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EFFECT OF STORAGE TEMPERATURE AND CULTIVARS ON SEED GERMINATION OF *Lilium*×*formolongi* HORT.

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Lilium×*formolongi* Hort.

Storage temperature

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Coefficient of the velocity of germination

ABSTRACT

An experiment was carried out to evaluate the influences of seed storage temperature and variation due to cultivars on the germination of the *Lilium*×*formolongi* hort. seeds. The seeds of four cultivars of *Lilium*×*formolongi* Hort. viz. Augusta, Raizan Herald (RH), Eorayeon 1ho, and Eorayeon 2ho was treated with four temperature ranges 5°C, -2°C, -20°C and 20°C (Room temperature, Control) up to 6 months to execute the experiment. In total 500 seeds of each cultivar including four temperature regimes and four replications for each temperature treatment and each replication represented by 30 seeds have been provided 10 days chilling treatment before to test the germination to get the uniformity in germination. The laboratory experiment was carried out in a completely randomized design (CRD) to assess the different parameters of germination viz. germination percentage (GP), mean germination time (MGT), germination index (GI), time taken to 50% germination (T50), and coefficient of the velocity of germination (CVG) at four temperature regime of four cultivars. The ANOVA analysis revealed significant variability among the four cultivars for all germination parameters in all temperature treatment levels. The results of the experiment demonstrated that the storage temperature has a great influence on germination percentage (GP), mean germination time (MGT), germination index (GI), time taken to 50% germination (T50), and coefficient of the velocity of germination (CVG) with comparisons to the normal temperature stored seeds (20°C). The significant variation among these parameters for each cultivar demonstrated the significance of varied requirements like stored temperature for the long term storage of that particular cultivar.

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1 Introduction

Germination is the first and most important stage in the plant life cycle when the growth of a new plant begins from a seed. The germination process which starting from imbibitions, activation, and succeeding manifestation lasts for the plant life cycle. In this context, seed germination can be defined as one of the most important and first stages of the plant growth cycle which denotes protrusion of the radicle from the tissue enclosing it (Bewley & Black, 2013; Baskin & Baskin, 2014). In other broader aspects; seed germination is a complex physiological process regulated by genetic and environmental factors including temperature, water potential, oxygen, light, and pH (Guo et al, 2020). Among the external factors affecting seed germination, the temperature is one of the primary important factors which play vital roles in affecting speed, rate, and percentage of germination (Marcos Filho, 2015).

The measurement of germination can provide valuable information about the percentage, rate, and time taken for germination of seeds, time taken for germination of 50% seeds, germination index, uniformity, and speed of germination. The germination rate provides the measure of the time course of seed germination. In other aspects, it is an estimate of the viability of seed or population of seeds. Another important aspect of seed germination is, of course, the rate of germination which is defined as the inverse of time to 50% germination (Bewley & Black, 2013) and it is usually time courses taken for the germination rate of 50% of total seed population usually denoted by T50. The mean germination time (MGT) is another way to calculate germination speed and which is nowadays widely used by a broad range of seed scientists and other researchers related to plant biologists (Soltani & Soltani, 2015). Likewise germination index and coefficient of the velocity of germination are other parameters deserving prime importance for the study of seed germination.

In east Asia especially in Japan and Korea, *Lilium × formolongi* Hort. is mainly seed propagated lily for cut flower production, seeds used to sow during winter (December to February) to get the flower in summer (July-August) (Ho et al., 2006, Xuan & Kim, 2014). The seeds usually harvested in the months of late September to early October have to store 3-4 months before to sow in the plastic house to prepare for transplanting in the main field in and around the third week of April (Rai et al., 2018). To keep the seed vigor constant without deteriorating the seed quality and germination percentage is a challenging job for the lily grower farmers. To understand the better storage temperature of seeds of *Lilium × formolongi* Hort., it is necessary to investigate a wide range of temperature which demonstrate higher germination percentage and constant the other biological aspects of seeds. In Japan and Korea, this type of seed propagated lilies is grown on a relatively small scale (Anderson et al., 2012; Kang et al., 2013). For this purpose up facing longiflorum hybrids viz. Augusta F1,

Raizan herald is developed by Daicxhii Japan (Lim & Van Tyul, 2007). Likewise, in Korea to fulfill the demand of seed market Eorayeon 1ho and Eorayeon 2ho were developed by KNU, a floricultural breeding laboratory(Xuan et al.,2009a; Xuan et al. 2009b). Seed propagated white trumpet lilies *Lx formolongi* Hort. were introduced for commercial cut flower production in the 1990s (Sato et al., 2010). Commercial *Lx formolongi* cultivars include Augusta F1, Raizan Herald (Sakigake Raizan)(Ho et al., 2006; Sato et al.,2010) while Eorayeon 1ho and Eorayeon 2ho were introduced in the first decade of the twenty-first century (Anderson et al., 2012). The objective of this experiment was to evaluate the effect of different temperatures on the seeds germination of four different cultivars of *L. ×formolongi* Hort.

2 Materials and Methods

2.1. Seeds materials and temperature treatment

The seeds of four cultivars of *Lilium × formolongi* Hort. viz. Augusta, Raizen Herald, Eorayeon 1ho, and Eorayeon 2ho were stored at different temperatures viz. -20°C, -2°C, 5°C, and 20°C (room temperature-Control) up to 6 months. After 6 months, treated seeds were surface sterilized with recommended fungicide Bhenoram @1g/L overnight separately covering the 500seeds of each cultivar in gauze. This was followed by the inoculation of these sterilized seeds on the wet filter containing petri dish, each petri dish has 30 seeds and each treatment replicated four times. After keeping the seeds, all Petri dishes were well labeled with cultivars' names, treatments, and replication accordingly. Then Petri dishes were kept inside the chamber maintaining temperature 5°C. After 10days of chilling treatment, observation on various germination parameters was recorded every day and with the help of forceps germinated seeds were discarded from the Petri dishes accordingly. After observation, distilled water was added over the tissue paper to maintain the moisture using the plastic bottle water sprayer.

2.1.1. Germination percentage (GP)

The germination percentage is a very usual term obtained by the number of germinated seeds divided by the total number of seeds and multiplied by 100. After 10 days of chilling treatment, the number of germinated seeds of each cultivar and temperature treatment was recorded every after 24h at morning 10 o'clock (AOSA, 1990). After 2 weeks of germination observation, the germination percentage was calculated by dividing the number of germinated seeds by the total number of seeds, multiplied by 100 (Tanveer et al., 2010; Cokkizgin & Cokkizgain, 2010).

$$GP = S/T \times 100$$

Where, GP=Germination percentage, S=Number of germinated seeds, T =Number of total seeds

2.1.2. Mean germination time (MGT)

It is one of the common method or ways to calculate germination speed, in this, the mean germination time (MGT) was calculated adopting the formula as given by Ellis & Roberts, 1980 as follows,

$$MGT = \sum (Dn) / \sum n$$

Where n stands for the number of seeds germinated each day and D for the day of counting.

2.1.3. Germination index (GI)

It emphasizes both germination speed and germination percentage. The GI was calculated using the formula given by the Association of Official Seed Analysis (AOSA, 1983) as follows;

$$GI = \sum (GT/Tt)$$

2.1.4. Time taken to 50% Germination (T50)

The time to reach 50% germination (T50) was estimated using the formula of Coolbear et al. (1984) and later on, modified by Farooq et al. (2005),

$$T50 = t_i + [(N/2 - n_i)(t_j - t_i)] / (n_j - n_i)$$

Where N is the number of final germination count and n_i , n_j cumulative number of seeds germinated at adjacent days t_i and t_j when $n_i < N/2 < n_j$

2.1.5. Coefficient of the velocity of germination (CVG)

The CVG indicates the rapidity of the germination of seeds. It increases when the number of germinated seeds increases and the time required for germination decreases. The CVG was estimated using the formula given by Maguire (1962) as,

$$CVG = \frac{(G_1 + G_2 + \dots + G_n)}{(1 \times G_1 + G_2 + \dots + n \times G_n)}$$

Where G stands for the number of germinated seeds and n stands for the last day of germination

2.2. Statistical Analyses

The raw data recorded were first arranged and prepared using MS-Excel 2013. Then the estimation of all parameters related to germination as mentioned from 2.2 to 2.6 was performed following the formula accordingly. The replication wise calculated mean value of all parameters viz. Germination percentage (GP), Mean germination time (MGT), Germination index (GI), Time taken to 50% germination (T50), and Coefficient of the velocity of germination (CVG) of each cultivar for different temperature treatment level viz. 20°C (normal temperature taken as control), 5°C,

-2°C and -20°C were used for the estimation of Analysis of Variance (ANOVA), F test adopting the TNAU-STAT- Statistical package (Manivannan, 2014) for the completely randomized design (CRD). The Duncan's Multiple Range Test has been estimated at the 5% level of significance for the separation of estimated means of the treatments using the TNAU-STAT- Statistical package (Manivannan, 2014)

3 Results

3.1 Germination percentage (GP)

The estimated germination percentage (GP) (Table 1) demonstrated that for the Augusta F1, the highest germination percentage (95.8%) was recorded for the seed stored at 5°C while the lowest germination percentage (7.5%) was recorded for seeds stored at 20°C. Moreover, the remaining temperature treatment i.e. -2°C and -20°C seeds have also possessed more than 80% GP. The GP of seeds stored at 20°C was recorded lower besides Eorayeon 1ho that has possessed 80% GP. The GP of seeds stored both at 5°C and -2°C were recorded more than 90% for all cultivars and more than 80% GP for seed stored at -20°C for 3 cultivars besides Eorayeon 2ho has possessed 75.8 GP.

3.2 Mean Germination Time (MGT)

In this experiment besides stored temperature, we have provided 10 days of chilling treatment of the seeds at 5°C then germination observation of the seeds was taken every day up to 2 weeks (14 days). The mean germination time (MGT) was found higher for the seed stored at 20°C (Control) ranging from 5.8 days (Eorayeon 1ho) to 12.4 days (Augusta) (Table 1). The temperature treatment of the seeds of all the four cultivars at 5°C, -2°C, and -20°C has significantly decreased the MGT. The lowest MGT (2.4 days) were found for Raizan Herald seeds stored at -20°C and thereby among their temperature treatment regime besides control highest MGT (5.5 days) were found for Augusta F1 seeds stored at -2°C. Among the four cultivars, Augusta F1 has demonstrated higher MGT for almost temperature range.

3.3 Germination index (GI)

It is one of the most comprehensive measurement parameters which comprises combining germination percentage and speed (spread, duration, and high/low events). The estimated GI (Table 1) demonstrated that normal temperature (control 20°C) was found lower than other temperature treatments for all cultivars. The GI was found lower for the seed stored at -2°C than the seeds stored at 5°C and -20°C for all cultivars. Among these 3 temperatures treated seeds lowest GI was recorded for Augusta F1 (6.8) at -2°C while the highest GI was recorded for Eorayeon 2ho (14.7) at 5°C.

Table 1 Effect of storage temperature and cultivars on various traits of seed germination (Seed germination percentage; Mean Germination Time; Germination Index; Time taken to 50% germination and Coefficient of the velocity of germination) in *Lilium×formolongi* Hort.

Cultivars	Seed germination percentage			Mean Germination Time			Germination Index			Time taken to 50% germination			Coefficient of the velocity of germination							
	Seed storage temperature			Seed storage temperature			Seed storage temperature			Seed storage temperature			Seed storage temperature							
	20° C	5° C	-2° C	20° C	5° C	-2° C	20° C	5° C	-2° C	20° C	5° C	-2° C	20° C	5° C	-2° C					
Augusta	7.5 ^d	95.8 ^{ab}	92.5 ^{ab}	80.0 ^{ab}	12.4 ^a	3.9 ^{def}	5.5 ^{cde}	3.4 ^{ef}	0.2 ^g	12.4 ^{abc}	6.8 ^{de}	9.8 ^{cd}	11.9 ^a	2.2 ^{de}	4.5 ^{cd}	2.4 ^{de}	8.1 ^e	29.2 ^{bc}	18.4 ^d	29.2 ^{bc}
Raizan Herald	54.2 ^c	90.8 ^{ab}	94.2 ^{ab}	83.3 ^{ab}	7.6 ^{bc}	3.3 ^{ef}	3.3 ^{ef}	2.4 ^f	2.6 ^{fg}	10.2 ^{bcd}	10.3 ^{bc}	13.5 ^{ab}	6.7 ^{bc}	2.3 ^{de}	2.6 ^{de}	1.5 ^e	13.2 ^{de}	28.3 ^c	30.7 ^{bc}	43.8 ^a
Eorayeon 1 ho	80.0 ^{ab}	100.0 ^a	100.0 ^a	96.5 ^{ab}	5.8 ^{cd}	2.8 ^f	3.3 ^{ef}	2.7 ^f	5.0 ^{ef}	12.3 ^{abc}	9.5 ^{cd}	10.8 ^{bc}	5.2 ^c	2.2 ^{de}	2.8 ^{de}	2.2 ^{de}	17.3 ^d	37.9 ^{ab}	30.1 ^{bc}	36.9 ^{abc}
Eorayeon 2 ho	53.3 ^c	96.7 ^{ab}	96.7 ^{ab}	75.8 ^b	8.7 ^b	2.3 ^f	3.4 ^{ef}	2.7 ^f	2.0 ^{fg}	14.7 ^a	11.0 ^{bc}	10.7 ^{bc}	8.4 ^b	1.7 ^e	2.5 ^{de}	1.9 ^e	11.6 ^{de}	37.7 ^{ab}	29.9 ^{bc}	38.2 ^{ab}
GM	48.8	95.8	95.9	83.9	8.6	3.1	3.9	2.8	2.4	12.4	9.4	11.2	8.1	2.1	3.1	2.0	12.6	33.3	27.3	37.0
CV(%)	27.4	2.6	3.4	6.0	7.3	5.9	8.1	14.8	26.4	11.0	9.3	11.5	10.4	8.5	10.4	13.3	6.0	10.9	8.6	8.9
SE	6.7	1.2	1.6	2.5	0.3	0.1	0.2	0.2	0.3	0.7	0.4	0.6	0.4	0.1	0.2	0.1	0.4	1.8	1.2	1.6
SED	9.5	1.7	2.3	3.6	0.4	0.1	0.2	0.3	0.5	1.0	0.6	0.9	0.6	0.1	0.2	0.2	0.5	2.6	1.7	2.3
CD(P=05)	20.6	3.8	5.0	7.8	1.0	0.3	0.5	0.6	1.0	2.1	1.3	2.0	1.3	0.3	0.5	0.4	1.2	5.6	3.6	2.1
P Value(0.05)	0.000	0.001	0.032	0.000	0.000	0.000	0.000	0.023	0.000	0.005	0.000	0.008	0.000	0.002	0.000	0.003	0.000	0.003	0.000	0.000
F test (5%)	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

GM=General mean, CV=Coefficient of variation, SE=Standard error, SED=Standard error difference, CD=Critical difference; * denotes significant at 5% level of significance, F tab. For 3df/12df(5% level of significance)=3.49, F ratio(1)GP: for 20°C=20.3, 5°C=9.6, -2°C=4.1 & 20°C=12.5, (2)MGT: for 20°C=80.1, 5°C=56.3, -2°C=46.1 & 20°C=4.5, (3)GI: for 20°C=38.5, 5°C=7.2, -2°C=17.95 & 20°C=6.2, (4)T50: for 20°C=46.9, 5°C=9.3, -2°C=34.7 & 20°C=8.4 & (5)CVG: for 20°C=102.4, 5°C=8.4, -2°C=25.6 & 20°C=13.5; Mean separation among the columns by Duncan's

3.4 Time taken to 50% germination (T50)

The estimated times taken to 50% germination for all stored temperature of all four cultivars are given in Table 1. In comparison with room temperature (20°C), the estimated time is taken to 50% germination (T50) for the stored temperature 5°C, -2°C and -20°C for all the cultivar under evaluation has been reduced significantly. T50 was recorded shortest for Raizan Herald (i.e. 1.5 days) seeds stored at -20°C while the longest T50 was recorded for Augusta seeds stored at normal temperature (20°C). The seed of all cultivars stored in different temperature (i.e. 5°C, -2°C, and -20°C) has demonstrated shorter T50 duration than the all cultivars seeds those stored at room temperature (20°C) (Table 1) thereby indicating temperature treatment significantly decreasing the time taken for 50% germination (T50) for all four cultivars.

3.5 Coefficient of the velocity of germination (CVG)

The coefficient of the velocity of germination (CVG) demonstrated that temperature treatment has significantly increased the CVG of all cultivars. Among the various tested temperatures, the lowest CVG was reported at room temperature (20°C) while other temperature treatment (i.e. 5°C, -2°C & -20°C) demonstrated almost either 2 to 3 times higher CVG as compared to the room temperature (Control). Among three significant temperature treatments, the lowest CVG (18.4) was recorded for Augusta at -2°C while the highest CVG (43.8) was found for Raizan Herald at -20°C. Likewise, in comparisons with other cultivars, the CVG were found lower for Augusta for all stored temperature (Table 1).

4 Discussion

In this experiment, different aspects of seed germination viz. Germination percentage (GP), Mean germination time (MGT), Germination Index (GI), T50 (Time is taken for 50% seed germination), and the coefficient of the velocity of germination were tested for the seeds of selected cultivars of *L. ×formolongi* Hort. at different temperature viz. 20°C, 5°C, -2°C, and -20°C.

The temperature is one of the key environmental factors which influence the maximum germination rate and percentage of germination (Ketabi et al., 2016). Many researchers so far have been focused on the impact of temperature relating to the germination of the concerned crop species viz. *Striga hermonthica* (Del.) Benth. (Aflakpui et al., 1998), *Lilium formosanum* Wall. (Weng & Hsu, 2006), *Hosta yingeri* SB Jones (Kim et al., 2015), *Conanthera spp.* (Tecophilaeaceae) (Cuadra et al., 2019) and *Pinus bungeana* Zucc. ex Endl. (Guo et al., 2020). Till now, the main concerns of the researchers are the impact of temperature on seed germination but the current study was not only focused on seed germination but also evaluate the effect of storage temperature on various genotypes of *L. formolongi*.

The germination percentage is one of the most important factors for the evaluation of seed quality and viability. The variation among the cultivars of *L. ×formolongi* Hort for the same stored temperature of the seeds denoted the variation due to the genotype (Han & Jia, 2008). On the other hand, the seed storage temperature has a great influence on the germination percentage which ranged from 6.5% to 100%. The germination percentage also differs for the species of the crops (Roh & Sim, 1996; Xiaoyu et al., 2003; Weng & Hsu, 2006; Han & Jia, 2008; Mascarello et al., 2011; He et al., 2011). Lee et al. (1999) evaluate the effect of priming on seed germination and concluded that there is no significant difference in the final germination percentage with primed seeds and control seeds in *Lilium ×formolongi*.

The mean germination time is another important factor to be considered while testing the germination of seeds. In this experiment, the MGT has changed according to the cultivars and storage temperature. Such type of variation is very important for researchers to make decisions for the long term storage of seeds in a particular temperature with maintaining the seed quality and vigor for the future usages purpose (Kulshrestha et al., 2013).

Another most comprehensive measurement parameter that combines both GP and speed is the germination index (GI). According to Kader, (2005), GI is an analysis method which can describes both the GP/speed relationship and it is the most accurate as well. In this experiment, the seed is stored in different temperature treatment viz. 5°C, -2°C, and -20°C had possessed higher GI than seed stored at normal temperature i.e. 20°C thereby signified the importance of temperature treatment to the seeds (Kader, 2005).

The coefficient of the velocity of germination (CVG) is determined by the speed of seed germination in other words it indicates germination. According to Jones & Sanders (1987), the CVG increases when the number of germinated seeds increases, and the time required for germination decreases. Theoretically, the highest CVG possible is 100 with a GI of one. It might achieve when all the seeds germinated on the first day (Jones & Sanders, 1987). On the other hand, the coefficient of the velocity of germination (CVG) does not focus on the final percent of germination but emphasizes the time required for reaching it (Kader, 2005). We have achieved significantly higher CVG for temperature treated seeds i.e. seed stored at 5°C, -2°C, and -20°C than seed stored at normal temperature (20°C). Moreover, the CVG were found lower for Augusta than the other three cultivars for almost temperature treated seeds.

The temperature is important for maintaining seed vigor in other words temperature is responsible for the deterioration of flower seeds. The stored temperature of the seed deserves vital importance at each 5°C reduction in seed temperature doubles the life of the seed (Harrington, 1972).

In this way, in this experiment different five parameters of germination test viz. germination percentage (GP), mean germination time (MGT), germination index (GI), time is taken to 50% germination (T50), and coefficient of the velocity of germination (CVG) has been estimated of all four cultivars of *L. × formolongi* Hort. The significant ANOVA analysis for all those parameters of germination of all four cultivars for all temperature treatment provided the validity of this experiment.

Among the four cultivars, lowest GP was found for the seeds stored at 20°C which ranges from 7.5% to 80% while for all stored temperature of seed viz. 5°C, -2°C, and -20°C all cultivars have possessed 83.3% to 100% germination thereby signifies the importance of stored temperature of seed to get higher GP. We have observed the germination of the seeds up to 2 weeks, the mean germination time for four cultivars stored at normal temperature ranges from 5.8 days to 12.3 days which indicates taking longer duration than temperature treated seeds i.e. 5°C, -2°C, and -20°C which demonstrated shorter mean germination time ranging from 2.4 days to maximum 5.5 days. Similar types of results we have observed for other parameters viz. GI, T50, and CVG, which demonstrated the significance of storage temperature than normal storage temperature.

In this way, the storage temperature has a great influence on germination percentage (GP), mean germination time (MGT), germination index (GI), time is taken to 50% germination (T50), and coefficient of the velocity of germination (CVG) which varied for each cultivar of *L. × formolongi* Hort. For the long time storage of the seeds of these cultivars, we can prefer 5°C, -2°C, and -20°C than normal room temperature i.e. 20°C.

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Conflict of interest

We all the authors hereby declare that there is no conflict of interest among us.

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