., 2021; Gostin, 2023). Based on shape, straight, spiral, hooked, branched, or unbranched trichrome can also be classified (Werker et al., 1985; Shahzad et al., 2020). Adebooye et al. (2012) has reported the conical type of trichome in *Trichosanthes cucumerina* (Cucurbitaceae). Plant trichome types and morphology studies in *L. amabile Pallibin* is our first attempt for the type study in Lilium. Based on the form and shape of the trichomes on the adaxial and abaxial surfaces of the leaves, we have identified four types of trichomes namely conical, cylindrical, grandular, and hooked types of trichomes (Figure 3.A-D). Out of the four types of trichomes, the conical type is widespread and found in more than 90% of all populations.

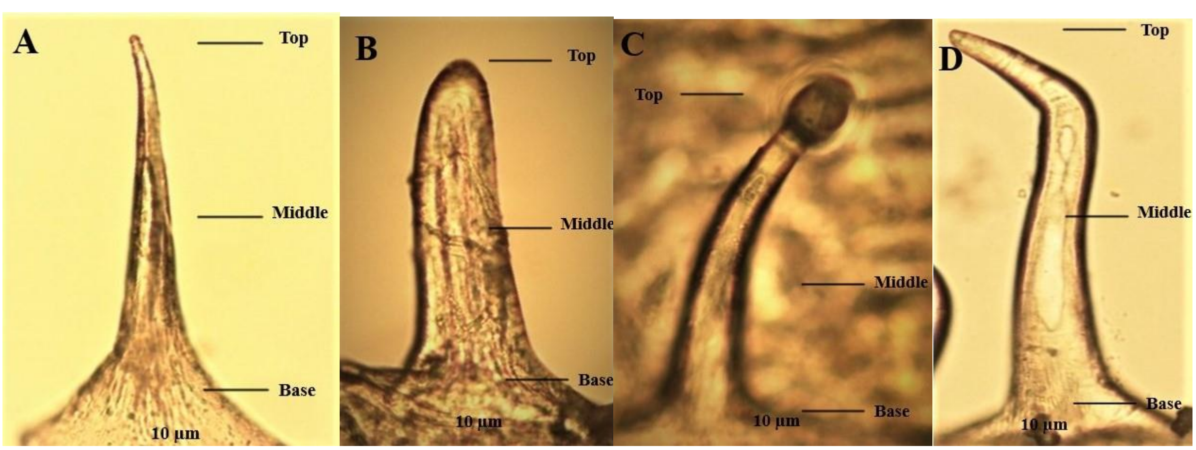

Figure 3. Different types of trichomes in *L. amabile* Palibian. (A. Conical hair, B. Cylindrical hair, C. Glandular hair, D. Hooked hair). Out of the four types of trichomes, the conical type is widespread and found in more than 90% of all populations.

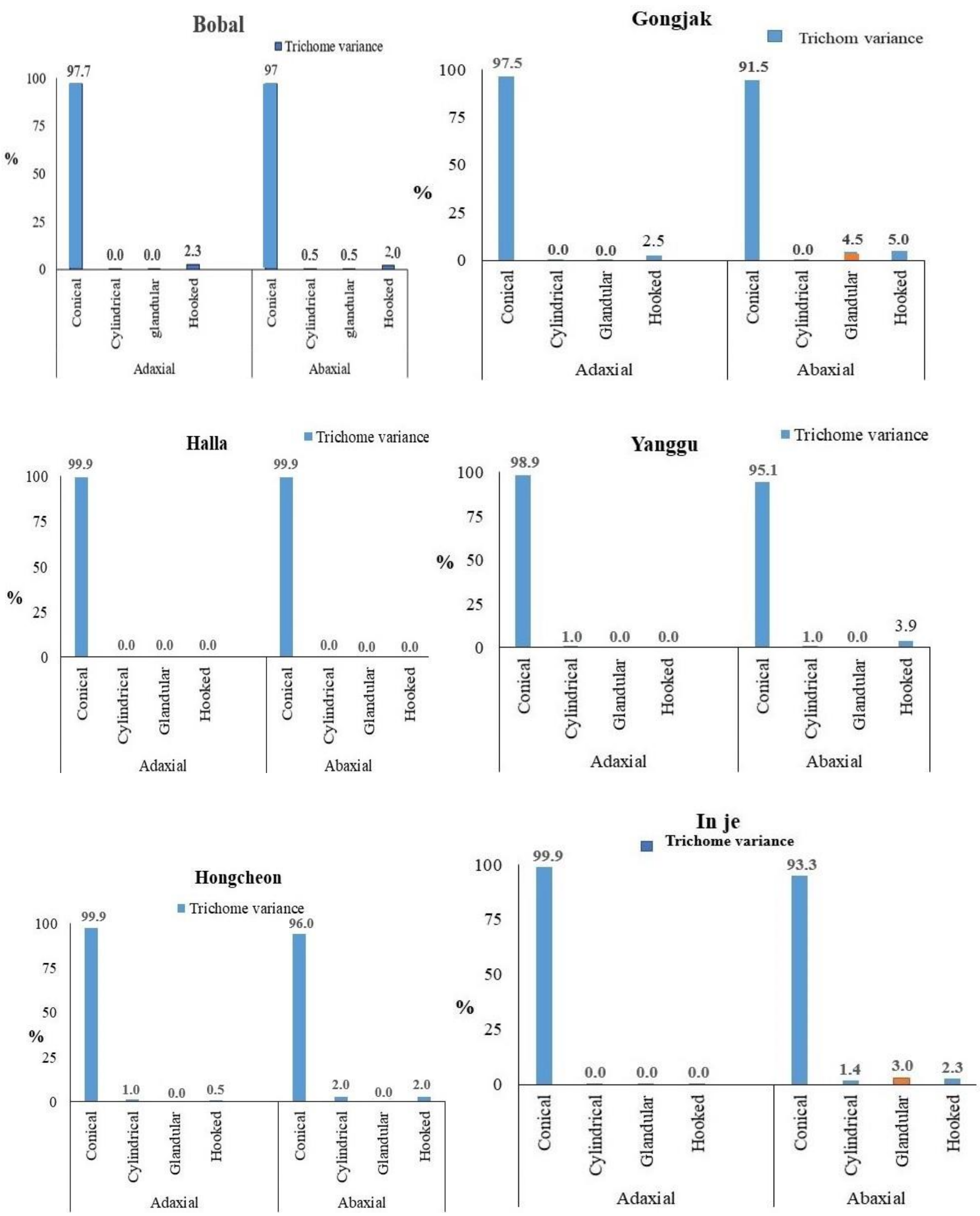

Figure 4. Types and distribution of trichomes in the populations (Bobal, Gongjak, Halla, Yanggu, Hongcheon, and Inje)

3.1.2. Distribution of trichome type in the populations

After the conical, hooked trichomes were found in common, the margin was tiny, ranging from 2% to 4.9%, and was not found in all populations. In Halla san populations, there is only a conical type of trichome, and the other three types were absent; moreover, in the populations viz. Halla, Yanggu, Hongcheon, and Inje, the hooked types of trichomes were found only in the abaxial surface of the leaves, which ranged from 2% to 3.9% and were absent in the adaxial surface of the leaves. The second one, the cylindrical type of trichomes, was not commonly found in all populations. It was absent in the Bobal and Halla populations. It was found both in the adaxial and abaxial surfaces of the leaves of Yanggu populations, but its margin was shallow, only 1%. In Gongjak populations, it was found only on the adaxial surface of the leaves, and the margin was only 1%.

Likewise, it was found in the abaxial surface of the Hongcheon and Inje populations, but the margin was shallow, 2% and 1%, respectively. Furthermore, glandular types of trichomes were found only on the abaxial surface of the leaves of Gongjak and Inje populations, the margins of which were recorded at 4.5% and 3%, respectively (Figure 4). Previous studies about plant trichomes in cotton demonstrated that trichome patterns and density vary within natural populations (Hauser, 2014).

3.2. Distribution of trichome on the leaf surfaces in the populations

The estimated leaf trichome density demonstrated the prevalence of variability among the six different geographical sites, viz. Bobal, Gongjak, Halla, Yanggu, Honghceon, and Inje, for both adaxial and abaxial leaf surfaces. The trichome density for the adaxial leaf surface appeared to be highest for the populations taken from the Gongjak san site, with 2884.8. In contrast, the lowest trichome density was found for the populations taken from Bobal San in 1953.4. On the other hand, trichome density for the abaxial leaf surface was found to be highest for Gongjak san populations (same as for adaxial) with 2029.8. In contrast, the lowest measurement was found for Halla san populations at 1578.4. Evidence shows that the adaxial leaf surface's trichome density was higher than the abaxial leaf surface for all six populations. The results contrasted with the previous studies in 11 Solanaceae species demonstrated the abaxial leaf surface has significantly higher tricome density with compared to the adaxialsurface (Watts & Kariyat, 2021b). The distribution of trichome density for both adaxial and abaxial leaf surfaces for the populations of different geographical sites (Figure 5).

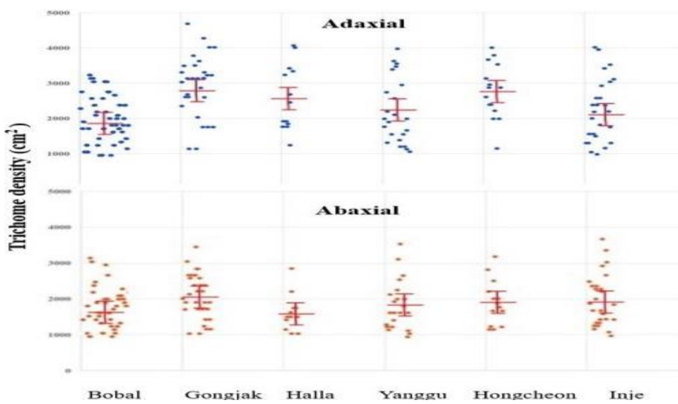

Figure 5. Variation of trichome density on the leaf surface (Adaxial and Abaxial) for the populations of different geographical sites.

3.3. Length and width variation among populations of different geographical regions

The longest trichome for the adaxial leaf surface was found in populations of Halla san (i.e., 33 µm), while the shortest trichome was found in populations of Hongcheon measuring 21.5 µm. On the other hand, the longest trichome for the abaxial leaf surface was measured in the populations of Halla san with a value of 61.1µm. In comparison, the shortest trichome in the abaxial leaf surface was found in the Hongcheon san geographical site populations with 39.5 µm. Likewise in the case of trichome width for the adaxial leaf surface widest trichomes were found in the populations of Halla san had possessed 12.2 µm and the narrowest trichome in adaxial leaf surface were measured 9.2 µm in the populations of Hongcheon. For the abaxial leaf surface, the widest trichome was found in the populations of Inje san at 15 µm while the narrowest trichome in the abaxial leaf surface was found in the populations of Hongcheon san measured at 9.2 µm. The length of trichomes was significantly higher in the abaxial leaf surface, while trichome width was not so different in the adaxial and abaxial leaf surfaces. Trichome dimensions especially density and length and width varied with location and across species (Watts & Kariyat, 2021a). The distribution of trichome length and width in adaxial and abaxial leaf surfaces was given in Figure 6 and 7, respectively.

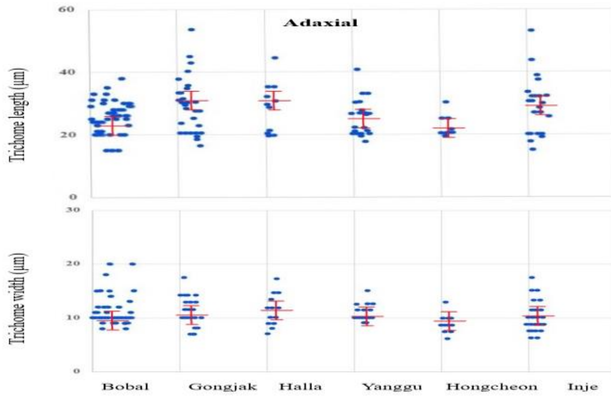

Figure 6. Trichome length and width (Adaxial surface) for populations of six different geographical regions

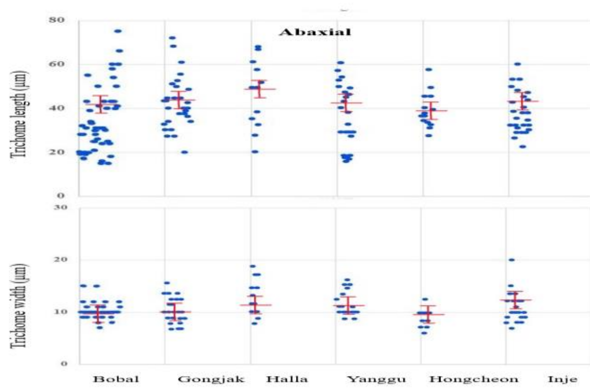

Figure 7. Trichome length and width (Abaxial surface) for populations of six different geographical regions

Table 2. Number of the observed trichomes on leaves of the studied plants from different populations

Plan Pt	Trichome density (cm^2)		Length (µm)		Width (µm)	
	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial
Bobal	1953.4±686.4^{d}	1723.8±554.2^{c}	23.6±4.0^{cd}	44.4±11.6^{c}	9.6±1.0^{cd}	9.7±1.0^{cd}
Gongjak	2884.8±890.6^{a}	2029.8±620.4^{a}	32.1±8.7^{ab}	44.7±11.6^{c}	10.8±2.2^{b}	10.6±2.0^{c}
Halla	2572.3±948.7^{bc}	1578.4±513.8^{d}	33.0±5.5^{a}	61.1±13.1^{a}	12.2±2.1^{a}	12.7±2.8^{b}
Yanggu	2185.0±876.0^{cd}	1745.6±665.7^{c}	24.7±7.0^{c}	48.7±10.2^{b}	10.5±0.7^{b}	10.5±0.7^{c}
Hongcheon	2788.9±810.9^{b}	1866.0±625.3^{b}	21.5±3.3^{d}	39.5±7.5^{d}	9.2±1.2^{d}	9.2±1.2^{d}
Inje	2167.4±924.0^{cd}	1963.3±740.1^{ab}	29.2±1.8^{b}	47.7±12.8^{b}	11.0±2.1^{ab}	15.0±3.5^{a}

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

3.4. Variation and relationship between B chromosome and trichome density

3.4.1. Variation in density due to chromosome number and B chromosome in *L. amabile*

Trichome densities were found varied in different plants which have possessed different chromosome number and B chromosome structure. The trichome density was highest on the adaxial surface of the leaves in 24+2B populations (i.e., 2919.09 µm) and lowest in the populations having chromosome number 24 (i.e., 2066.83 µm). On the abaxial surface of the leaves, the highest density of trichomes was found for the populations of 25+2b (i.e., 2327.50 µm), and the lowest was found in the population shaving chromosome number 24 (i.e., 1755.18 µm) (Figure 8). In this sort of study, the impact of chromosome/B chromosome in *L. amabile* first in its record, and variation was observed with the variation of chromosome number /B chromosome. The density of the plant trichomes decreases as leaf age increases (Adebooye et al., 2012).

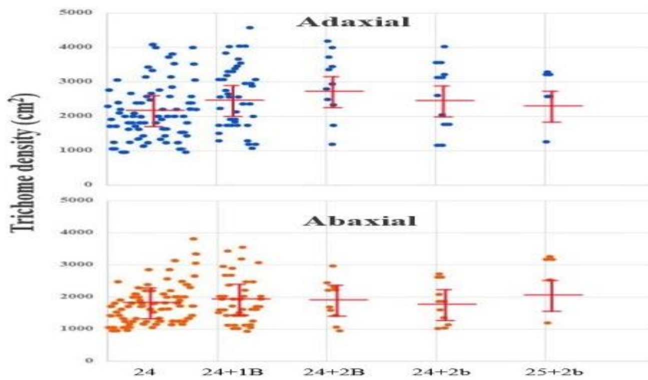

Figure 8. Trichome density distribution in plant populations having different chromosome/B chromosome

3.4.2. Variation in trichome length and width due to chromosome/B chromosome *in L. amabile*

The estimation of trichome length and width in plant populations having different chromosome/ B chromosomes (Table 3). For the adaxial surface of the leaves, the longest trichome was found in 24+2B populations (45.25 µm), while the shortest trichome was found in the populations of 25+2b (22.25 µm). Likewise, for the abaxial surface of the leaves, the longest trichome was found in the populations having chromosome number 24+2B (54 µm), while the shortest trichome was found in the populations of 25+2b (34.25 µm). Likewise, in the case of trichome width, in the adaxial surface of the leaves, the widest trichomes were found in the populations having chromosome number 24+2B (11.75 µm), and the narrowest trichome were found in the populations of 25+2b (9.25 µm). In the case of the abaxial surface of the leaves, the widest trichome was found in both the populations having chromosome 24 and 24+2B (11.75 µm for each), and the lowest trichome width was measured in the populations with chromosome number 24+2b (9 µm).

Table. 3. Number of the observed trichomes on leaves of the studied plants having different chromosome types

Plant	**Density (cm2)**		**Length (µm)**		**Width (µm)**	
	Adaxial	Abaxial	adaxial	abaxial	adaxial	abaxial
24	2066.83±814.98d	1755.18±596.97d	27.08±6.72c	50.83±12.10b	10.88±1.90ab	11.75±2.88a
24+1B	2554.44±899.93cd	1859.89±678.82cd	30.81±4.39b	47.94±13.31c	10.81±2.46ab	10.69±2.09b
24+2B	2919.09±931.86a	1900.00±606.81b	45.25±5.56a	54.00±4.69a	11.75±1.26a	11.75±1.26a
24+2b	2565.00±989.76c	1892.08±630.76bc	23.25±4.72cd	34.25±6.95d	10.25±0.50b	9.00±1.41d
25+2b	2739.17±775.09b	2327.50±673.76a	22.25±2.06d	39.25±14.52cd	9.25±0.96c	9.75±0.50c

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

4. Conclusions

This study examines variations in trichome density, length, width, and chromosome/B chromosome status in plant populations in *Lilium amabile Palibian* of different ecological-geographical regions viz. Bobal, Gongjak, Halla, Yanggu, Hongcheon, and Inje. The study showed significant variations in trichome density, length, width in six different geographical sites from where sample populations were drawn. Variations were also observed in trichome parameters (i.e. trichome density, trichome length, and trichome width) due to leaf surface i.e. Adaxial and Abaxial. Four distinct types of trichomes were identified namely, unicellular conical, cylindrical, glandular hair, and hooked hair. All leaves contained glandular and non-glandular trichomes, but the density and distribution varied. Changes in B-chromosomes in plants do not significantly affect the shape, size, and density of the plant trichome. The habitat factors on the population, such as elevation, sunlight, soil, and water supply, influence the density, shape, and size of trichomes. It is concluded that the changes in B-chromosomes in plants do not have a strong effect on the shape, size, and density of plant trichomes.

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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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