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Cultivation and Propagation of *Iris laevigata* Fisch., an Endangered Ethno-medicinal Plant of Imphal Valley Manipur, India

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Abstract

Iris laevigata Fisch., is restricted geographically in Manipur, north eastern India. An experiment was planned with Factorial Randomized Block Design during 2018 to 2020 (3 seasons) in the study plots of ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat. The study encompasses on the growth and development of *I. laevigata* seedlings collected from Ipa Thoukok Complex: influenced by spacing, trimming and correlations amongst the growth parameters, so as to validate the most favourable conservation method for this endangered plant. Among the treatments, $T_6 = S_2T_3$ (45 cm × trimming 40 DAT) was found the best treatment. $T_9 = S_3T_3$ (60 cm × trimming 40 DAT) was second, $T_3 = S_1T_3$ (30 cm × trimming 40 DAT) was the third and lowest was found in case of $T_1 = S_1T_1$ (30 cm × 0 trimming, *i.e.*, without trimming). Strong positive correlation between leaf surface area and plant height on the growth and development was found to be effective among other correlations.

Keywords: *Iris laevigata* Fisch., endangered, growth and development, correlation, soil nutrient, water quality and depth

1. Introduction

Water Iris Plant: *Iris laevigata* Fisch., belongs to the family Iridaceae, grows in moist habitats like marshy grassy slopes, meadowlands, bogs and lakes. The plant is commonly known as Water Iris, *Kombirei* in Manipuri, Japanese iris, rabbit-ear iris, or shallow-flowered iris, is native to Japan. Water Iris is distributed from Assam to Myanmar. In Manipur the plant grows naturally in Lamphel pat, Yaral pat, Loktak Lake, etc., as an endemic water plant presently in the endangered status under the RET (Rare Endangered and Threatened) category. The genus Iris comprises more than 300 herbaceous species, growing in the temperate regions of the northern hemisphere (Rodionenko, 1987).

Regarding *Iris* species, Kostrakiewicz (2000) studied the analysis of spatial horizontal structure of *I. sibirica* L. population on the station in Stanisławice near Bochnia. The studied species growing in wet habitats belongs to rare, legal protected plants in Poland. The distribution of the individuals within all phytocenosis as well as on 100 m^2 selected area was presented.

Earlier in Manipur, *I. laevigata* was wrongly identified by some researchers as *Iris bakeri* Wall. (Deb, 1961a, 1961b; Sinha, 1996). However, one scientist H. B. Singh identified *Kombirei* after confirmation from RBG, Kew (Sobhapati, 2017). Four species of *Iris* were reported in Manipur. Unfortunately this beautiful flower, which has very close connections with the traditions of Manipuris since time immemorial, is facing a great threat due to lack of attention. Height is the major distinct differences between *I. laevigata* (106.68 cm) and *I. singuinea* (60.96 cm) besides their leaves. *Singuinea* has no straight leaves unlike *Laevigata*. Even though other species such as *I. wattii*, *I. singuinea*, *I. kumaon* grow wild in the State, this flower grows only in the wetlands of Lamphelpat and Loktak Lake. Interestingly, *I. singuinea* which is used as real *Kombirei* (*I. laevigata* Fisch.) during annual *Cheiraoba* festival in April is being cultivated in private nurseries in view of the huge public demand (Sobhapati, 2017).

Lee et al. (2018) reported the effect of water levels and soil nutrients on the growth of endangered *I. laevigata* seedlings in Korea. The study was conducted to examine the effects of environmental conditions such as water levels and soil nutrient conditions on the growth and survival of *I. laevigata* seedlings. Complete submergence lowered the total number of leaves, biomass, and survival rates. A rise in soil nutrients increased overall seedling growth and increased tiller numbers via the promotion of asexual reproduction. The water level must be lower than the seedling height for effective growth and management of *I. laevigata*.

Earlier, water Iris grows naturally, when the monsoon comes in Manipur during March-April, however, without people's care these plants could complete its mature stage and blooms flower. One can understand that *Kombirei* will be available in the marshy areas, when we need the flower. But nowadays, because of urbanization, drying up of lakes, expansion of agricultural lands, etc., it is high time to take care and artificial human protection and conservation of the plant.

In this regard, an understanding of the life cycle of a plant, such as the method of reproduction, seed dispersion, germination, and survival conditions of seedlings is essential for the stable establishment of the species (Mahoney & Rood, 1998). Here it can be mentioned that, water level is the most important factor affecting seedling survival and the establishment of submerged plants (Nicol & Ganf, 2000; Fraser & Karnezis, 2005; Kwon et al., 2007; Casanova & Brock, 2000).

From China, Wang et al. (2017) observed the plant growth parameters between reproductive ramets and non-reproductive ramets and reported that leaves of reproductive ramets stopped growing around the time that flowering began (in late May), while the leaves of non-reproductive ramets would continue growing until late August, dramatically increasing the leaf area available for photosynthesis.

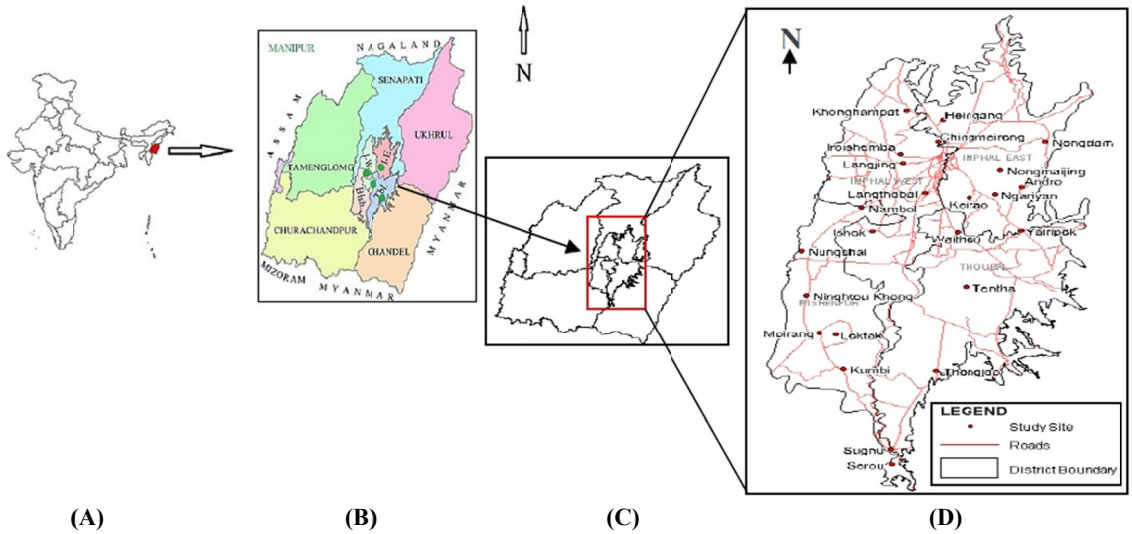
In Manipur, there is continuous exploitation, habitat-degradation, unsustainable harvesting and over-exploitation bringing substantial loss of the habitat of *I. laevigata*, is still going on. Most problematic one is during the annual *Cheiraoba* festival, *i.e.*, Annual New Year, which falls during the month of April, two *Cheiraoba* festivals are being observed: one on the day of *Shajibu nongma panba* for Meiteis, and another one, as *Charak puja* observed by the *Meitei* following Hinduism religion. On these two days *Kombirei* flower is being offered to the God as one of the most important constituents. On this day people purchase from the local market at Rs. 10 to 50 per bunch of *Kombirei* flower. People use to over harvest from the marshy natural home of *Kombirei* or from the market.

Some researchers from Manipur State worked on the distribution and mapping of some endangered plants and their status under the RET category (Devi & Das, 2016; Singh et al., 2017, 2020; Devi et al., 2021). So far, no scientific work has been done regarding *I. laevigata*, which is endangered plant under the RET category. Here, we focused on the growth and development of *I. laevigata* seedlings with specific objectives of this paper as follows: (1) influenced by spacing, (2) trimming and (3) correlations amongst the growth parameters of the plant, so as to validate the most favourable conservation method for this endangered plant.

2. Materials and Methods

2.1 Study Site

The present work has been taken up to cultivate Water Iris plant, which is under the RET category, so as to conserve the plant species by following modern tools of agricultural practices under ICAR, Lamphelpat, Imphal; field condition of Ipa Thoukok Complex (Figure 1).



(A) **(B)** **(C)** **(D)**

Figure 1. Study area: (A) Map of India showing the location of Manipur; (B) Map of Manipur showing districts; (C) Map of Manipur showing the location of Imphal valley; (D) Map of Imphal valley and study sites ICAR, Lamphelpat

2.2 Experimental Design

Factorial Randomized Block Design was made with a Plot Size of 4.0 m × 5.0 m. The Layout Plan for the cultivation and propagation of *Iris laevigata* Fisch., was done during 2018 to 2020 (3 seasons) in the study plots of ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal. Treatments: Two factors: Factor 1: Spacing 3 levels (S_1 = 30 cm, S_2 = 45 cm, S_3 = 60 cm); Factor 2: Trimming 3 levels (T_1 = 0 trimming, T_2 = trimming 20 DAT (Days after Transplantation), T_3 = trimming 40 DAT) (Table 1).

Table 1. Layout Plan for the Cultivation and Propagation of *Iris laevigata* Fisch

Plot Size: 4.0 m × 5.0 m

Design: Factorial Randomized Block Design

Treatment:

Factor 1: Spacing 3 levels: (S_1 = 30 cm, S_2 = 45 cm, S_3 = 60 cm);

Factor 2: Trimming 3 levels: (T_1 = 0 trimming, T_2 = trimming 20 DAT, T_3 = trimming 40 DAT)

Rows (R)			Treatments
R_1	**R_2**	**R_3**	
T_5	T_6	T_3	$T_1 = S_1T_1$ (30 cm × 0 trimming)
T_3	T_4	T_5	$T_2 = S_1T_2$ (30 cm × trimming 20 DAT)
T_9	T_8	T_7	$T_3 = S_1T_3$ (30 cm × trimming 40 DAT)
T_6	T_9	T_6	$T_4 = S_2T_1$ (45 cm × 0 trimming)
T_1	T_1	T_1	$T_5 = S_2T_2$ (45 cm × trimming 20 DAT)
T_4	T_7	T_4	$T_6 = S_2T_3$ (45 cm × trimming 40 DAT)
T_8	T_5	T_2	$T_7 = S_3T_1$ (60 cm × 0 trimming)
T_2	T_3	T_9	$T_8 = S_3T_2$ (60 cm × trimming 20 DAT)
T_7	T_2	T_8	$T_9 = S_3T_3$ (60 cm × trimming 40 DAT)

2.3 Physico-chemical Soil and Water Composition

Soil and water samples of the experimental plots were analyzed at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal.

2.4 Meteorological Data

Meteorological Data of the experimental farm was recorded from ICAR Complex during the tenure of the research program. Soil and water analysis of the experimental farms were analysed at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal. In Manipur there are four marked seasons namely, spring (March-May), rainy (June-Aug.), summer (Sept.-Nov.) and winter (Dec.-Feb.); because of the fluctuation and changing environment, each and every seasons overlapping each other (Figure 2).

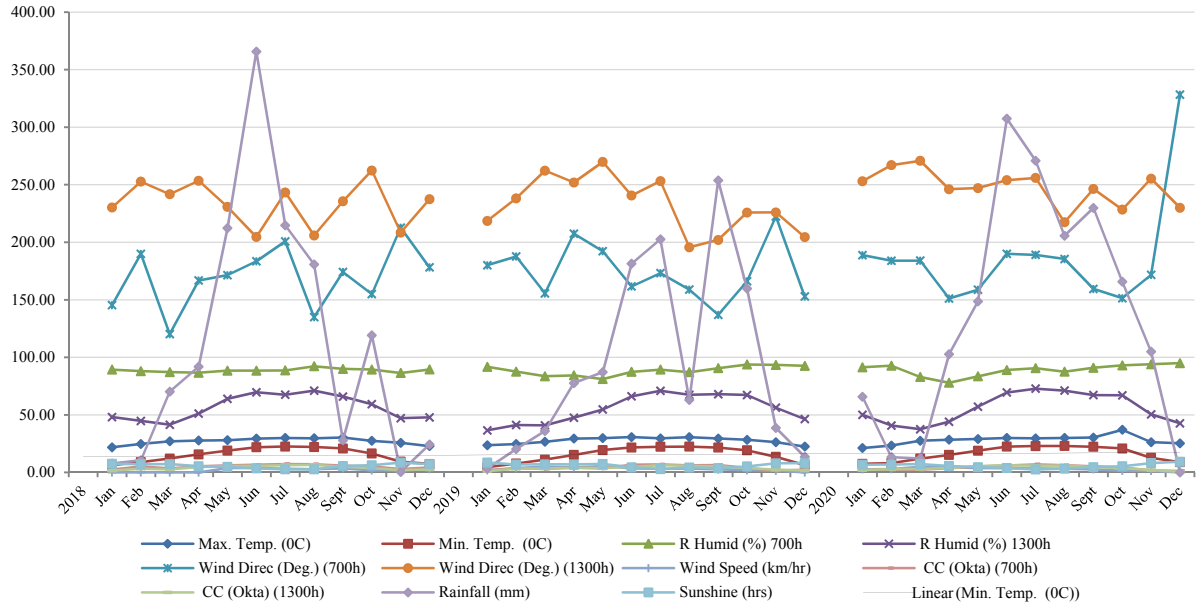


Figure 2. Weather data of *Iris laevigata* Fisch., experimental farm at ICAR Research Complex, Lamphelpat, Imphal from 2018 to 2020

2.5 Plant Growth Parameters

Plantation of water iris was done in the month of July, trimming started after 20 and 40 DAT, avoid cutting of leaves completely as the rhizomes will need some foliage to collect nutrients and recover from the transplant shock. The leaf will develop erectly after the transplantation in the field. The leaf surface area, plant height, number of leaves, number of plants per clump, number of flowers per pike, number of rhizomes per clump, etc., will be measured. The correlations between plant height and leaf surface area and other growth parameters will be calculated. Flowering will be during the month of March to May and harvesting follows.

2.6 Statistical Anlysis

All the analyses were carried out using SPSS 22.0 for Windows (SPSS, Inc., Chicago, Illinois, USA). The experiment was laid out under factorial randomized block design. The data were analyzed by Fisher's analysis of variance (ANOVA) technique and then results were interpreted.

3. Results and Discussion

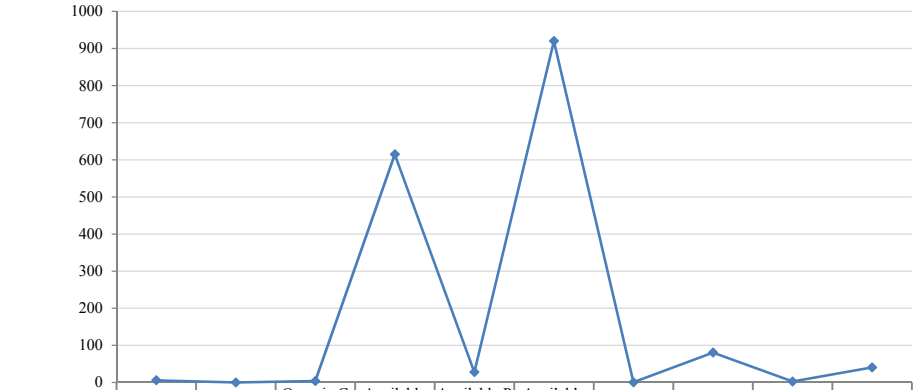
In Table 2, among the treatments, $T_6 = S_2T_3$ (45 cm × trimming 40 DAT) was found the best treatment. $T_9 = S_3T_3$ (60 cm × trimming 40 DAT) was second, $T_3 = S_1T_3$ (30 cm × trimming 40 DAT) was the third and lowest was found in case of $T_1 = S_1T_1$ (30 cm × 0 trimming, *i.e.*, without trimming). From the results it can be concluded that, in water iris cultivation spacing of 45 cm with trimming at 40 DAT treatment T_6 was the best and whereas, lowest value of treatment T_1 in which 30 cm spacing without trimming is unfavorable for the growth of water iris. The plant can grow luxuriantly in the ICAR Experimental Farm, Lamphelpat with the Treatment No. $T_6 = S_2T_3$ (45 cm × trimming 40 DAT) (Table 2).

Soil pH value was 5.8; E.C. was 0.048; organic carbon was 3.76%; available N, P and K were found to be 615, 28 and 920 kg/ha respectively. Some micronutrients *viz.*, Cu, Fe, Zn and Mn ranges from 0.4, 80.4, 2.5 and 40.50 mg/kg respectively. Water pH value was 7.26; E.C. was 0.48; P and K ranges from 0.014 and 14.7 ppm respectively.

Table 2. Influence of spacing and trimming on growth and development of *Iris laevigata* Fisch.

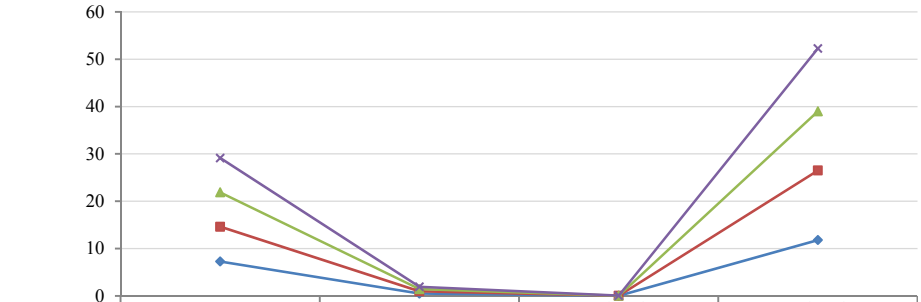
Treatment	Plant height (cm)	No. of leaves/ plant	No. of plants/ clump	Days to spike emergence	Days to flower initiation	No. of spikes/ plant	No. of flowers/ spike	Spike length (cm)	No. of rhizomes/ clump	Weight of rhizome (g)
Year (Y)										
Y_1-2018	56.207	15.488	6.363	247.934	9.635	3.302	3.180	48.474	6.363	94.074
Y_2-2019	56.905	15.423	7.626	247.749	9.693	3.556	3.780	55.327	7.626	99.249
Y_3-2020	58.329	17.643	8.437	249.737	9.869	4.129	3.375	54.564	8.437	100.881
S.E(m)±	**1.510**	0.364	0.201	1.427	0.286	0.133	0.114	0.867	0.201	1.873
C.D. at 5%	**NS**	1.036	0.573	**NS**	**NS**	0.379	0.325	2.464	0.573	**NS**
Spacing (S)										
S_1(30 cm)	48.170	12.509	5.437	253.883	10.129	3.410	3.282	48.774	5.437	79.241
S_2(45 cm)	60.659	16.762	6.941	243.983	9.251	3.854	3.411	54.417	6.941	103.725
S_3(60 cm)	62.613	19.284	10.048	247.553	9.817	3.723	3.640	55.173	10.048	111.237
S.E(m)±	1.510	0.364	0.201	1.427	0.286	0.133	0.114	0.867	0.201	1.873
C.D. at 5%	4.294	1.036	0.573	4.059	**NS**	**NS**	**NS**	2.464	0.573	5.327
Trimming (T)										
T_1(No trimming)	51.936	14.011	6.578	259.572	10.907	3.011	2.961	47.983	6.578	92.442
T_2(20 DAT)	56.864	15.961	7.400	246.933	9.503	3.634	3.492	51.902	7.400	97.926
T_3(40 DAT)	62.641	18.582	8.448	238.913	8.788	4.341	3.881	58.480	8.448	103.836
S.E(m)±	1.510	0.364	0.201	1.427	0.286	0.133	0.114	0.867	0.201	1.873
C.D. at 5%	4.294	1.036	0.573	4.059	0.813	0.379	0.325	2.464	0.573	5.327
Interaction (SXT)										
S_1T_1	43.179	11.411	4.956	266.103	11.803	2.800	2.609	42.799	4.956	70.970
S_1T_2	47.813	12.467	5.511	250.677	9.807	3.460	3.429	48.851	5.511	79.476
S_1T_3	53.517	13.650	5.844	244.869	8.778	3.971	3.809	54.673	5.844	87.279
S_2T_1	53.839	13.258	5.822	259.699	10.574	3.058	2.886	48.799	5.822	99.169
S_2T_2	59.770	16.163	6.644	240.660	8.757	3.709	3.382	53.071	6.644	103.940
S_2T_3	68.368	20.864	8.356	231.591	8.422	4.794	3.967	61.382	8.356	108.066
S_3T_1	58.791	17.365	8.956	252.914	10.343	3.177	3.388	52.350	8.956	107.187
S_3T_2	63.009	19.253	10.044	249.463	9.944	3.733	3.666	53.783	10.044	110.361
S_3T_3	66.039	21.233	11.144	240.280	9.163	4.258	3.868	59.386	11.144	116.164
S.E(m)±	2.616	0.631	0.349	2.472	0.495	0.231	0.198	1.501	0.349	3.245
C.D. at 5%	**NS**	1.794	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
C.V	**13.731**	11.693	14.002	2.985	15.262	18.889	17.221	8.531	14.002	9.926

Soil and Water samples for the cultivation of water iris should be maintained as follows: Soil pH value at 5.8; E.C. at 0.048; organic carbon 3.76%; available N, P and K should be maintained as 615, 28 and 920 kg/ha respectively. Some micronutrients *viz*., Cu, Fe, Zn and Mn required from 0.4, 80.4, 2.5 and 40.50 mg/kg respectively. Water pH value should be maintained at 7.26; E.C. at 0.48; P and K should be maintained from 0.014 and 14.7 ppm respectively. Some micronutrients like, Cu, Fe, Zn and Mn content in water samples were found to be in trace amounts. In some places where water iris could not grow were low water level, eutrophic condition, alkaline pH of water and soil; and low nutrients (Figures 3 and 4).



	pH	E.C.	Organic C (%)	Available N (Kg/ha)	Available P (Kg/ha)	Available K (Kg/ha)	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn(mg/kg)
Series 1	5.8	0.048	3.76	615	28	920	0.4	80.4	2.5	40.5

Figure 3. Soil analysis of the Experimental Fields of *Iris laevigata* Fisch. Lamphelpat



	pH	E.C.	P (ppm)	K (ppm)
Mean	7.26	0.48	0.01	13.3
3	7.25	0.47	0.01	12.5
2	7.36	0.48	0.014	14.7
1	7.26	0.49	0.008	11.8

Figure 4. Water analysis of the Experimental Fields of *Iris laevigata* Fisch., Lamphelpat

As water iris is an aquatic plant with almost all the aerial half is above water in amphibious manner, maintenance of water level is mandatory for seedling growth. Submerged and flooding of water levels with eutrophic conditions are badly affected to the plant. So, any marshy and waterlogging areas are not suitable for Iris cultivation. Lacoul and Freedman (2006) supported this view and opined that "rapid seedling growth is required to quickly escape the stressful environment of submergence and low nutrient seedlings". Lee et al. (2018) reported the effect of water levels and soil nutrients on the growth of endangered *Iris laevigata* seedlings in Korea. A rise in soil nutrients increased overall seedling growth and increased tiller numbers via the promotion of asexual reproduction. The water level must be lower than the seedling height for effective growth and management of *I. laevigata*.

Environmental factors of Manipur State favours for the natural growth of water iris. The plant is a perennial plant with rhizomatous stalk keep drying as a dormant phase during winter (Dec.-Feb.), when monsoon (March-May) comes during the end of February month starts sprouting and has reached the mature stage and flowering begins during the first week of April and continues till May end. As there were fluctuations of meteorological data during the three crop seasons (2018 to 2020) of water iris maximum rainfall reach maximum during the month of July, however, in the year 2019 rainfall was meagre and attain maximum in October. Maximum and minimum temperatures also fluctuate every year. If so fluctuations could be seen during three crop seasons. Even though, the normal temperatures did not cross an average of 32 ^{o}C every year, the plant maintains its growth (Figure 2).

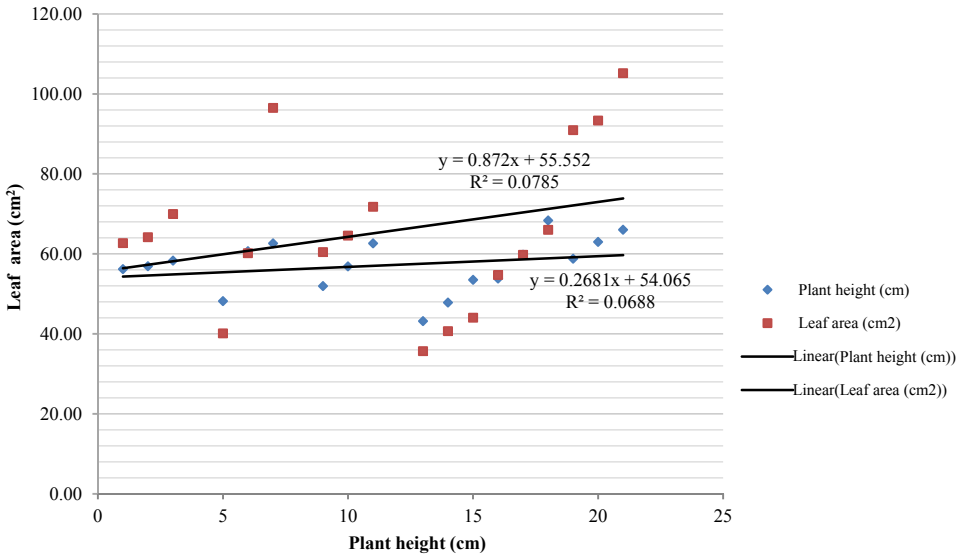


Figure 5. Correlation between leaf surface area and plant height on the growth and development of *Iris laevigata* Fisch.

Strong positive correlation between leaf surface area and plant height on the growth and development was found to be effective among other correlations (Figure 5). This work is in conformity with Wang et al. (2017), he reported that leaves of reproductive ramets stopped growing around the time that flowering began (in late May), while the leaves of non-reproductive ramets would continue growing until late August, dramatically increasing the leaf area available for photosynthesis.

4. Conclusion

From the above mentioned facts, scientific method of cultivation as an objective of conservation measures was done at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal. QPMs (Quality Planting Materials) were adopted in the present research program. Plant Growth Analysis and good harvesting techniques were also adopted to conserve the plant and the findings should be reached to the farmers. Water iris should be planted at a spacing of 45 cm during the month of July, trimming at 40 DAT (days after transplantation), flowering during March to May and harvesting of rhizomes followed.

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