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THE EFFECT OF TEMPERATURE AND STRATIFICATION TIMES ON SEEDS GERMINATION OF SOME *GLADIOLUS* SPECIES

Petronica AMIŞCULESEI, Maria APOSTOL, Neculai MUNTEANU, Lucia DRAGHIA

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author emails: amisculesei_petronica@yahoo.com, lucia@uaiasi.ro

Abstract

In this paper, the germination of seeds from three species of the genus Gladiolus was studied: G. imbricatus, G. byzantinus and G. tristis. The seeds used had different origins: the seeds of G. imbricatus and G. byzantinus come from the plants from the Floriculture collection of at the Faculty of Horticulture ("Ion Ionescu de la Brad" Iasi University of Life Sciences), and the seeds of G. tristis come from specialized companies. The seeds were stratified at 4°C for 44, 58, 74, 86 and 98 days. The seeds were germinated in Petri dishes in the germination chamber at different temperatures to trigger the germination (13-14°C, 17-19°C and 20-22°C). Data obtained showed that the germination percentage depend of Gladiolus specie, temperature and number of days of stratification. Thus, at G. byzantinus, the best germination rate was obtained at the temperature of 13-14°C, after 58 days of stratification; in the case of 74 days stratified seeds, the germination temperature did not affect the percentage of germinated seeds. In G. tristis, germination was favored by temperatures between 17-19°C. The seeds of G. imbricatus germinatedin a reduced percentageonly after 98 days of stratification, but regardless of the temperature.

Key words: *germination, gladiolus, seed, stratification, temperature.*

INTRODUCTION

The *Iridaceae* Juss. family comprises a large number of herbaceous plants, perennials (geophytes), with underground organs rhizomes, corms and less often bulbs (Ciocârlan, 2009). The main center of origin of these plants is southern Africa, to which are added Central and South America, including the tropical part of Mexico and the Caribbean (Alves et al., 2011), so they are cosmopolitan, spreading from temperate regions.to the tropical ones (Ștefan and Oprea, 2007). The number of genera and species included in the family *Iridaceae*, varies, depending on different botanical classifications made over the time: approx. 2000 species distributed in 60-70 genera (Goldblatt et al., 2008, cited by Alves et al., 2011 and Raycheva et al., 2020), approx. 1800 species and 78 genera (Skrypec&Odintsova, 2020), approx. 1800 species and 92 genera (Ștefan & Oprea, 2007).
The *Gladiolus* L., with representatives studied in this paper, is one of the important genera of the *Iridaceae* family, considering from the ornamental point of view. The similarity between some species and the morphological variations determined by the environmental conditions have sometimes led to taxonomic confusions or uncertain delimitations, being treated, by many authors, as synonyms or placed in lower taxonomic ranks (Mifsud & Hamilton, 2013; Yetişen et al., 2014). According to some authors, the *Gladiolus* genus includes approx. 150 species (Reddy et al., 2019; Dahlgren, 1985 cited by Yetişen et al., 2014), followed by more than 250-260 species (Băla & Sala, 2021; Mifsud & Hamilton, 2013; Azimi, 2020; Sağiroğlu & Akgül, 2014) or even 311 species (Raycheva et al., 2020). Their origin is in different regions of South and tropical Africa, Asia, southern Europe and part of the Mediterranean basin (Cantor & Tolety, 2011; Mifsud & Hamilton, 2013; Reddy et al., 2019; Raycheva et al., 2020; Yetişen et al., 2014), many of them being spread throughout the world (Lewis et al., 1972, cited by Fernández et al., 2005).
Gladiolus species are petaloid monocotyledonous plants with oval or globular corms as underground storage organs, covered with several layers of fibrous tunics (De Hertogh & Le Nard, 1993; Cantor, 2016; Cantor & Pop, 2008). All species of gladiolus can be propagated sexually or asexually (Cantor, 2016), but seed production is higher than corm production (González et al., 2003). For commercial

production, gladioli are vegetatively propagated by corms and cormels (Aljaser & Anderson, 2021), although this method is characterized by a low rate of propagation (each season a mother corm normally produces 1 to 2 new corms and several cormels). The production of corms and cormels is also severely affected by the high percentage of spoilage, during storage and the attack of *Fusarium* (Memon et al., 2016; Riaz et al., 2010). In recent years, numerous studies have been conducted to improve *in vitro* regeneration protocols, both in cultivated varieties of gladiolus and in wild species, in order to increase the rate of multiplication, to obtain free material from viruses and other pathogens (Kumar et al., 2018; Memon et al., 2016; Dutta Gupta & Prasad, 2010) and the conservation of rare wild species (Rakosy-Tican et al., 2012).

Sexual reproduction in gladiolus is important for breeders and to the recovery and maintenance of endangered germplasm (Fernández et al., 2005). Gladiolus species form winged or unwinged seeds (Mifsud & Hamilton, 2013). In general, seed-bearing plants bloom in the second year (Cohat, 1993, cited by Fernández et al., 2005) and do not always maintain the typical characteristics of the species or cultivar (Kumar et al., 2018).

For the wild gladiolus species, seed propagation is one of the important propagation pathways, due to its relatively good yield compared to corm propagation, especially when *ex situ* propagation is desired to restore threatened populations. For example, the vegetative spread of *G. imbricatus* is limited by the fact that in one season it, usually, forms only one new tuberobulb (Kostrakiewicz-Gierałt, 2017).

Studies on the germination of gladiolus seeds have been performed on both *G. hybridus* cultivars and wild species, in order to clarify a number of issues related due to the peculiarities of germination and to establish optimal seed propagation technologies. It is known that the germination of seeds of some plant species, especially perennials, is influenced by many factors, the literature focusing on issues related to seed origin, separate and simultaneous interaction of environmental factors, seed storage conditions, dormancy period, morpho-anatomical, physiological and biochemical features of the seeds etc. (Fernández et al., 2005).

Widespread in spontaneous plants, seeds dormancy appears as a mechanism of survival under various environmental conditions, specific to the areas of origin and is genetically imprinted. Entering or leaving seeds dormancy is influenced by external or internal factors (Toma & Jităreanu, 2007). Baskin & Baskin (2004) classified the types of seed rest in physiological, morphological, morphophysiological, physical and combined, ones. Some of the biological reasons for dormancy, listed by ISTA (1966, 1993), are the hard and impermeable coating, the immature or latent embryo, the absence of the endosperm and the fleshy part of the fruit. The dormancy period varies from species to species (Olmez et al., 2007). The interruption of the seeds dormancy and the improvement of the germination is achieved by applying different treatments on the seeds: stratification, vernalization, physical, chemical, mechanical and hormonal treatments etc. (Toma & Jităreanu, 2007; Draghia, 2011; Basra et al., 2003; Afzal et al., 2004). Seed treatment before sowing is a known method and an effective way to increase the germination rate of seeds, as well as the growth rate in some species, for example, with small embryos or species with gradual seed development (Sivritepe, 2000). This treatment is also effective for better seed germination of vegetables and herbs (Arif et al., 2007).

Carpenter et al. (1991) showed that seed germination in *G. grandiflorus* was independent of light, but strongly in fluenced by temperature, themostsuitable being a constant of 20°C or an alternation of temperature between 20-25°C or 25-30°C. The same authors mention that reducing the humidity of the seeds and treating them for seven days, with temperatures of -20^{0}C, does not influence the germination.

Dutt et al. (2000) treated the seeds of some cultivars of *G. hybridus* with GA_3 solution (100, 150 and 200 ppm) and distilled water for 24 hours. Compared to distilled water treatment, the GA_3 treatments significantly increased seed germination rates and shortened the germination time.

In order to improve the germination rate and reduce the time required for germination at *G. alatus* seeds were applied by bathing for 48 hours, before sowing in KNO_3 solutions with different concentrations (1, 2, 3, 4 and 5%) and in distilled water, the control being untreated seeds (Ramzan et al., 2010). The best germination (over 80%) was obtained at the seeds kept in distilled water (92%) and only in 1% and 2% KNO_3 solutions. Hydration of the seeds in distilled water shortened both the minimum time required for germination of 50% of the seeds and the total time of germination. Similar experiments with *G. alatus* were performed by Mushtaq et al. (2012), who used KNO_3 treatments in concentrations of 0.25-5%, the better results were reported by treatments with distilled water and lower concentrations of KNO3 (0.25-0.75%) (Mushtaq et al., 2012).

The seeds stratification is also an efficient method of interrupting of dormancy and improving germination. The stratification ensures the good conditions for cold of the seeds and is achieved by an exposure to 1^0-5^0 (10°)C and humidity, a certain period of time, depending on the species.The experiments performed on *G. palustris,* both in the laboratory and in the field, indicated a better germination rate when the seeds were subjected to cold stratification schemes (Brunzel, 2010).

Seeds germination studies have been performed in other species of the Iridaceae family. For example, *Iris*s eeds are used to weaken the strength of the skin by mechanical action (Blumenthal et al., 1986). *I. ensata* seeds germinate in a proportion of approx. 94% if these are cold layered for 60 days (Xiao et al., 2010). In *Freesia* species it was found that the seeds germinate at temperatures between 15^0C and 18^0C, in about 21 days. In the Changjiang area of China, the seeds are sown directly in cold beds, which can be kept warm in winter (Long, 1996).

The objective of this paper was o determine the influence of temperature and stratification time on seed germination of some *Gladiolus* species from different geographical areas, including Romania.

MATERIALS AND METHODS

The study of seeds germination was performed on three species of the genus *Gladiolus*: *G. byzantinus, G. imbricatus* and *G. tristis*.

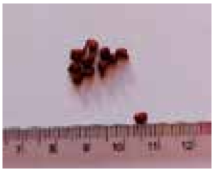
G. byzantinus Mill. it also appears with the synonymous name *G. communis* L. subsp. *byzantinus* (Mill.) A. P. Ham. However, the classification of the taxon as a subspecies of *G. communis* (Hamilton, 1976, quoted by Tison & Girod, 2014; Raamsdonk & Vries, 1989) is not supported by many authors, who do not recognize it as a distinct subspecies. Mifsud & Hamilton (2013) consider that *G. Byzantinus* is a sterile hexaploid (2n = 90), which is not found in nature and isjust a simple garden variety described by Philip Miller Hamilton (2013); Tison & Girod (2014) consider it is a polymorphic hybrid (*G.* x*byzantinus*), found, mainly, in the western Mediterranean basin, southern Spain and northern Africa, Italy and Sicily, reaching the coasts of Greece (Lopez-Espinoza et al., 2003). The plants form flowers talks of approx. 0.5-1 m tall, with bright purple-red flowers arranged unilaterally, which bloom in May-June.

G. imbricatus L., native to Central and Eastern Europe, the Mediterranean, the Caucasus and Western Siberia (Kose et al., 2019), is a declining species in Europe and on the red lists in many countries (Moora, 2008). In Romania it appears as a wild species, being reported in areas throughout Moldova, Muntenia, Transylvania, Banat, Oltenia, Maramureș (Oprea, 2005). It was cultivated *ex situ* from a local seeds populationin the Floriculture field at the Faculty of Horticulture in Iași. It grows up to 30-80 (100) cm in height and forms unilateral inflorescences with pink-lilac flowers and eeds of approx. 1.8 mg (Kose et al., 2019; Jõgarand Moora, 2008). *G. imbricatus* seeds weigh 1.8 mg (on average) and mature in August-September (Zelená, 1967; Goldblatt & Manning, 2008, cited by Kubíková & Zeidler, 2011), but in July-August in Iasi conditions. The seeds go through a dormancy that is interrupted during the winter (Chrtek et al., 2007, cited by Kubíková & Zeidler, 2011). Klimeš et al. (1997) characterized the limited vegetative spread, and the productions of corms are very small, respectively, one corm in a growing season (cited by Szczepaniak et al., 2016).

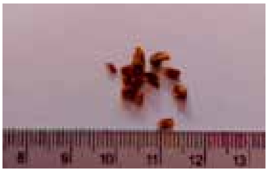
G. tristis L. is native to South Africa, but is widespread in areas of Australia and the coastal region of California. It has floral stems 60-70 cm high and inflorescences with 2-10 fragrant flowers, which bloom in spring (Gonzalez et al., 1998; Milandri et al., 2008).

The study of seed germination of the three species of *Gladiolus* were carried out in the period 2020-2021, in laboratory conditions, of the Horticultural Research Center (former Fruit Research Laboratory "Prof. Dr. Gica Grădinariu") of the Faculty of Horticulture from "Ion Ionescu de la Brad" University of Life Sciences in Iasi Romania.

The seeds used in the experiment came from plants from the collection of the discipline of Floriculture (*G. byzantinus* and *G. imbricatus*) and from trade (*G. tristis*) (Figure 1).



a.



b.

c.

Figure 1. Gladiolus seeds: a. *G. byzantinus*; b. *G. imbricatus*; c. *G. tristis*

The experiments were bifactorial, the experimental factors being represented by the stratification period, with five graduations (44, 58, 74, 86 and 98 days) and the germination temperature, with three graduations (20-22°C, 17-19°C and 13-14°C), resulting in 15 experimental variants (Table 1).

Table 1. The experimental scheme

Variant	Specification (no. days of stratification/ t^0C germination)
V_1	44 / 20-22
V_2	44 / 17-19
V_3	44 / 13-14
V_4	58 / 20-22
V_5	58 / 17-19
V_6	58 / 13-14
V_7	74 / 20-22
V_8	74 / 17-19
V_9	74 / 13-14
V_{10}	86 / 20-22
V_{11}	86 / 17-19
V_{12}	86 / 13-14
V_{13}	98 / 20-22
V_{14}	98 / 17-19
V_{15}	98 / 13-14

The seeds were stratified at a temperature of 4 - 6°C, starting on 11.10.2020, for different periods, according to the experimental scheme.

At the end of each stratification period, the seeds were placed in Petri dishes on a layer of filter paper moistened with 10 mL of distilled water, then placed in the germination chamber at the three temperature levels: 13-14°C, 17-19°C and 20-22°C (Figure 2).

a.

b.

Figure 2. Stratification (a) and seeds germination (b)

The experiments were organized in a randomized blocks design with three repetitions; each repetition contains 100 seeds.

Observations and determinations on seed germination were performed daily, but in the summary tables, the data were entered every two days.

The dynamics of germination was determined from the beginning of germination. The total percentage of germinated seeds corresponded to the moment when, at 2-3 consecutive determinations, the same percentage of germination was obtained.

Germination rate is the percentage of seeds that have germinated overnight. Based on the individual rates, the average germination rate was calculated for each variant.

Germination rate (V_G) is the percentage of seeds germinated at a given date and was calculated according to the formula:

$$V_G = \frac{Gi}{n} \text{ (1), where:}$$

G_i = germination at a certain date;

n = number of days in which G_i germination was performed.

Germination rate (CV_G) is the rate of germination relative to the final germination of the seed and was calculated according to the formula:

$$C_{VG} = \frac{Gi}{GF \text{ x } n} 100 \text{, where:}$$

Gi and *n* have the same meanings as in the previous formula;

G_f= final germination.

The results of the final percentage of germination were compared to the average of the variants (considered control), and the interpretation was made using the analysis of the variance, with the LSD test (Saulescu and Saulescu, 1967).

RESULTS AND DISCUSSIONS

Gladiolus byzantinus

The observations on *G. byzantinus* (Table 2). indicate that seeds germination was influenced by the stratification period and less by the germination temperature. Seeds that were stratified 44 days had the lowest germination percentage, between 16 (V_1 and V_3) and 20% (V_2). With the increase of the stratification period, the percentage of germinated seeds also increased, so that at a stratification period of 58 days the percentage of germinated seeds reached 55% (V_4), 56% (V_5) and 51% (V_6). In the case of variants with stratified seeds 74 days (V_7, $V_8$and V_9), the percentage of germinated seeds was 74% higher than in the stratified 44 days and 38% higher than in the stratified 58 days. In variants with stratified seeds 86 and 98 days (V_{10}-V_{14}), the percentage of germinated seeds was over 96% (100% when the germination temperature was maintained at 17-19ºC).

The duration of germination was 11 days, for stratified seeds for 44, 58 and 74days (V_1 - V_9), seven days for stratified seeds for 86 days (V_{10}, V_{11} and V_{12}) and only five days for stratified seeds for 98 days (V_{13}, V_{14} and V_{15}). Differences were also found in the time taken from seed removal to stratification to germination: 12 days for 44 days stratified seeds (V_1-V_3); two days for 74, 86 and 98 days stratified seeds (V_7 - V_{15}); eight days for 58daysstratified seeds (V_4, V_5 and V_4) (Table 2).

Table 2. *G. byzantinus* germination dinamics

Var.	Date of removal from the stratification	Data/germinated seeds (%)																					
		05.12.	07.12.	09.12.	11.12.	13.12.	15.12.	17.12.	19.12.	21.12.	23.12.	25.12.	27.12.	29.12.	31.12.	02.01.	06.01.	08.01.	10.01.	12.01.	20.01.	22.01.	24.01.
V_1		12	12	14	14	14	16																
V_2	23.11.20	10	14	16	18	18	20																
V_3		8	12	12	14	14	16																
V_4							12	22	29	39	49	55											
V_5	07.12.20						10	19	28	38	51	56											
V_6							8	21	29	40	48	51											
V_7											10	24	54	78	84	90							
V_8	21.12.20										12	26	50	60	87	94							
V_9											14	20	50	64	83	86							
V_{10}																	10	18	82	96			
V_{11}	04.01.21																32	50	94	100			
V_{12}																	32	50	88	96			
V_{13}																					10	82	98
V_{14}	18.01.21																				32	94	100
V_{15}																					32	88	96

The dynamics of the germination rate in *G. byzantinus* (Table 3) indicate certain variations determined, in particular, by the duration of the stratification. Stratification for 44 days led to maximum values of the rate of emergence in the first day, respectively 12% in variants V_1, 10% in V_2 and 8% in V_3; towards the end of the period, regardless of the temperature, the germination rate was identical (2%). The 58 days stratified seeds had a relatively constant germination rate during the first nine days, and on the eleventh day the lowest rate (5-7%). The 74 days stratified seed variants (V_7-V_9) had high germination rates in the first days, with maximum values on the sixth day after germination, after which they decreased. Long-term stratification (86 and 98 days) resulted in a higher germination rate, indicating faster and more uniform germination. The maximum values were recorded on the fifth day from the beginning of germination on variants V_{10}-V_{12} and on the third day on variants V_{13}-V_{15}. The highest germination rate (72%) had the seeds of variant V_{13}, after two days from the beginning of germination and four days after they were removed from the stratification. In the case of these variants, in which the total percentage of germinated seeds was over 96%, on the last day of determinations, the germination rate dropped sharply to low values (6-16%).

Table 3. *G. Byzantinus* germination rate dynamics

Var.	Data/germination rate dynamics (%)																					Average
	05.12.	07.12.	09.12.	11.12.	13.12.	15.12.	17.12.	19.12.	21.12.	23.12.	25.12.	27.12.	29.12.	31.12.	02.01.	06.01.	08.01.	10.01.	12.01.	20.01.	22.01.	24.01.	
V_1	12	0	2	0	0	2																	2.7
V_2	10	4	2	2	0	2																	3.3
V_3	8	4	0	2	0	2																	2.7
V_4						12	10	7	10	10	6												9.2
V_5						10	9	9	10	13	5												9.3
V_6						8	13	8	11	8	3												8.5
V_7										10	14	30	24	6	6								15.0
V_8										12	14	24	10	27	7								15.7
V_9										14	6	30	14	19	3								14.3
V_{10}																10	8	64	14				24.0
V_{11}																32	18	44	6				25.0
V_{12}																32	18	38	8				24.0
V_{13}																				10	72	16	32.7
V_{14}																				32	62	6	33.3
V_{15}																				32	56	8	32.0

Germination rate and seed rate coefficient was analyzed in Table 4. The rate was different from one variant to another, as well as during the germination period.

In variants V_1-V_3, with stratified seeds 44 days, it is found that the highest values of velocity and coefficient of velocity were recorded at the first observations since the onset of germination, when the velocity was 4-4.7%, and the coefficient of velocity was between 23.3-25%. In the following days, the values were constantly decreasing, to values of 1.5-1.8% of the velocity and 9.1% of the coefficient of velocity. Stratification for 58 days (V_4-V_6) resulted in an increase in velocity, which was 6.3-7.3% at the beginning of germination and towards the end of the period 4.6-5.1%. And the coefficient of velocity was maintained at higher values, between 45.8% and 25.5%. Unlike the other variants, the stratification for 74days (V_7-V_9) was highlighted by a relatively constant level of velocity and coefficient of velocity, which oscillated, in most cases, between values of approx. 9-11%. The evolution of the two indicators in the case of 89 days stratified seeds (V_{10}-V_{13}) was characterized both by larger differences due to germination temperature and by day-to-day fluctuations, with alternative maxima and minima.

Thus, germination at temperatures above 20°C was slower in the first days, and the maximum velocity and coefficient of velocity were recorded on the fifth day. Instead, the temperature below 20°C led to lower fluctuations in velocity and coefficient of velocity but also with maximums on the fifth day. For the stratified variants 98 days, in the five days that the germination lasted, the analyzed indicators were at the maximum level on the third day (velocity between 20.7-29.3%, and the coefficient of velocity between 27.9-31.3%). On the last day, when germination is completed (96-100%), the velocity and the coefficient of velocity had very small variations (19.2-20%).

Table 4. *G. byzantinus* germination velocity and coefficient of velocity dynamics

Var.	V/Cv	Data/ velocity/coefficient of velocity dynamics																						Average
		05.12.	07.12.	09.12.	11.12.	13.12.	15.12.	17.12.	19.12.	21.12.	23.12.	25.12.	27.12.	29.12.	31.12.	02.01.	06.01.	08.01.	10.01.	12.01.	20.01.	22.01.	24.01.	
V_1	V	0.0	4.0	2.8	2.0	1.6	1.5																	2.4
	Cv	0.0	25.0	17.5	12.5	9.7	9.1																	14.8
V_2	V	0.0	4.7	3.2	2.6	2.0	1.8																	2.9
	Cv	0.0	23.3	16.0	12.9	10.0	9.1																	14.3
V_3	V	0.0	4.0	2.4	2.0	1.6	1.5																	2.3
	Cv	0.0	25.0	15.0	12.5	9.7	9.1																	14.3
V_4	V						0.0	7.3	5.8	5.6	5.4	5.0												5.8
	Cv						0.0	45.8	36.3	34.8	34.0	31.3												36.4
V_5	V						0.0	6.3	5.6	5.4	5.7	5.1												5.6
	Cv						0.0	31.7	28.0	27.1	28.3	25.5												28.1
V_6	V						0.0	7.0	5.8	5.7	5.3	4.6												5.7
	Cv						0.0	43.8	36.3	35.7	33.3	29.0												35.6
V_7	V										0.0	8.0	10.8	11.1	9.3	8.2								9.5
	Cv										0.0	8.9	12.0	12.4	10.4	9.1								10.6
V_8	V										0.0	8.7	10.0	8.6	9.7	8.5								9.1
	Cv										0.0	9.2	10.6	9.1	10.3	9.1								9.7
V_9	V										0.0	6.7	10.0	9.1	9.2	7.8								8.6
	Cv										0.0	7.8	11.6	10.6	10.7	9.1								10.0
V_{10}	V																0.0	6.0	16.4	13.7				12.0
	Cv																0.0	6.3	17.1	14.3				12.6
V_{11}	V																0.0	16.7	18.8	14.3				16.6
	Cv																0.0	16.7	18.8	14.3				16.6
V_{12}	V																0.0	16.7	17.6	13.7				16.0
	Cv																0.0	17.4	18.3	14.3				16.7
V_{13}	V																				0.0	27.3	19.6	23.5
	Cv																				0.0	27.9	20.0	24.0
V_{14}	V																				0.0	20.7	20.0	20.4
	Cv																				0.0	31.3	20.0	25.7
V_{15}	V																				0.0	29.3	19.2	24.3
	Cv																				0.0	30.6	20.0	25.3

Gladiolus imbricatus

The results obtained for this species have shown that the duration of statification has an important role in seed germination. According to the literature, the germination of *G. imbricatus* seeds takes place in cool and humid spring conditions, after periods of warm and cold stratification (Clothier, 2003; Kostrakiewicz-Gierałt, 2017).

Table 5. *G. imbricatus* germination dinamics

VAR.	Date of removal from the stratification	Data/germinated seeds (%)									
		07.12.	21.12.	04.01.	18.01.	01.02.	25.01.	27.01.	29.01.	31.01.	02.02.
V_1		0.0	0.0	0.0	0.0	0.0					
V_2	23.11.2020	0.0	0.0	0.0	0.0	0.0					
V_3		0.0	0.0	0.0	0.0	0.0					
V_4		0.0	0.0	0.0	0.0	0.0					
V_5	07.12.2020	0.0	0.0	0.0	0.0	0.0					
V_6		0.0	0.0	0.0	0.0	0.0					
V_7		0.0	0.0	0.0	0.0	0.0					
V_8	21.12.2020	0.0	0.0	0.0	0.0	0.0					
V_9		0.0	0.0	0.0	0.0	0.0					
V_{10}		0.0	0.0	0.0	0.0	0.0					
V_{11}	04.01.2021	0.0	0.0	0.0	0.0	0.0					
V_{12}		0.0	0.0	0.0	0.0	0.0					
V_{13}							6.0	20.0	27.0	34.0	40.0
V_{14}	18.01.2021							9.0	22.0	34.0	-
V_{15}								8.0	19.0	25.0	32.0

In our experience, the 44, 58, 74, and 86 days stratified seeds (V_1-V_{12}) did not germinate, regardless of the germination temperature to which they were exposed (Table 5). The 98 days statified seeds (V_{13}, V_{14} and V_{15}) germinated in a proportion of 32-40%, the maximum germination being obtained at temperatures above 20ºC (V_{13}), and the minimum at 13-14ºC, the temperature level directly influencing proportionally the percentage of germinated seeds. After removal from stratification, seed germination was triggered earlier (at seven days) by exposure to temperatures of 20-22°C, while at temperatures below 20°C (V_{14}-V_{15}), germination began after nine days (Table 5).

Table 6. *G. imbricatus* germination rate dynamics

VAR	Data/Germination rate dynamics (%)										Average
	07.12.	21.12.	04.01.	18.01.	01.02.	25.01.	27.01.	29.01.	31.01.	02.02.	
V_1	0.0	0.0	0.0	0.0	0.0						0
V_2	0.0	0.0	0.0	0.0	0.0						0
V_3	0.0	0.0	0.0	0.0	0.0						0
V_4	0.0	0.0	0.0	0.0	0.0						0
V_5	0.0	0.0	0.0	0.0	0.0						0
V_6	0.0	0.0	0.0	0.0	0.0						0
V_7	0.0	0.0	0.0	0.0	0.0						0
V_8	0.0	0.0	0.0	0.0	0.0						0
V_9	0.0	0.0	0.0	0.0	0.0						0
V_{10}	0.0	0.0	0.0	0.0	0.0						0
V_{11}	0.0	0.0	0.0	0.0	0.0						0
V_{12}	0.0	0.0	0.0	0.0	0.0						0
V_{13}						6.0	14.0	7.0	7.0	6.0	8.0
V_{14}							9.0	13.0	12.0		11.3
V_{15}							8.0	11.0	6.0	7.0	8.0

The highest germination rate was recorded nine days after interruption of stratification and germination of V_{13} seedlings and 11 days for V_{14} and V_{15} seedlings (Table 6). It is also observed that the temperature of 17-19°C shortened the germination period correlated with a higher average germination rate (14.7%) compared to the other two thermal levels, at which the average rate was 8.0% (Table 6). Germination velocity and coefficient of velocity (Table 7) were favorably influenced by more moderate germination temperatures below 20ºC, although the highest number of germinated seeds was 20-22ºC. The average values of the two indicators were higher at V_{14}, with the germination temperature of 17-19ºC, followed by V_{15} (13-14ºC) and, lastly, V_{13} (20-22ºC).

Table 7. *G. imbricatus* germination velocity and coefficient of velocity dynamics

VAR.	V/ Cv	Data/velocity/coeff. of velocity 07.12.	21.12.	04.01.	18.01.	01.02.	25.01.	27.01.	29.01.	31.01.	02.02.	Average V/Cv
V_1	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
V_2	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
V_3	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
V_4	V	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_5	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_6	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_7	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_8	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_9	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{10}	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{11}	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{12}	V	0.0	0.0	0.0	0.0	0.0						0.0
	Cv	0.0	0.0	0.0	0.0	0.0						0.0
V_{13}	V						0.0	6.7	5.4	4.9	4.4	5.3
	Cv						0.0	16.7	13.5	12.1	11.1	13.3
V_{14}	V							0.0	7.3	6.8		7.05
	Cv							0.0	21.6	20.0		20.8
V_{15}	V							0.0	6.3	5.0	4.6	5.6
	Cv							0.0	19.8	15.6	14.3	16.5

Gladiolus tristis

From the analysis of the seed germination dynamics in *G. tristis* (Table 8), it is observed that the major influence on this process had the germination temperature, given that, regardless of the stratification period, at temperatures of 20-22°C, the seeds did not germinate. In the other two temperature variants, the final germination was between 78-86%. During the same stratification period, the best germination was recorded at 13-14ºC, respectively 2-4% more than at 17-19ºC. It can also be seen that the temperature of 13-14ºC caused a reduction time in germination time, especially at long layering periods (V_{12} and V_{15}).

The results obtained are similar to those reported by González and Lopez (2005), who stated that the optimal temperatures for seed germination in *G. tristis* var. *tristis* and *G. tristis* var. *concolor* are between 13-15°C.

Table 8. *G. tristis* germination dinamics

Var.	Date of removal from the stratification	02.12.	04.12.	06.12.	08.12.	10.12.	12.12.	16.12.	18.12.	20.12.	22.12.	24.12.	26.12.	28.12.	30.12.	02.01.	04.01.	06.01.	06.01.	08.01.	11.01.	13.01.	15.01.	17.01.	24.01.	26.01.	28.01.	30.01.
		Data / germinated seeds (%)																										
V_1		0	0	0	0	0	0																					
V_2	23.11.2020	8	24	55	58	71	79																					
V_3			15	33	55	68	81																					
V_4								0	0	0	0	0	0															
V_5	07.12.2020							10	26	54	60	70	82															
V_6									16	30	56	60	68	86														
V_7															0	0	0	0	0									
V_8	21.12.2020													10	26	54	60	70	78									
V_9															16	30	56	60	70	82								
V_{10}																					0	0	0					
V_{11}	04.01.2021																				10	30	70	80				
V_{12}																						20	40	82				
V_{13}																									0	0	0	0
V_{14}	18.01.2021																								10	26	66	80
V_{15}																										20	46	84

The germination rate differed between variants, but also within the same variant, during the germination (Table 9). In the studied variants, the maximum rate was reported on the fifth day after germination. Comparing the average germination rates from the variants with the same layering time, it is found that the values were higher at 13-15°C. Stratification for 86 and 98 days increased the average germination rate to 20-28%(Table 9).

Table 9. *G. tristis* germination rate dynamics

Var.	02.12.	04.12.	06.12.	08.12.	10.12.	12.12.	16.12.	18.12.	20.12.	22.12.	24.12.	26.12.	28.12.	30.12.	02.01.	04.01.	06.01.	06.01.	08.01.	11.01.	13.01.	15.01.	17.01.	24.01.	26.01.	28.01.	30.01.	Average
	Data/ germination rate dynamics (%)																											
V_1	0	0	0	0	0	0																						0.0
V_2	10	16	31	3	13	8																						13.5
V_3		16	18	22	13	13																						16.4
V_4							0	0	0	0	0	0	0															0.0
V_5							10	16	28	6	10	12																13.7
V_6								16	14	26	4	8	18															14.3
V_7														0	0	0	0	0										0.0
V_8													10	16	28	6	10	8										13.0
V_9														16	14	26	4	10	12									13.7
V_{10}																				0	0	0						0.0
V_{11}																				10	20	40	10					20.0
V_{12}																					20	20	42					27.3
V_{13}																								0	0	0	0	0.0
V_{14}																								10	16	40	14	20.0
V_{15}																									20	26	38	28.0

The rate of germination highlights the way in which the experimental factors analyzed ensure the germination of a certain number of seeds at a given time. The experimental data presented showed that the highest germination rate of the variants was recorded on the fifth day after the onset of germination (Table 10). In the variants with stratified seeds 44, 58 and 76 days (V_2, V_3, V_5, V_6, V_8, V_9), characterized by longer germination time (9-11 days), after this peak speed, the values were decreasing towards the end of the period. The variants with stratified seeds 86 and 98 days and which were germinated at 13-15°C (V_{12} and V_{15}) had the maximum velocity at the last determinations, respectively on the fifth day. Of all the variants, the highest germination rate (16.4-16.8%) had the stratified seeds 86 and 98 days, germinated at 13-15°C, and the lowest (7.2%) the seeds from variant V_2, stratified 44 days and germinated at 17-19°C.

The coefficient of velocity varied between 8.8-20% and recorded the highest values (20%) in the conditions in which the stratification was 86--98 days, and germination temperatures of 13-15°C (Table 10).

Table 10. *G. tristis* germination velocity and coefficient of velocity dynamics

Var.	V/ Cv	Data/velocity/coefficient of velocity																											
		02.12.	04.12.	06.12.	08.12.	10.12.	12.12.	16.12.	18.12.	20.12.	22.12.	24.12.	26.12.	28.12.	30.12.	02.01.	04.01.	06.01.	06.01.	08.01.	11.01.	13.01.	15.01.	17.01.	24.01.	26.01.	28.01.	30.01.	Average
V_1	V	0	0	0	0	0	0																						0
	Cv	0	0	0	0	0	0																						0
V_2	V	0	8	11	8.3	7.9	7.2																						7.07
	Cv	0	9.8	13.4	10.1	9.6	8.8																						8.62
V_3	V		15	11	11	9.7	9																						11.1
	Cv		17.4	12.8	12.8	11.3	10.5																						13
V_4	V							0	0	0	0	0	0																0
	Cv							0	0	0	0	0	0																0
V_5	V							0	8.7	10.8	8.6	8	7.5																7.23
	Cv							0	0	13.2	10.5	10	9.1																7.05
V_6	V								0	10	11.2	9	7.6	7.8															7.53
	Cv								0	11.6	13	10	8.8	9.1															8.75
V_7	V													0	0	0	0	0	0										0
	Cv													0	0	0	0	0	0										0
V_8	V													0	8.7	10.8	8.6	7.8	7.1										7.17
	Cv													0	11.1	13.8	11	10	9.1										9.17
V_9	V														0	10	11.2	8.6	7.8	7.5									7.52
	Cv														0	12	13.7	11	9.5	9.1									9.13
V_{10}	V																				0	0	0	0					0
	Cv																				0	0	0	0					0
V_{11}	V																				0	10	14	11.4					11.8
	Cv																				0	12.5	17.5	14.3					14.7
V_{12}	V																					0	13.3	16.4					14.9
	Cv																					0	16.3	20					18.2
V_{13}	V																								0	0	0	0	0
	Cv																								0	0	0	0	0
V_{14}	V																								0	8.7	13.2	11.4	11.1
	Cv																								0	10.8	16.5	14.3	13.9
V_{15}	V																									0	15.3	16.8	16.1
	Cv																									0	18.3	20	19.2

In order to highlight the extent to which the duration of the stratification and the temperature influenced the total percentage of germinated seeds, the statistical-mathematical processing, respectively the analysis of the variance was used. The evaluation considered both the cumulative action of the two experimental factors and their separate action. The cumulative action (Table 11) indicates a favorable influence, with very significant positive differences compared to the average, on variants V_7-V_{15} from *G. byzantinus* and only on variants V_{13}-V_{15} from *G. imbricatus*. In contrast, in *G. tristis*, the positive influence of the two factors was manifested in all variants, except those in which the germination temperature was maintained at 20-22^{0}C.The comparison of the results from the variants with seeds subjected to the same stratification duration allowed to highlight the individual influence of this experimental factor: 44 days, 58 days, 74, 86 days and 98 days. Negative differences compared to the average were in the stratified variants 44 and 58 days (*G. byzantinus* and *G. imbricatus*) as well as 74 and 86 days (*G. imbricatus*). The positive differences were determined by the stratification of 74 and 86 days (*G. byzantinus*) and 98 days (*G. byzantinus* and *G. imbricatus*). The non significant influence of the stratification duration was highlighted in *G. tristis* (Table12).

Table 11. The cumulative influence of experimental factors (temperature and stratification) on seeds germination

Var.	*G. byzantinus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. imbricatus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. tristis* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.
V_1	16	22.4	-55.3	000	0	0	-7.1	000	0	0	-54.3	000
V_2	20	28.1	-51.3	000	0	0	-7.1	000	79	145.5	24.7	xxx
V_3	16	22.4	-55.3	000	0	0	-7.1	000	81	149.2	26.7	xxx
V_4	55	77.1	-16.3	000	0	0	-7.1	000	0	0	-54.3	000
V_5	56	78.5	-15.3	000	0	0	-7.1	000	82	151.0	27.7	xxx
V_6	51	71.5	-20.3	000	0	0	-7.1	000	86	158.4	31.7	xxx
V_7	90	126.2	18.7	xxx	0	0	-7.1	000	0	0	-54.3	000
V_8	94	131.8	22.7	xxx	0	0	-7.1	000	78	143.6	23.7	xxx
V_9	86	120.6	14.7	xxx	0	0	-7.1	000	82	151.0	27.7	xxx
V_{10}	96	134.6	24.7	xxx	0	0	-7.1	000	0	0	-54.3	000
V_{11}	100	140.2	28.7	xxx	0	0	-7.1	000	80	147.3	25.7	xxx
V_{12}	96	134.6	24.7	xxx	0	0	-7.1	000	82	151.0	27.7	xxx
V_{13}	98	137.5	26.7	xxx	40	563.4	32.9	xxx	0	0	-54.3	000
V_{14}	100	140.3	28.7	xxx	34	478.9	26.9	xxx	80	147.3	25.7	xxx
V_{15}	96	134.6	24.7	xxx	32	450.7	24.9	xxx	84	154.7	29.7	xxx
$\bar{x}$	71.3	100.0	0.0	control	7.1	100.0	0.0	control	54.3	100.0	0.0	control
	LSD 5% = 1.8; 1% = 2.4; 0.1% = 3.3				LSD 5% = 1.5; 1% = 2.0; 0.1% = 2.6				LSD 5% = 3.1; 1% = 4.1; 0.1% = 5.5			

Table 12. Seeds germination obtained under the influence variation of stratification duration

Stratif. (days)	*G. byzantinus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. imbricatus* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	*G. tristis* Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.
44	17.3	24.3	-54.0	000	0	0	-7.1	000	53.3	98.2	-1.0	ns
58	54.0	75.7	-17.3	000	0	0	-7.1	000	56.0	103.1	1.7	x
74	90.0	126.2	18.7	xxx	0	0	-7.1	000	53.3	98.2	-1.0	ns
86	97.3	136.5	26.0	xxx	0	0	-7.1	000	54.0	99.5	-0.3	ns
98	98.0	137.5	26.7	xxx	35.3	497.2	28.2	xxx	54.7	100.7	0.4	ns
x	71.3	100.0	0.0	control	7.1	100.0	0	control	54.3	100.0	0.0	control
	LSD 5% = 1.4; 1% = 2.1; 0.1% = 3.1				LSD 5% = 1.2; 1% = 1.8; 0.1% = 2.7				LSD 5% = 1.6; 1% = 2.4; 0.1% =3.6			

The study of the individual influence of temperature on the total percentage of germinated seeds (Table 13) was performed by grouping the results obtained at variants with the same thermal level: 20-22°C (V_1, V_4, V_7, V_{10}, V_{13}), 17-19°C (V_2, V_5, V_8, V_{11}, V_{14}) and 13-15°C (V_3, V_6, V_9, V_{12}, V_{15}). Compared to the average experience, the differences were non significant in *G. byzantinus* for 20-22°C and in *G. imbricatus* for 17-19°C and 13-15°C. Very significant positive differences were recorded at 17-19°C (*G. byzantinus* and *G. tristis*) and at 13-14^{0}C (*G. tristis*). The negative differences were at 13-14^{0}C (*G. byzantinus*) and at 20-22°C (*G. tristis).*

Table 13. Seeds germination obtained under the influence variation of temperature

Temp. (^{0}C)	*G. byzantinus*				*G. imbricatus*				*G. tristis*			
	Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.	Total germ. (%)	% from $\bar{x}$	Diff. from $\bar{x}$	Sign.
20-22^{0}C	71	99.6	-0.3	ns	8	112.7	0.9	x	0	0	-54.3	000
17-19^{0}C	74	103.8	2.7	xxx	6.8	95.8	-0.3	ns	79.8	146.9	-25.5	xxx
13-14^{0}C	69	96.8	-2.3	00	6.4	90.1	-0.7	ns	83.0	152.9	28.7	xxx
$\bar{x}$	71.3	100.0	0.0	control	7.1	100.0	0.0	control	54.3	100.0	0.0	control
	LSD 5% =0.8; 1% =1.4; 0.1% =2.6				LSD 5% =0.8; 1% =1.3; 0.1% =2.4				LSD 5%=3.0; 1%=4.9; 0.1%=9.2			

CONCLUSIONS

Germination of *G. byzantinus* seeds is mainly influenced by the duration of stratification and less by the germination temperature. At 74 days of cold stratification, the percentage of germinated seeds increases to over 80%, and at 86 and 98 days, germination reaches 96-100%, simultaneously with a decrease in germination time to 5 days (compared to 11 -14 days for short layering options).

The influence of stratification duration is evident in *G. imbricatus*, germination being triggered only at 98 days of stratification. Better germination is favored by temperatures of 20-22^{0}C.

In *G. tristis*, the factor with major influence on germination was temperature, at 20^{0}C the germination is blocked, regardless of the stratification duration. The higher germination rate and the shorter germination time was obtainedat 13-15^{0}C.

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