Temperature and Relative Humidity Govern Germination and Storage of Gladiolus Seed

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Additional index words. *Gladiolus grandiflorus*, seed moisture content, storage temperatures, statistical models

Abstract. *Gladiolus* (*G. grandiflorus*) seed germination was light-independent, but temperature influenced the germination rate. Constant 20C promoted higher total germination (97%), fewer days (4.3) to 50% of final germination, and shorter span of days (4.8) between 10% and 90% germination than other constant temperatures, although similar results were achieved by alternating 12-h cycles of 20 to 25C. Total germination was unchanged after seed treatment for 7 days at 10 to -20C, but longer germination periods were required after treatments below -10C. Reducing seed moisture contents from 11.8% to 4.2% caused no reduction in total germination, but moisture contents below 6.6% delayed achieving 50% of final germination and extended the periods from 10% to 90% of germination. Temperature and relative humidity (RH) during storage were important in retaining seed viability, with RH having a larger effect. Smallest declines in total germination during 12 months of storage occurred at 11% and 33% RH at 15C. The statistical analysis estimated the optimum seed storage at 14C and 26% RH.

Optimum seed storage and germination of gladiolus are important to gladiolus hybridizers. Since seeds are harvested in the early fall and not planted until the following spring, they are stored for 6 to 7 months at 5 to 15C (Griesbach, 1972). Kelly (1953) reported seeds will survive short storage periods below 0C if harvested when mature and dried before storage. At 20C, 14 to 21 days are required to germinate 50% to 70% of the seeds (Pickell, 1972). Freshly harvested gladiolus seeds are enclosed in a waxy, oily wing that can delay germination (Griesbach, 1972); therefore, most seeds are "dewinged" before planting. The seed of many of the late-flowering species, e.g., *G. maculatus* 'Sweet', can be germinated only after 6 months of storage (Lewis et al., 1972). Of the 180 species of gladiolus, almost 90% are endemic to southern Africa and many are very rare or endangered (Lewis et al., 1972). Only seed may be exported to permit hybridization with other species. Leaves, flowers,

and corms may be infected with gladiolus smut (*Urocystis gladiolicola* Aino), which prohibits their entry into Europe and North America (Magie and Poe, 1972). The development of long-term seed storage recommendations is important for the preservation of this genus. Objectives of this research were to determine the optimum environmental conditions for the storage and germination of gladiolus seeds.

Light and temperature studies. Gladiolus seeds from controlled self-crosses among inbred lines of cv. Dr. Magie were obtained from a breeding program. Seeds were harvested 5 weeks after pollination and prepared by removing the winged appendages and dusting with 3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-W-isoinidole-1,3(2H)-dione (captan) before germination. In all experiments, there were four blocks with 100-seed replicates for each treatment. The seeds of each replicate were germinated in a 9-cm petri dish on a double layer of Whatman no. 1 filter paper moistened with 5 ml of distilled water (dw). Imbibed seeds were kept in darkness or in continuous incandescent light, 50 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ of photosynthetically active radiation (PAR), in incubators at a constant 10, 15, 20, 25, 30, or 35 $\pm$ 1C. Daily germination counts were made of seeds with visible radicle protrusion through the testa. Total absolute germination percent (G), days to 50% of final germination (T_{50}), and ger-

mination span measured in number of days between 10% and 90% germination ($T_{90} - T_{10}$), were calculated as recommended by Furutani et al. (1985). We used a randomized complete block design arranged as a 2 $\times$ 6 factorial, with data tested by analysis of variance (ANOVA). Polynomial equations relating G, T_{50} , and $T_{90} - T_{10}$ to temperature were fitted using SAS (1982).

In a second study, treatments with four blocks of 100-seed replicates were established following cleaning and seed storage of 14 days at 5C and 45% RH. Seeds were germinated in dark incubators at alternating temperatures (12-h) regimes of 15 to 20C, 15 to 25C, 15 to 30C, 15 to 35C, 20 to 25C, 20 to 30C, 20 to 35C, 25 to 30C, or 25 to 35C. The G, T_{50} , and $T_{90} - T_{10}$ were calculated from the daily germination counts and data were analyzed by Tukey's honestly significant difference test at $P = 0.05$.

Seed moisture content. After the preparation of freshly harvested seeds, 100-seed lots were weighed, placed in open 9-cm petri dishes, and dehydrated in forced-draft ovens at 40C for 0, 8, 16, 24, 48, 72, 96, or 120 h. Following cooling after dehydration, seeds were reweighed and immediately placed in 5-ml sealed glass vials, held for 7 days, then germinated in darkness at 20C. Daily germination counts were recorded, G, T_{50} , and $T_{90} - T_{10}$ calculated, and data analyzed by the least significant difference test (LSD) at $P = 0.05$. After cleaning, four lots of 100-seeds were weighed, dehydrated in a forced-draft oven at 105C for 48 h, and reweighed after cooling to determine the seed initial moisture content.

Storage temperature. After seed preparation, moisture contents were reduced by placing seeds in 15 $\times$ 2.5-cm petri dishes on wire screens supported by segments of tubing 1 cm above a chemical desiccant. Constant 22% RH was maintained in the sealed petri dishes by adding 50 ml of saturated potassium acetate to the bottom of each dish (Copeland, 1976). The refrigerated incubators maintained 15C during 1 week of seed dehydration. Seeds were placed immediately in 10-ml glass vials, that were sealed and immersed in polyethylene glycol-water (v/v) in controlled-temperature baths

Table 1. Germination of gladiolus 'Dr. Magie' seed under alternating temperatures.

Temperature cycle* (°C)	Germination		
	G ^y	T_{50} ^x	$T_{90} - T_{10}$ ^w
15-20	48	7.1	7.3
15-25	81	5.4	5.6
15-30	57	22.2	11.4
15-35	27	27.8	30.9
20-25	96	4.0	4.6
20-30	85	6.9	7.9
20-35	51	11.3	11.7
25-30	96	7.9	9.8
25-35	22	13.9	14.4
Tukey's HSD, 5%	7	2.6	3.4

*Temperature alternated every 12 h.

^yPercent absolute final germination after 38 days.

^xDays to 50% relative germination.

^wDays from 10% to 90% relative germination.

Received for publication 24 Oct. 1990. Florida Agricultural Experiment Station Journal Series no. R-01104. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Fig. 1. Germination curves for gladiolus 'Dr. Magie' seed exposed to light or darkness at 20C, illustrating total absolute germination (G), days to 50% relative germination (T_{50}), and germination span in days between 10% and 90% relative germination ($T_{90} - T_{10}$).

(Guy and Carter, 1984) at 10, 5, 0, -5, -10, -15, or -20C. Bath temperatures were lowered 3C/h to final temperatures, held for 7 days, then increased 4C/h to 10C. Following temperature treatments, seeds were germinated in dark 20C incubators. Daily germination counts were made, G, T_{50} , and $T_{90} - T_{10}$ calculated, and data analyzed using LSD at $P = 0.05$.

Temperature and relative humidity interaction during seed storage. Freshly har-

vested seeds were prepared as described previously, dusted with captan, and placed in storage at 11%, 33%, 52%, 75%, or 95% RH and 5, 15, or 25C for 3, 6, 9, or 12 months. Humidity treatments were achieved in 15 × 2.5-cm petri dishes as described previously, except different desiccants were used. Incubators with refrigeration and heating capacities maintained constant temperatures during seed storage. Seeds were germinated in darkness at 20C immediately

after storage. The design was a randomized complete block arranged as a 5 × 3 × 4 factorial. The data were analyzed by ANOVA, and transformed values using the arcsin $\sqrt{\% G}$ transformation were fitted by regression to produce plots of the arcsin $\sqrt{\% G}$ surface for combinations of relative humidity, temperature, and storage duration.

Imbibed seeds receiving light or held in darkness had similar G, T_{50} , and $T_{90} - T_{10}$ values (Fig. 1). No light × temperature indicators were found; therefore, data for light and dark treatments were combined for analysis in Fig. 2. Temperature influenced seed germination, with 20C promoting the highest G (Fig. 2A). The germination percentage curve, expressed as a function of temperature, was cubic (Fig. 2A). Days to T_{50} were similar for germination temperatures of 15, 20, 25, or 30C, but were greatly increased at 10 and 35C (Fig. 2B). The best fitting curve for days to 50% germination was cubic (Fig. 2B). The shortest span between $T_{90} - T_{10}$ occurred at 20C with progressively longer periods as temperatures increased above or declined below 20C. The trend curve for $T_{90} - T_{10}$ was quadratic (Fig. 2C).

Highest total germination percentages were at 20 to 25C or 25 to 30C for seeds receiving alternating temperatures at 12-h intervals (Table 1). Seeds given 12-h diurnal periods having one temperature at 15 or 35C had reduced G. Similar G values resulted from constant 20C (Fig. 2A) or alternating 20 to 25C or 25 to 30C (Table 1). Shortest T_{50} and $T_{90} - T_{10}$ occurred at 15 to 20C, 15 to 25C, or 20 to 25C (Table 1), with periods similar to those at constant 20C (Fig. 2B, C). Alternating temperature combinations of 20 or 25C with 35C caused large delays in achieving T_{50} and longer periods for $T_{90} - T_{10}$ (Table 1). Bewley and Black (1986) reported that temperature controls the rate of enzyme reactions and protein denaturation during seed germination, causing excessively high temperatures to be deleterious to germination. This offers a plausible explanation for the

Fig. 2. Effect of temperature on gladiolus 'Dr. Magie' seed germination, from combined data for treatments in light and darkness, using polynomial equations relating (A) percent total germination, (B) mean days to 50% germination (T_{50}), and (C) germination span ($T_{90} - T_{10}$) days to temperature, as fitted using SAS (1985).

Fig. 3. Comparisons of gladiolus seed germination T_{50} (●), and $T_{90} - T_{10}$ (■) in darkness after reducing seed moisture contents for 7 days.

Fig. 4. Germination of gladiolus seed in darkness after low temperature seed storage for 7 days, comparing treatment T_{50} (●), and $T_{90} - T_{10}$ (■).

Fig. 5. Three-dimensional (A) and contour plots (B) using the $\text{arc sin}\sqrt{\% \text{ Germination}}$ to compare the relationship between relative humidity and temperature during 12 months of seed storage on germination. Contour lines (B) represent estimated percentages of total germination after seed storage.

low G and delayed germination at constant or alternating 35C.

Gladiolus seeds were tolerant of extensive desiccation, with similar G values at all seed moisture contents from 11.8% to 4.2% (data not presented). Reducing seed moisture from 11.8% to 7.8% left T_{50} and $T_{90} - T_{10}$ unchanged, but lower levels caused significant delays in germination (Fig. 3). Seeds having 4.2% to 6.6% moisture were found to require longer periods for seed rehydration. Seeds were more tolerant of desiccation than Pridham and Thompson (1930) found for gladiolus corms.

Seeds were cold-tolerant, and G values of seeds held at 10 to -20C were similar (data not presented). Both T_{50} and $T_{90} - T_{10}$ were unchanged when seeds with 9.6% moisture content were stored at 10 to -5C for 7 days, but -10 to -20C lengthened the periods of germination (Fig. 4). Seeds were more cold-tolerant than gladiolus corms during storage (Emsweller, 1930). Pridham (1930) found that corms were severely injured or killed during storage below -1C. Emsweller (1930) reported the successful storage of corms between 3.5C and 30C, with earlier and fewer days to flowering from corms stored at 30C.

The three-dimensional plot and contour plot (Fig. 5 A, B) from the statistical analysis predicted that optimal seed storage occurs at 14C with 26% RH, as based on the maximum value of $\text{arc sin}\sqrt{\% G}$. The estimated germination after 12 months of storage from this temperature-relative humidity combination was 99.9%. Lower G occurred at all temperatures when relative humidity levels exceeded 39%. The relative humidity level during seed storage was found to have a larger

Fig. 6. (A) The arcsin $\sqrt{\%G}$ Germination surface for combinations of relative humidity and storage period, and (B) plots of the effect of storage period on arcsin $\sqrt{\%G}$ Germination for 11% (—), 39% (-----), 67% (---), and 95% (- · - · -) relative humidity.

effect on G than storage temperature (Fig. 5B). Plots evaluating storage periods of 3, 6, 9, or 12 months at different RH levels indicated that the least decline in G occurred during storage at 11% or 39% RH, with progressively larger declines at 67% and 95% RH (Fig. 6 A, B). Seeds dehydrated at 105C following 12 months of storage at 11% or 33% RH were found to contain 8.1% and 9.6% moisture contents, respectively, levels that did not reduce G values or increase T_{50} or $T_{90} - T_{10}$ in the dehydration study (Fig. 3).

These studies help us better understand the frequent low total and irregular germination of gladiolus seed and the rapid loss of viability during storage. The data reported should assist in developing recommendations for the improved germination, handling, and storage of gladiolus seeds. Breeders depend on seeds to gain access to native gladiolus species and need long-term seed storage for breeding lines. Our results demonstrate that

gladiolus seed can be stored at 14C and 26% RH for 12 months with only a small loss in germination. Since gladiolus seed is tolerant of subfreezing temperatures, further lengthening of the seed storage period may be possible.

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