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Research article

Phenology of Wild and *In-vitro* Propagated Benguet Lily (*Lilium philippinense* Baker) Grown in the Greenhouse (BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad)

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Abstract

Benguet lily (*Lilium philippinense*) is an endemic lily found in the mountains of the Cordilleras with a high potential of being utilized as cut flower and as potted plant since it has a high resemblance to commercially produced liliums. During the past decade, however, its population continuously declined these prodded the conduct of studies aimed to conserve, domesticate and propagate the plant through tissue culture Unfortunately, the growth and development of Benguet Lily collected from the wild and those that were invitro propagated maintained under greenhouse condition has not been adequately described. The aim of the present study is to evaluate and compare the performance of the wild and the *in-vitro* propagated *Lilium philippinense* when both are grown in the greenhouse.

The wild and *in-vitro* propagated Benguet lily plants exhibited similar growth phases in the greenhouse. The major growth phases include shoot emergence, flower bud emergence, flower opening, capsule emergence, capsule maturation, dehiscence of seeds and plant senescence. The duration of the growth phases and corresponding climatic condition were different for the two types of plants.

Shoot emergence in both plant type coincides with a relatively cooler temperature and humid condition. More humid and warmer temperature favors flower bud emergence in wild plants while cooler and humid condition favors it *in-vitro* propagated plants. Flower opening of both plant type was favored by cold and more humid condition. Capsule emergence and maturation in wild plants is favored by comparably cold and humid condition while warmer and drier condition favors capsule emergence and maturation in *in-vitro* propagated plants. The differences observed in the transition and duration of each growth phase between appeared to be highly attributable to the difference in maturity of the planting material used. The high adaptability of *L. philippinense* to both field and greenhouse conditions points to a high potential for commercialization.

Keywords: Phenology; Benguet Lily; *Lilium philippinense*

Introduction

Lilium philippinense is a native plant of the mountains of Benguet and nearby Mountain Province, and was first chronicled in 1873. In the 1880's, it was reported that populations of this species occur in clusters of the hundreds. However, years later, the cluster had only 10 to 50 individuals or less [1]. The decline in the population of *L. philippinense* in the wild was affirmed by Madulid [2] and was attributed to overcollection of farmers and plant hobbyists.

In an effort to save and preserve this species, *in-vitro* propagation technique was developed by several researchers [3-6]. Unfortunately, the growth and development of Benguet Lily collected from the wild and those that were *in-vitro* propagated then maintained under greenhouse condition has not been adequately desribed. The aim of the present study is to evaluate and compare the performance of the wild and the *in-vitro* propagated *Lilium philippinense* when both are grown in the greenhouse.

Materials and Methods

Time and place of study

The experiment was conducted in May 2015 to June 2016 in the BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad (Figure 2 a and b). It is situated in the inner part of Benguet with geographical coordinates of 16o 27' 18" north longitude and 120o 35' 15" east latitude and an elevation range from 500 to 1700 m above sea level.

Plant selection and management

One hundred wild Benguet Lily plants at shoot emergence stage were dug-out from their natural habitat on the mountain sides of Moatong, Poblacion, Bokod. They were transplanted to 5 x 8 polybags containing Alnus compost as soil medium and were placed in the greenhouse at the BSU Floriculture Project Area. Likewise, one hundred *in-vitro* propagated plantlets were transplanted to polybags containing the same soil media. These plantlets were regenerated from bulbs that were collected from the natural habitat in Moatong, Poblacion, Bokod and cultured *in-vitro* from October 2014 to March 2015 using the modified *in-vitro* propagation protocol of Ayban [6]. The bagged plants (Figure 1 a and b) were maintained in the same greenhouse, and were irrigated three times a week at full saturation level while other cultural practices like weeding were done as needed.

Figure 1: Wild (a) and *In-vitro* propagated (b) *L. philippinense* in polybags and grown under greenhouse condition in the BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad.

Figure 2: Experimental set-up in BSU Floriculture Project Area at Cabanao, Poblacion, La Trinidad. (a) The greenhouse used in the study. (b) The experimental set up in the greenhouse.

Climatic data

Daily temperature and relative humidity were taken throughout the study using a generic weather forecaster with built in digital thermometer which records two readings, one at 6:00 in the morning and another at 3:00 in the afternoon. The average of the two readings was taken as the temperature and relative humidity for the day. The daily temperature and relative humidity were averaged to obtain the monthly average temperature and relative humidity, respectively. Daily photoperiod was obtained from the nearest PAG-ASA weather station.

Soil analysis

Soil samples in this study were obtained in the composite pile of Alnus compost used as a potting media before shoot emergence

and after plant senescence. Four samples were brought to the BSU Soil Science Service Laboratory for analysis.

Identification of phenological phases

One hundred samples each from wild plants and *in-vitro* propagated plants were observed for the highly distinguishable phenological phases using the modified phenological scale described by Meier [7]. A phase was said to begin when 10% of the sample plants are in that particular phase, while a phase is said to cease when 10% of the sample plants are still in that phase. The peak of a phenological phase is when 80% of the sample plants have reached the phase. The number of days to the different growth phases was determined using the start of shoot emergence as a point of reference.

The phenological phases in both wild and *in-vitro* propagated plants were plotted against the different climatic data obtained to generate the phenological calendar from shoot emergence during the current season to shoot emergence the following growing season.

Results

Climatic condition during the growing season

Daily temperature, relative humidity, and photoperiod were recorded and averaged monthly from May 2015 to June 2016 (Figure 3 and 4) while data from January to April was obtained from the PAG-ASA BSU Weather Station. The lowest temperature (16.230C) was recorded in February 2016 and warmest (22.130C) in April 2016. The highest relative humidity of 93.7% was observed in August 2015 (Figure 3) while the lowest of 73.59% was in April 2016. The shortest photoperiod was in December, 11 hours 9 min. while the longest was in June 2015, 13 hours 3 minutes (Figure 4).

Figure 3: Temperature and relative humidity obtained under greenhouse condition in BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad from January 2015 to June 2016 with January to April 2015 as secondary data from PAG-ASA BSU Weather Station.

Figure 4: Photoperiod recorded under greenhouse condition in BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad from January 2015 to June 2016 with January to April 2015 as secondary data from PAG-ASA BSU Weather Station.

La Trinidad belongs under the Type I climate with a distinct wet and dry season.

Dry season is from November to April while the wet season starts from May to October. The average temperature ranges from 11.7°C to 23.20C with an average daily temperature of 18.550C. In the month of December however, temperature dropped to as low as 70C while the warmest can be as high as 23.2°C during the months of March, April, and May. For the duration of the study, there were 198 rainy days with August and September being the rainiest months, while July and December were recorded as the most and least humid months, respectively (www.dilg.car).

Greenhouse cultivation is advantageous because it helps to maintain an optimal plant growth environment and protects the crops from varying outdoor conditions.

However, the values of temperature and relative humidity inside the greenhouse covered with diffusive plastic were very similar to outside conditions, reaching small differences, on average. 1.80C above the outside temperature ad 4.4% lower than external pH [8].

Soil analysis

Analysis of the Alnus compost used as the bagging medium was done at the Department of Soil Science Service Laboratory of Benguet State University. Results of the analysis are presented in Table 1. After the experiment, the soil became slightly acidic (lower pH) while organic matter and available N were significantly reduced. P level was almost unchanged while K increased slightly. The pH levels recorded at the beginning and end of the study fall within the ranges for both Asiatic and longiflorum hybrid groups [9].

	Soil Ph	O.M (%)	N (ppm)	P (ppm)	K (ppm)
Start of the study	6.83	1.66	83	20	73
End of the study	6.48	0.11	50	18	8.8

Table 1: Analysis of Alnus compost used as a bagging medium for plants grown under greenhouse condition.

Phenology, growth and development of transplanted wild and *in-vitro* propagated *Lilium philippinense* in greenhouse condition

The duration in the greenhouse of the different growth phases observed in transplanted wild and acclimatized *in-vitro* propagated *L philippinense* plants are presented in Figures 7 and 8. The percentage of plants under the different growth phases is presented in Figure 9. The growth phases observed were (a) shoot emergence, (b) flower bud emergence, (c) flower opening, (d) capsule emergence, (e) capsule maturity, and (f) senescence. Furthermore, differences in the duration and climatic condition of the different growth phases were also observed.

Emergence of the Shoot/New Leaf

Shoots of transplanted wild *L. philippinense* emerged in the fourth week of May or 21 DAT. At this time, temperature, relative humidity, and photoperiod were at 19.2^{0}C, 85.2% and 12H 55 minutes, respectively. After 42 DAT, 68% of the plant population had initiated new leaves and the growth phase lasted until the 56th DAT. In contrast, the shoots of *in-vitro* propagated plants emerged one week later at 28th DAT. On the fourth week of July, 72% of the *in-vitro* propagated plants had their leaves unfurled.

Flower bud emergence and development

The flower bud of the wild *L. philippinense* (Figure 5a) was observed on the third week of June, 35 days after shoot emergence (DASE), during which the average temperature obtained was 20.3^{0}C and relative humidity was 84.6%. By 63 DASE, all the transplanted wild *L. philippinense* had initiated flower buds.

The *in-vitro* propagated plants, on the other hand, took a longer time to change phase. Flower buds (Figure 5b) started to emerge on the third week of October or 154 DASE, during which the temperature, relative humidity, and photoperiod had decreased to 18.7^{0}C, 87% and 11 hours 55 minutes. As temperature, relative humidity and photoperiod gradually decreased in the second week of January or 231 DASE, more *in-vitro* propagated plants initiated flower buds. The slowest plants to develop had their flower buds out by 280 DASE at a temperature of 16.5^{0}C, relative humidity of 74.8% and photoperiod 11 hours 10 minutes.

The mature flower buds of wild plants (Figure 5c) open on the second week of July, 21 days after flower bud emergence (DAFBE) where temperature and relative humidity was 89.7% and 19.4^{0}C, respectively. More matured flower buds opened at more humid condition of 94.3% during the second week of August which was 42 DAFBE. Flower opening in wild plants ended at 77 DAFBE (Figure 5e).

Unlike in wild plants, mature flower buds of *in-vitro* propagated plants (Figure 5d) opened on the third week of November (21 DAFBE) where the temperature and relative humidity was 18.7^{0}C and 87%, respectively. More flower buds in *in-vitro* propagated bloomed on the third week of January where temperature further decreased to 17.6^{0}C and relative humidity to 80.7% respectively. Flowers of the remainder of the population opened at 147 DAFBE (Figure 5f).

Figure 5: Flowering phase of wild and *in-vitro* propagated *L. philippinense* under greenhouse condition in BSU Floriculture Project Area in Cabanao, Poblacion, La Trinidad. Flower bud emergence (a and b), Mature flower bud (c and d) and Flower opening (e and f).

Capsule emergence and development

Capsule emergence occurs when tepals wilt and fall off. For the wild *L. philippinense*, initial observations of the flower wilting which marks the start of capsule emergence were on the first week of August, and ended on the fourth week of August at comparable climatic condition to that obtained during the flower opening phase (Figure 6a). On the other hand, tepals of *in-vitro* propagated *L. philippinense* started to fall off during the first week of November in which the temperature and relative humidity were down to 18.6^{0}C and 83.4%, respectively (Figure 6b). More immature capsules were observed during the second week of February when the temperature was at its coldest at 16.7^{0}C and relative humidity decreased to 75. 8%. As the environment got warmer on the second week of April or 329 DASE, fewer immature capsules were observed.

The onset of capsule maturation in both wild and *in-vitro* propagated Benguet Lily is marked by the change of color from dark green to lighter green and eventually to light brown. Initial capsule maturation in wild *L. philippinense* (Figure 6c) was observed 105 DASE during which the recorded average temperature was 19.70C, relative humidity of 90.7% and photoperiod at 12 hours 25 min. respectively. Capsule maturation peaked during the second week of October where 70% of the remaining plant populations had reached maturity. At full plant maturity, the capsule splits and releases its seeds while the other upper plant parts wither, marking the beginning of the senescence stage at 140 DASE. On the other hand, the *in-vitro* propagated *L. philippinense* capsules was observed to mature 238 DASE on the third week of January when temperature and relative humidity decreased, and photoperiod increased (Figure 6d). As the temperature increases during the second week of April to 22^{0}C and relative humidity decreases to 73.9%, the remaining immature capsules of *in-vitro* propagated matured after 350 DASE.

Figure 6: Capsule emergence and maturation phase in wild (a-c) and in-vitro propagated (b-d) L. philippinense grown under greenhouse condition in BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad, Benguet. Capsule emergence (a-b) and Capsule Maturation (c-d).

Figure 7: Phenological calendar of wild and *in-vitro* propagated *L. philippinense* under greenhouse condition in BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad with the corresponding climatic condition. Temperature (a) and Relative humidity (b) S.E- Shoot Emergence; FBE- Flower Bud Emergence; FO- Flower Opening: CE-Capsule.
Emergence; CM – Capsule Maturation; SD Dehiscence of Seeds
D- Dormancy

Figure 8: Phenological calendar of wild and *in-vitro* propagated *L. philippinense* under greenhouse condition in BSU Floriculture Project Area, Cabanao, Poblacion, La Trinidad with the corresponding photoperiod. S.E- Shoot Emergence; FBE- Flower Bud Emergence; F- Flower Opening: CE-Capsule Emergence; CM – Capsule Maturation; SD- Dehiscence of Seed; D- Dormancy.

Figure 9: Percentage of plants in the different growth phases at the indicated time and temperature under greenhouse condition i n BSU Floriculture Project Area in Cabanao, Poblacion, La Trinidad. Wild (a) and *In-vitro* propagated (b) *L. philippinense*.

For both plants at capsule maturity, the different plant parts start to wither, and the capsule opens and dehisces the seeds. At this point, the bulb enters the dormancy phase until the next growing season.

Discussion

In general, La Trinidad has a cold weather condition with relatively low temperatures reaching at a mean average of 15^0C. The average minimum temperature for the last 5 years is at 14.68^0C and the maximum temperature averaged to 23.6^0C (latrinidad.gov.ph). During the time of the study, the months of January, February until the first week of March were the coldest, and the warmest were the last week of March and April. La Trinidad falls under Type I climate condition where the rainy season is from May to October and the dry season is from November to April. Thus, highest relative humidity was observed during the months of August with a monthly average of 93% and the lowest of 84% observed during the month of April. For photoperiod, longest day was observed during the month of June while the shortest was observed during the month of December.

In this study, the environmental condition inside the greenhouse from May 2015 to June 2016 was monitored daily and averaged to obtain the monthly climatic condition. There was little variation between internal and external conditions during the study. Similar to the external climatic condition, the lowest temperature was obtained in February with an average of 16.230C and the highest temperature was in April with an average of 230C. De Andrade., *et al*. [10] reported that climatic conditions inside the greenhouse were 8.5% higher than the external climatic condition. In addition, Boueri and Lunardi [11] reported that the temperature in a protected environment is 17% or 3.30C higher than the outside environment, except for relative humidity, which is lower inside the greenhouse than outside. These variations are mainly dependent on the type of ventilation that is present in the facility.

On the other hand, the lowest relative humidity was obtained in April 2016 with an average of 73.59% and the highest was in August 2015 with an average of 87.8%. The longest day was in June 2015 with an average of 13 hours and 3 minutes and shortest day was in December with an average of 11 hours 9 minutes.

The Alnus compost used in the study compares to the recommended soil for growing commercial *Lilium* species - highly porous with a good water-holding capacity and pH of 6 to 6.5 [12]. At the start of the experiment, the soil pH was 6.83 (slightly acidic), with 1.66% of organic matter, 83 ppm of available nitrogen, 20 ppm of phosphorus and 73 ppm of potassium. After the study, a significant decrease in the soil pH to 6.48, the organic matter content to 0.11% and the available NPK decreased to 50 ppm, 1.8 ppm and 8.8 ppm, respectively.

Transplanted wild and *in-vitro* propagated *L. philippinense* showed similar growth phases, wild *L. philippinense* in the greenhouse developed earlier compared to *in-vitro* propagated plants. Wild plants were observed to be well adapted to the cooler temperature (19.2^0C) and more humid condition (90% RH) in the greenhouse. This high adaptability of *L. philippinense* to both field and greenhouse conditions points to a high potential for commercialization.

Other factors that affect the growth and development of plants in the greenhouse include temperature, specifically that to which dormant bulbs are exposed and initial bulb weight. Flowering in most Liliums is highly dependent on the exposure of dormant bulbs to low temperature to break dormancy and promote flower initiation [13,14]. Okubo., *et al*. [15] reported that in *Lilium formosanum* and *Lilium brownii* var. *colchesterii* bulbs that were

stored at 10^0C for 4, 6, and 8 weeks and grown in a greenhouse at 12^0C, flowered after 170, 138, and 144 days while untreated bulbs did not flower.

For wild plants grown in the greenhouse, exposure of bulbs to low temperature occurred in the natural habitat during the months of January to February which was four months before they were collected. During that time, average temperature recorded was 19.2^0C in January and 18.3^0C in February, which was low enough to break the dormancy and promote flowering in the wild plants. Transplanted wild plants developed flower buds and flowered earlier compared to *in-vitro* propagated plants wherein both coincided with humid condition.

In-vitro propagated plants had a longer period of growth and development than the wild plants. This is closely attributed to the juvenile state of *in-vitro* propagated bulblets. Lian., *et al*. [16] reported that when directly planted, small bulblets produced *in-vitro* emerged slowly, were ununiform, and had low survival.

Heavier bulbs tend to produce plants that grow faster and develop earlier than smaller sized bulbs. Langens-Gerrits., *et al*. [17] specifically mention sufficiently larger bulbs > 300 mg sprouts with a stem instead of a rosette plant. Today, *in-vitro* bulbs are considered juvenile bulblets that will flower after two to three vegetative growing cycles in the soil, except for *Lilium longiflorum* which flowers in the first growing season after tissue culture [17,18].

Mojtahedi., *et al*. [19] observed a longer developmental phase in *in-vitro* propagated *L. longiflorum* cv. "Ceb-dazzle" which flowered after 16 months. Misra and Datta [20] observed the same for in-vitro propagated Asiatic hybrid plantlets that were planted in pots and maintained under field condition. They first formed a rosette of leaves for six months, then the stem became visible only after nearly 16 to 18 months. In addition, *in-vitro* propagated *Allium sativum* was observed to have longer vegetative development during the first growing season [21-23].

Conclusion

Wild and *in-vitro* propagated *L. philippinense* exhibited similar growth phase but differs in the duration of each phase. The growth phases observed were shoot emergence, flower bud emergence, flower opening, capsule emergence, capsule maturation, dehiscence of seeds, dormancy and shoot emergence for the following growing year. In terms of duration of the growth phase, *in-vitro* propagated plants have a longer transition to each phase compared to wild plants that develops earlier.

Shoot emergence in both coincide with a relatively cold warm temperature and humid condition while in flower bud emergence, wild *L. philippinense* was favored by warmer and humid condition while in *in-vitro* propagated plants it was favored by humid condition but at a colder condition. Flower opening of both plant type was favored by colder and more humid condition compared during flower bud emergence phase. Capsule emergence and maturation of both occurred at lower climatic condition compared to that obtained during the flower opening.

Results provided a baseline information on the phenology of wild and *in-vitro* propagated plants under greenhouse condition. Notably, high adaptability of *L. philippinense* to diverse conditions points to a high potential for commercialization.

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