

# Features of Seed Germination in Different Ecological Groups of the Species of the Section *Violidum*, subgenus *Nomimium*, genus *Viola* L. (*Violaceae*)

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**Abstract**—This article presents the results of seed germination in different ecological groups in the species (22 populations) of the section *Violidum*, subgenus *Nomimium*, genus *Viola*. Conditions for seed germination, as well as germinating ability, germination energy, intensity of germination energy, longevity of seeds and profitability of their ability, and the period when the seed germinating ability decreases by 50% ( $P_{50}$ ) have been determined. The seeds of most species of xeromesophytes and mesophytes massively germinate at +23...+25°C within a period of 10 days. For other species of these ecological groups, cold stratification is favorable. The hygromesophytes are characterized by prolonged seed germination (up to 2 months). The petrophyte seeds have shallow physiological dormancy (B1). The seeds of other species are, as a rule, nondormant.

**Keywords:** section *Violidum*, subgenus *Nomimium*, genus *Viola*, biology of germination, germinating ability, germination energy, longevity of seeds, ecological groups

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## INTRODUCTION

The study of the biology of seed germination is one of the main areas of studying the latent period. Results of this study are useful both for practical purposes, i.e., for creating and maintaining living collections and seed stock, and for dealing with theoretical issues, including the systematic and phylogenetic directions and the identification of the evolutionary and adaptive potential of the genus in general. These studies have been conducted for a long time, but they are generally focused on agricultural, decorative, and drug species (Nikolaeva, 1956, 1958, and 1977; Vitality of seeds, 1978; Ishmurova and Tkachenko, 2009; and Telewski and Zeevaart, 2002). The features of seed germination, depending on the area and origin of species, were considered based on the example of the genus *Euonymus* (Nikolaeva, 1956), *Fraxinus* (Nikolaeva, 1958), *Berberis* (Nikolaeva and Alekseyeva, 1984), *Pinus* (Nikolaeva, 1990), and *Alnus* (Banaev et al., 2006). The authors note that the species of the East Asia area, as well as the tertiary relicts, are characterized by the absence of the dormancy period in seeds, and the seeds of Mediterranean species require the period of cold stratification. The germinating ability of seeds and the period of their storage, taking into account their belonging to the ecological group, was studied by A.A. Svetlakova (2000) based on the example of the genus *Delphinium*. The author established that the highest indicators of the seed germinating ability and storage duration were identified in xerophytes, and

the lowest indicators were identified in mesophytes. Therefore, endogenous factors that influence seed germination are associated with genus phylogeny and are reflected in the areal width and ecological association.

Little is known about the biology of germination of the seeds of the species of the genus *Viola* L. (Semenova, 1987; Filippova, 1987; and Deno, 1998). The longevity of seeds, which is an important feature for the characteristic of the biology of seed germination, has been determined as economic longevity only for *V. Wittrockiana* Gams (Flower crop seeds, 1984).

The purpose of this work is to reveal the features of germination of the seeds of the species of the section *Violidum*, subgenus *Nomimium*, genus *Viola* L., taking into account their belonging to the ecological group and areal peculiarities.

## MATERIALS AND METHODS

The object of research was the collection of the genus *Viola*, which was established in 1978 in the Central Siberian Botanical Garden (the city of Novosibirsk). In 2013 there were 56 species of 120 populations presented in the collection. The most numerous taxonomic group among the species of this genus is the section *Violidum*, subgenus *nomimium*. Fourteen species (22 populations) of this section germinate in the collection.

Most of the studied introduction populations are of natural origin and represent the species of Siberian flora

(Table 1). The studied species belong to different ecological groups: hygromesophytes (*V. selkirkii*), mesophytes (*V. dactyloides*, *V. czemalensis*), mesoxerophytes (*V. irtutiana*), and other species belong to xeromesophytes. Four of the 14 species have a restricted ecological association and belong to lithophytes and calciphiles (*V. alexandrowiana*, *V. czemalensis*, *V. milanae* (lithophytes), and *V. jooi* (calciphile). The area of the studied species is different (Table 2). *V. czemalensis* (Altai endemics) and *V. jooi* (Western Europe endemics) are the most restricted endemics, while *V. selkirkii* is the most common species.

Six species are of Eastern Asia areal type (*V. dactyloides*, *V. gmeliniana*, *V. incisa*, *V. irtutiana*, *V. mandshurica*, and *V. variegata*, with *V. dactyloides*, *V. incisa*, and *V. irtutiana* growing in a disjunctive area). The *V. dissecta* is a North Asian species. The *V. milanae* and *V. alexandrowiana* are hemiendemics (Southern Siberia and Eastern Siberia, respectively). The names of taxa are given according to Zuev classification (2012).

The experience was carried out starting April 2011 according to classical techniques (Methodological guidelines, 1980; Ishmuratova and Tkachenko, 2009; Florya, 1987) modified for the species of the genus *Viola* (Elisafenko, 2012). We used general harvest seeds (July–September), which were mostly from the fruits of cleistogamous (autogamous) flowers and were harvested from the introduction populations of the crops of 2000–2012, that were stored in paper packets in room conditions. Seeds were germinated within the period when they were freshly harvested (within a week after collection), within the first year of storage (3–8 months of storage), within the second year of storage (15–20 months of storage), etc.

Seeds were germinated in Petri dishes with a diameter of 9 cm that have a combined bed (quartz sand and a paper filter) in the 4-fold sequence, 100 seeds per dish. The combined bed slows down the loss of moisture, and small violet seeds can be clearly seen on the paper filter. The seeds were considered germinated if there was a rootlet that exceeded the seed length. Germinated seeds were calculated daily within 10 days after the beginning of germination and, afterwards, every second day. The duration of the experiment was no less than 30 days.

Seeds were germinated in laboratory (room) conditions characterized by natural daily temperature variations (the experiment began in March at +25...+28 °C). For the seeds of some violet species, germination requires stratification (cold stratification or two-stage stratification with temperature change, i.e., thermal and cold stratification) (Elisafenko, 2001). In this case, the stage of thermal stratification began in autumn or winter months, with seeds being placed in a climate chamber with a photoperiod of 16.5 h and daily temperature variations close to the natural conditions of the summer period (+27°C during the light period and +17°C during the night period). The duration of the first stage was 1 month. At the second stage,

i.e., during cold stratification, seeds were placed in a refrigeration unit at +4...+6°C for 1 to 3 months.

To process the results, it was necessary to take into account the year of seed harvesting, the period of seed storage, the regime of the experiment, the date of the beginning of the experiment, the duration of the experiment (days), the period before seed germination (days), the period of germination (days), germinating ability (%), and germination energy (%). The germination energy was determined on the 5th day after the seeds began to germinate. In addition, we determined the intensity of germination energy (%), i.e., the percent of germinated seeds on the 5th day after the beginning of germination (Elisafenko, 2012). To characterize the longevity of seeds, the biological longevity and profitability of introduction longevity of seeds (Dorogina and Elisafenko, 2014), as well as the period when the seed germinating ability decreases by 50% (P50), were determined. The latter feature is considered important for the characteristic of the biology of seed germination (Strel'tsov et al., 1988; Dowsett et al., 2012).

The results of seed germination are presented in the form of the range of the minimum and maximum values and the features are compared with respect to the maximum values.

## RESULTS AND DISCUSSION

The seeds of most species did not require stratification; they were germinated at room temperature in spring or in a climate chamber in winter, except for *V. czemalensis* and *V. cucullata* and freshly harvested *V. incise* and *V. jooi* seeds (Table 3). For *V. czemalensis* and *V. cucullata* seeds, the positive results of germination were obtained at variable temperature conditions: after thermal stratification (1 month), we had to use long cold stratification (2–3 months). This is not due to embryo immaturity or seed immaturity, since the freshly harvested seeds of these species are a well-developed differentiated embryo.

With respect to seed germination, the studied species can be divided into four groups:

1. Massive germination at room temperature (the majority of the species of the Section *Violidum*).
2. Prolonged germination at room temperature (*V. selkirkii*).
3. Single germination with the use of thermal stratification and massive germination after cold stratification (*V. cucullata*, freshly harvested seeds, and *V. incise* and *V. jooi* harvest in separate years).
4. Two-stage stratification is required. Single germination in the refrigeration unit and massive germination after cold stratification (*V. czemalensis*).

The period prior to seed germination depends on the storage period. In most species, the shortest period was observed in 1-year-old stored seeds and was  $5 \pm 2$  days, except for *V. incise* (10 days) and *V. czemalensis* (115 days). In freshly harvested seeds, this indicator is twice as high in most of species as that in 1-year-old

**Table 1.** Species of the section *Violidum*, subgenus *Nomimum*, genus *Viola* L.: the origin and the duration of storage in the collection of the Central Siberian Botanical Garden (the city of Novosibirsk)

Species	Origin	Year of introduction into the collection
<i>V. alexandrowiana</i> (W. Becker) Juz.	The Republic of Buryatia, city of Arshan, rocks	1986
	Irkutsk oblast, 30 km southwest of Irkutsk, the village of Baklashi, right bank of Irkut River, valley mixed forest	1979
<i>V. czemalensis</i> Zuev	Republic of Altai, Chemal'skii raion, outskirts of Elanda village, Katun HPP station	2004
<i>V. cucullata</i> Ait	Italy represented by amateur gardeners	2001
<i>V. dactyloides</i> Schultes	Republic of Sakha, Olekminskii raion, village of Chapaevo, pine fores	1979
<i>V. dissecta</i> Ledeb.	Krasnoyarsk krai, Sharypovskii raion, village of Ivanovka, Lake Ingol, southern slope, grass meadow	1984
	Republic of Altai, Ongudaiskii raion, left bank of Katun River between the villages of Kuchegen' and Inya, stony shore	2009
	Republic of Altai, Ongudaiskii raion, estuary of Chuya River, left bank of Katun River, gravel in the flood plain	2006
	Republic of Altai, Maiminskii raion, city of Gorno-Altai, Tugoya Mountain, forest park	2009
<i>V. gmeliniana</i> Roem et Schultes	Irkutsk oblast, Republic of Buryatia, outskirts of village of Zun-Murino, steppified meadow	2009
<i>V. incisa</i> Turcz.	Krasnoyarsk krai, Sharypovskii raion, village of Ivanovka, lake Ingol, southern steppe slope	1982
<i>V. ircutiana</i> Turcz.	Republic of Buryatia, Tunkinskii raion, village of Tolgoi, left bank of the Khyry-Khobok, edge of birch forest, grass meadow	1986
<i>V. irinae</i> N.Zolot.	Novosibirsk oblast, Kolyvanskii raion, outskirts of village of Kolyvan', Ob floodplain, outskirts of the holiday village of Rybachii, pine forest	1993
	Republic of Altai, Maiminskii raion, city of Gorno-Altai, Tugoya Mountain, forest park	2009
<i>V. jooi</i> Janka	Germany, city of Dresden, botanical garden	2003
<i>V. mandshurica</i> W.Beckr.	Collection of the Central Siberian Botanical Garden, Herbarium lab	2003
<i>V. milanae</i> Vl. Nikit.	Republic of Tuva, Tes-Khemskaia raion, outskirts of village of Bert-Dag, valley of the Khyraalyg River, floodplain, and pebbles	2003
	Republic of Altai, Ongudaiskii raion, valley of Aigulak River, rocky steep slope, and steppe	2001
<i>V. selkirkii</i> Pursh	Republic of Buryatia, southeastern coast of Baikal, outskirts of village of Goryachinsk, fir forest	2004
	Novosibirsk oblast, Akademgorodok, village of Kirov, outskirts of the Central Botanical Garden, slope of ravine	2001
	Republic of Khakassia, descent from the Sayanskii pass, 800 m.a.s.l., floodplain of the Bol'shoi On River, mixed forest	2003
<i>V. variegata</i> Fisch.ex Link	Zabaikalskii krai, Baleiskii raion, village of Kunikan, river valley, steppe rocky-carbonate slope	1981

**Table 2.** Ecological characteristic of some species of the section *Violidum*, subgenus *Nomimum*, genus *Viola* L.

Species	Area	Ecological group, specialization
<i>V. alexandrowiana</i>	Eastern Siberia endemic	Mesophyte, facultative lithophyte
<i>V. czemalensis</i>	Altai endemic, south of Western Siberia	Mesophyte and lithophyte
<i>V. cucullata</i>	North American species: Canada	Xeromesophyte
<i>V. dactyloides</i>	North Asian endemic growing in a disjunctive area (all of Siberia, Far East, Japan, and China)	Mesophyte
<i>V. dissecta</i>	North Asian area (all of Siberia, Far East, Central Asia, Mongolia, and China)	Xeromesophyte
<i>V. gmeliniana</i>	East Asian species: Eastern Siberia, Far East, Mongolia, and Manchuria	Xeromesophyte
<i>V. incisa</i>	East Asian species growing in a disjunctive area: the south of Siberia, the middle part, and south of the Far East	Xeromesophyte
<i>V. ircutiana</i>	East Asian species growing in a disjunctive area: Eastern Siberia and Far East	Mesoxerophyte
<i>V. irinae</i>	Endemic in the south of Western Siberia	Xeromesophyte
<i>V. jooi</i>	Western Europe endemic: Romania and Ukraine	Mesophyte and calciphile
<i>V. mandshurica</i>	East Asian species: Amur oblast, Manchuria, and Sakhalin oblast.	Xeromesophyte
<i>V. milanae</i>	Southern Siberia endemic	Xeromesophyte and lithophyte
<i>V. selkirkii</i>	Eurasian and North American species: the entire area Europe and Siberia, Far East, Mongolia, Japan, China, and North America	Hygromesophyte
<i>V. variegata</i>	East Asian species: Eastern Siberia (Dauria), the middle part and the south of the Far East, Japan, China, Manchuria, and Korea	Xeromesophyte

stored seeds, except for *V. mandshurica*. The further storage of seeds did not influence the duration of the period prior to germination in five species (*V. alexandrowiana*, *V. czemalensis*, *V. cucullata*, *V. dissecta*, and *V. irinae*). For the other species with storage time, this indicator increased 2 times in the third year of storage in *V. ircutiana*; in the fourth year of storage in *V. mandshurica*, *V. selkirkii*, and *V. variegata*; in the fifth year in *V. dissecta* and *V. milanae*; and in the sixth year in *V. dactyloides*. For *V. incisa*, this indicator increased insignificantly from the seventh year of storage. The period for *V. jooi* increased 2–5 times in the fifth year of storage prior to germination. In freshly harvested seeds, the period is 2 to 4 times shorter prior to the germination of hygromesophytes and mesophytes (from 4 to 9 days) than that in other ecological groups. In all species, the period prior to the germination of freshly harvested seeds is longer than that in 1-year-old stored seeds.

The seeds of most of the species germinated within 30 days, except for *V. gmeliniana* and *V. selkirkii*, which required 40 and 70 days, respectively. The period of seed germination was different between freshly harvested seeds and 1-year-old stored seeds only in six species. In *V. alexandrowiana*, *V. ircutiana*, *V. jooi*, and *V. milanae*, the period of germination of freshly harvested seeds was twice as long or more than that in 1-year-old stored seeds. In *V. dactyloides*, *V. irinae*, and *V. mandshurica*, freshly harvested seeds germinated faster (2 times) than 1-year-old stored

seeds. The freshly harvested seeds of *V. incisa* germinated at different regimes in different harvest years and, accordingly, the period of germination was different. In other species of *V. cucullata*, *V. dissecta*, *V. selkirkii*, and *V. variegata*, these indicators had no difference. The period of seed germination after 1 year of storage had no significant differences in all species, except for *V. selkirkii*, in which it decreased 3 times. The largest period of seed germination after 1 year of storage was recorded in hygromesophytes (*V. selkirkii*) (more than 35 days), while the seeds of the species of other ecological groups germinated within a month.

The maximum germinating ability of seeds reached 90–100% in 10 of the 14 studied species (*V. cucullata*, *V. dactyloides*, *V. dissecta*, *V. gmeliniana*, *V. incisa*, *V. ircutiana*, *V. irinae*, *V. mandshurica*, *V. selkirkii*, and *V. variegata*) after 1 year of storage. The lowest value was in *V. jooi* (40%). In five species the germinating ability of freshly harvested seeds was lower than that in 1-year-old stored seeds (*V. alexandrowiana* (Buryatia), *V. incisa*, *V. ircutiana*, *V. milanae* (Altai), and *V. selkirkii*); in six species these indicators had no differences (*V. alexandrowiana* (Irkutsk), *V. cucullata*, *V. dactyloides*, *V. dissecta*, *V. irinae*, *V. mandshurica*, and *V. variegata*).

In *V. jooi*, when 1-year-old stored seeds were germinated, the maximum laboratory germinating ability was 40%. However, freshly harvested seeds of this species germinated only after two-stage stratification, and the germinating ability reached 100%. It was recorded

**Table 3.** Characteristic of germination of the seeds of the section *Violidum*, subgenus *Nomimium*, genus *Viola*.

Germination regime	Seed storage period, years	Germinating ability, %	Period, days		Germination energy, %	Intensity of energy germination, %
			prior to germination	germination days		
<i>V. alexandrowiana</i> (Republic of Buryatia)						
32RC	4	36–38	5	21	19–23	50–64
32RC	3	38–49	4	5–20	27–41	81–100
32RC	2	47–58	4	15–22	25–36	48–62
32RC	1	67–76	4	9–22	45–67	67–92
32RC	0	9–22	10–17	7–32	1–10	11–67
<i>V. alexandrowiana</i> (Irkutsk oblast)						
32RC	4	36–58	5–6	18–23	15–41	34–71
32RC	3	26–38	5–6	11–23	6–22	16–73
32RC	2	45–56	4–6	11–28	24–34	49–67
32RC	1	56–80	4	11–28	40–73	71–94
55RC	0	60–78	8–10	32–45	13–36	17–60
<i>V. czemalensis</i>						
50RC + 66RU + 20RC	4	8–11	117–119	4–14	1–10	13–100
50RC + 66RU + 20RC	3	32–44	115	5–12	27–43	82–98
50RC + 66RU + 20RC	2	37–58	115	12–14	28–48	76–83
50RC + 66RU + 20RC	1	39–71	115–117	7–12	35–36	81–97
<i>V. cucullata</i>						
40RC + 216RU + 18RC	5	0–5	5	3		
40RC + 216RU + 18RC	4	0				
40RC + 216RU + 18RC	3	8–27	7–258	2–253	8–27	100
40RC + 216RU + 18RC	2	30–84	5–7	251–254	30–84	100
40RC + 216RU + 18RC	1	90–98	5–15	245–262	90–98	98–100
30RU + 80T	1	0	–	–		
30RU + 80RC	1	51	37	61	10	20
80T	1	0	–	–		
80RC	1	17	16	63	6	35
40RC + 216RU + 18RC	0	94–97	11–257	4–250	89–97	94–100
<i>V. dactyloides</i> (Republic of Sakhaà)						
35RC	7	0–1	12	1		
35RC	6	1–7	8–10	5–9	1–7	50–100
35RC	5	47–68	5–6	10–14	42–51	72–89
35RC	4	67–83	4–5	25–28	58–76	87–92
35RC	3	87–90	4	11–18	82–84	91–97
35RC	2	74–94	4	21–28	67–85	87–91
35RC	1	94–99	3–5	6–18	91–96	96–97
35RC	0	68–100	4–9	3–12	4–99	4–100
<i>V. dissecta</i> (Krasnoyarsk krai)						
38RC	8	33–36	7–9	13–22	17–26	50–72
38RC	7	19–34	8–9	18–26	11–16	41–63
38RC	6	33–65	6–8	15–28	12–36	23–55
38RC	5	63–74	7–9	17–30	20–51	30–73
38RC	4	84–90	4–5	13–21	71–84	84–93
38RC	3	90	6	26	82	91
38RC	2	96–100	4–6	16–28	75–91	75–93
38RC	1	96–100	3–6	10–17	79–92	82–98
38RC	0	85–100	4–10	10–22	19–91	20–91
38RC	0	89–100	4–8	24–37	52–89	55–89
<i>V. gmeliniana</i>						
50RC	2	68–100	3	2–39	50–100	69–100
50RC	1	94–96	3–4	7–37	90	94–96

Table 3. (Contd.)

Germination regime	Seed storage period, years	Germinating ability, %	Period, days		Germination energy, %	Intensity of energy germination, %
			prior to germination	germination days		
<i>V. incisa</i> (Krasnoyarsk krai)						
41RC	9	0–1	29	1		
41RC	8	7–17	13–19	15–21	2–9	18–53
41RC	7	24–51	13–15	14–18	12–37	50–81
41RC	6	75–79	12–13	10–17	56–72	75–91
41RC	5	87–95	11–13	12–19	78–82	86–91
41RC	4	71–98	11	7–23	66–93	92–97
41RC	3	95–100	11	7–21	91–99	95–99
41RC	2	68–100	11	6–27	59–100	87–100
41RC	1	81–94	9–11	15–28	47–84	58–92
48RC + 112RU + 14CI	0	56–63	26–139	5–118	54–63	95–100
30RC + 100RU + 14CI	0	23–78	25–132	6–115	22–77	94–100
30CI	0	66–72	5	9–16	56–59	79–85
<i>V. ircutiana</i>						
35RC	4	1–6	9–33	1–14	1–5	40–100
35RC	3	45–68	8–10	15–25	24–49	53–84
35RC	2	76–83	5–6	15–21	69–74	86–93
35RC	2	77–78	5–6	9–29	66–78	86–95
35RC	1	89–100	5	6–16	81–98	87–98
40RC	0	58–89	10	14–22	47–68	59–93
<i>V. irinae</i>						
60RC	4	55–67	5–7	32–41	26–30	39–53
60RC	3	84–95	4	21–37	62–76	74–80
60RC	2	66–77	4–6	39–41	40–52	53–79
60RC	1	95–100	3	24–32	92–95	94–98
60RC	0	99–100	9	12–15	53–86	53–85
<i>V. jooi</i>						
45RC	5	0–1	25	1		
45RC	4	23–43	6–7	22–39	6–21	19–58
45RC	3	40–49	3–5	13–40	32–43	67–95
45RC	2	12–19	6–11	26–34	1–10	6–53
45RC	1	34–41	5–13	24–34	2–14	6–34
80RC	1	0	–	–		
48RC + 130RU + 12CI	0	87–99	18–31	152–166	86–97	98–100
<i>V. mandshurica</i>						
45RC	6	0–1	29	1		
45RC	5	0–3	33	2	2	67
45RC	4	56–58	7–11	32–36	6–12	11–21
45RC	3	81–97	5–6	19–37	57–83	70–87
45RC	2	95–100	5–6	26–37	70–81	73–81
45RC	1	90–99	3	13–35	84–93	88–98
80T	1	2.22	76	1	2.22	
45RC	0	87–100	3–4	8–16	1–77	1–78
<i>V. milanae</i> (Republic of Tuva)						
38RC	8	1	10–11	1	1	
38RC	7	0				
38RC	6	2–9	8–15	1–14	2–3	33–100
38RC	5	14–17	7–8	11–18	9–13	53–93
38RC	4	60–70	3–4	13–28	39–59	62–91
38RC	1	87.5	3	13	80	91
38RC	0	95–100	7–8	17–23	14–23	15–23

Table 3. (Contd.)

Germination regime	Seed storage period, years	Germinating ability, %	Period, days		Germination energy, %	Intensity of energy germination, %
			prior to germination	germination days		
<i>V. milana</i> (Republic of Altai)						
38RC	4	21–45	4	12–30	14–29	58–78
38RC	2	67–73	4–5	19–30	36–44	52–60
38RC	1	96–100	3–4	9–21	79–93	82–92
80T	1	4	73	6	3	75
80RC	1	89	12	49	13	15
50RC	0	69–81	7–9	23–36	1–9	1–13
<i>V. selkirkii</i> (Republic of Buryatia)						
60RC	5	0				
60RC	3	74–89	9	32–45	22–44	25–52
60RC	2	67–84	7–9	41–45	17–43	25–58
60RC	1	77–97	4–6	35–51	9–72	12–74
60RC	0	62–76	1–18	37–58	1–2	2–3
<i>V. selkirkii</i> (Novosibirsk oblast)						
60RC	8	0				
60RC	7	0				
60RC	4	0–11	10–21	13–16	3–4	27–67
60RC	3	36–56	8–9	46–47	1–8	2–22
60RC	1	49–73	3–5	39–51	14–16	22–29
60RC	0	31–64	5–6	57–69	2	22
<i>V. variegata</i>						
35RC	6	0–1	6	1		
35RC	5	0–1	11	1		
35RC	4	13–24	7–13	20–26	5–8	24–46
35RC	3	85–95	5–6	14–28	39–64	46–67
35RC	2	89–96	4	23–29	62–79	69–88
35RC	1	98–100	4	7–26	85–92	85–94
35RC	0	100	10	16–20	14–34	14–34

RC, room conditions; T, thermostat; Cl, climate chamber; and RU, refrigeration unit.

that mesophytes and most xeromesophytes had close indicators of the germinating ability of freshly harvested seeds and 1-year-old stored seeds; in calciphiles (*V. jooi*), the germinating ability of freshly harvested seeds was higher than that in 1-year-old stored seeds; however, a different germination regime was required (Table 3). In the other germinating groups, i.e., mesoxerophytes (*V. ircutiana*), hygromesophytes (*V. selkirkii*), and lithophytes (*V. alexandrowiana* and *V. milanae*), the germinating ability of freshly harvested seeds was lower than that in 1-year-old stored seeds.

In the case of long storage of seeds, the germinating ability decreases in all species. In the case of dry storage of seeds in laboratory conditions, the period when the germinating ability decreased by 50% ( $P_{50}$ ) was 3 years in *V. cucullata* seeds; 4 years in most of the studied species (*V. alexandrowiana*, *V. czemalensis*, *V. ircutiana*, *V. jooi*, and *V. selkirkii* (Novosibirsk oblast)); 4 years in *V. variegata*; 5 years in *V. dactyloides*, *V. irinae*, *V. mandshurica*, and *V. milanae* (Tuva); 7 years in *V. dissecta*; and 8 years in *V. incisa*. The period during which more than 10% of germinating ability (profitability of introduction longevity)

was maintained corresponded to  $P_{50}$  in most of the studied species, except for *V. dissecta*, which had a profitability of introduction longevity exceeding  $P_{50}$  within 1 year and continuing for more than 8 years. The maintenance of germinating ability exceeding 0% (biological longevity) was 4–5 years for five species (*V. alexandrowiana*, *V. czemalensis*, *V. ircutiana*, *V. irinae*, and *V. selkirkii*) (Novosibirsk oblast). The highest biological longevity was found in *V. incise* (9 years).

As a result, a sharp decrease in the germinating ability up to 0% was observed in a number of species within 4–5 years ( $P_{50}$  and the biological longevity were coincident). Other species were divided into three groups: in the first group, the biological longevity was formed in the year following  $P_{50}$  (*V. jooi*, *V. mandshurica*, *V. dissecta*, and *V. incise*); in the second group, seeds did not germinate for 2 years after a 50% loss of germinating ability (*V. cucullata*, *V. variegata*, and *V. dactyloides*); the last group includes *V. milanae*, whose biological longevity (3 years after a 50% loss of germinating ability) was 8 years. We did not identify

**Table 4.** Characteristic of germination of the seeds of some species of the section *Violidum*

	Germinating ability	Germination energy	Intensity of germination energy
<i>V. alexandrowiana</i>			
$M \pm m$	52.4 ± 3.7	30.66 ± 3.6	58.0 ± 4.47
$V, \%$	31.22	52.43	34.50
min–max	26–80	6–73	16–94
<i>V. dactyloides</i>			
$M \pm m$	71.9 ± 5.9	62.5 ± 6.1	87.1 ± 3.7
$V, \%$	43.69	51.33	22.49
min–max	1–100	1–99	4–100
<i>V. incisa</i>			
$M \pm m$	72.1 ± 4.8	62.9 ± 5.1	80.0 ± 3.5
$V, \%$	39.9	48.8	25.9
min–max	7–100	2–100	18–100
<i>V. selkirkii</i>			
$M \pm m$	64.1 ± 4.0	17.7 ± 3.4	26.5 ± 3.9
$V, \%$	34.8	105.2	82.6
min–max	6–97	1–72	1.5–74

any relation of seed longevity and  $P_{50}$  with the ecological group of species.

The germination energy, as well as the germinating ability, changed depending on the period of seed storage (Table 3). In most of species, the germination energy of freshly harvested seeds was lower than that of 1-year-old stored seeds. In *V. alexandrowiana*, *V. milanae*, *V. selkirkii*, and *V. variegata*, these values were several times lower. These indicators are similar only in three species, i.e., *V. cucullata*, *V. dactyloides*, and *V. dissecta*. The intensity of germination energy, which reflects the dynamics of seed germination, is lower in freshly harvested seeds than that in 1-year-old stored seeds of most of the studied species. For many of them, these indicators are 30% different or more. In *V. cucullata*, *V. dactyloides*, and *V. irtutiana*, the dynamics of germination of freshly harvested seeds and 1-year-old stored seeds has no difference. In the case of long dry storage, the dynamics of seed germination is species-specific. Thus, a low variability of the intensity of germinating energy, depending on the period of seed storage, was previously recorded for *V. mirabilis* from the section *Mirabiles* (Elisafenko, 2012). However, in the section *Violidum*, we may observe an insignificant variability of values of this feature, depending on the period of storage (figure). *V. dactyloides* is characterized by the most common dynamics of seed germination. Based on the example of *V. dactyloides*, *V. alexandrowiana*, *V. incisa*, and *V. selkirkii*, we determined the arithmetic mean value, the coefficient of variation, and the range of values for germinating ability, as well as the energy of germination and the intensity of seed germination energy in the case of long storage in laboratory conditions (Table 4), and identified the coefficients of the correlation between these features, taking into account all replications (Table 5). The largest variation was found for germinating energy. Although the coefficient of the correlation between the germination energy and the intensity of

germination energy is high (0.48–0.92), the latter indicator, as well as the dynamics of seed germination, in the four species under consideration is less dependent on the storage period.

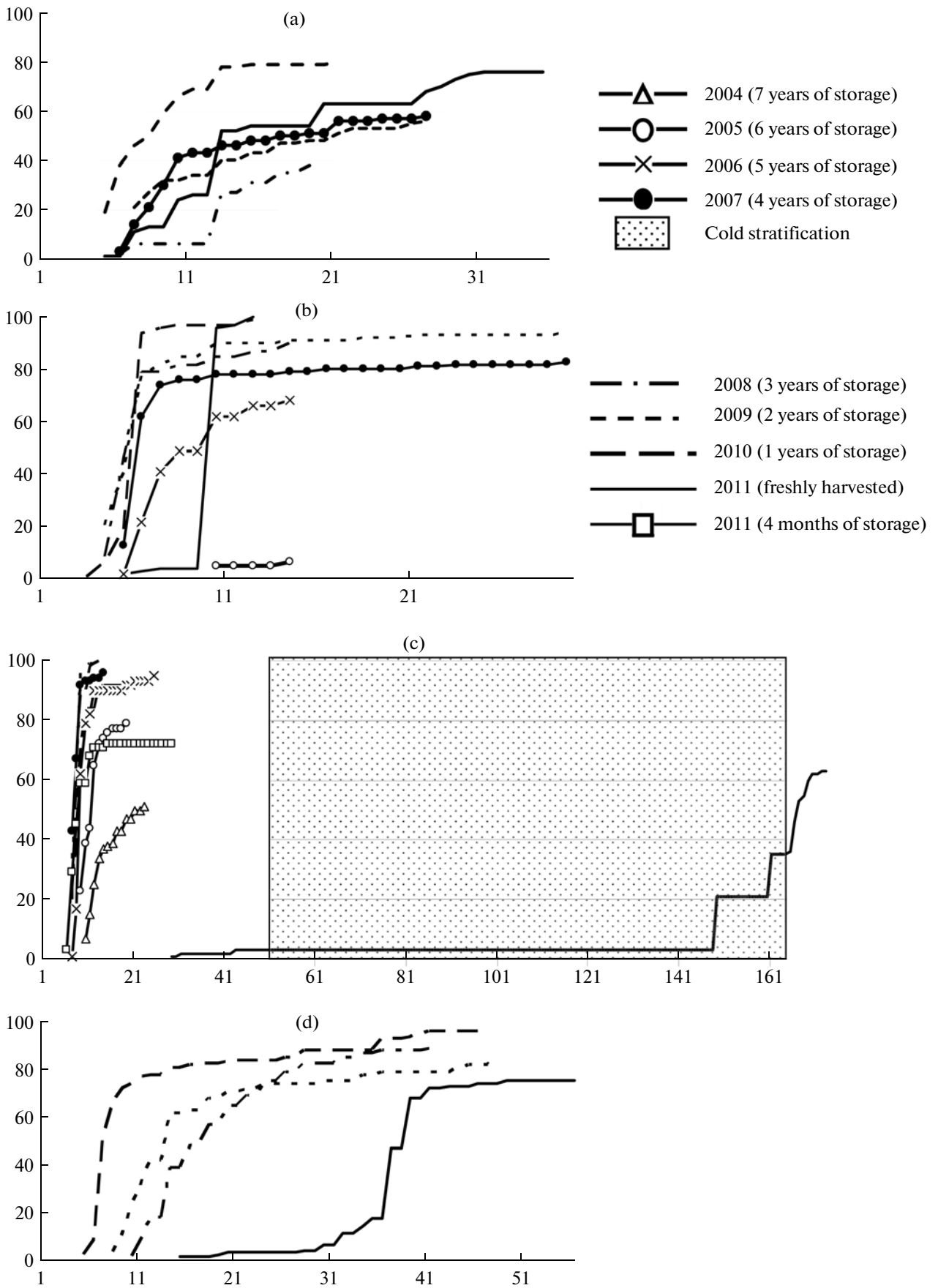
Therefore, differences between freshly harvested seeds and 1-year-old stored seeds have been found in all ecological groups with respect to the seed germination energy. It has been found that in lithophytes and mesoxerophytes, the intensity of germination energy is higher in 1-year-old stored seeds than in freshly harvested seeds. Hygromesophytes, mesophytes, and xeromesophytes had no difference in the dynamics of germination of freshly harvested seeds and 1-year-old stored seeds.

As a result, we have identified differences in different ecological groups with respect to the period when seeds begin to germinate, as well as to the period of seed germination and germinating ability. Hygromesophytes and mesophytes require a short period prior to seed germination (4–9 days), while the other ones require more than 10 days. The seeds of *V. Selkirkii* (hygromesophytes) have a specific long-term germination (70 days), while the other species germinate within approximately 30 days.

*V. selkirkii*, which is a representative of hygromesophytes, has the widest (Eurasian and North American) area among the studied species. One may assume that prolonged germination provides the survival of seedlings in unfavorable conditions and increases the likelihood for germination in different climatic zones; in addition, the association with moist habitats enables water supply in the optimal regime.

We have found that cold stratification is favorable for the germination of the seeds of ecologically specific species (lithophytes, i.e., *V. alexandrowiana*, *V. czemalensis*, and *V. milanae*, and the calciphile *V. jooi*). In some harvest years, without using cold stratification,





Dynamics of germination of the seeds of the Siberian species, genus *Viola* L.

The dynamics of seed germination. (A) *V. alexandrowiana*, Irkutsk oblast; (B) *V. dactyloides*, the Republic of Sakha; (C) *V. incisa*, Krasnoyarsk krai; and (D) *V. selkirkii*, Republic of Buryatia.

Days of the experiment are shown along the abscissa axis and the laboratory germinating ability is shown along the ordinate axis, %.

seeds failed to germinate, similarly to the freshly harvested seeds of *V. jooi* and *V. incisa*. All these species, beyond being ecologically specific, have a restricted area. All of them are endemics, from locally restricted to regional ones.

On the whole, for most of the species of the section *Violidum*, the highest indicators of the studied features are characteristic of the species of the group of xeromesophytes. Consequently, one may consider that moderately moist conditions are the most optimal for the genus. Mechanisms of plant adaptation for survival in different moisture supply conditions and plant adaptation for the edaphic factor are determined by the physiological processes during seed germination; thus, they are determined by the absence of seed dormancy for hygromesophytes and mesophytes and by the presence of physiological dormancy in freshly harvested seeds and seeds in separate harvest years, as well as by their prolonged germination, for lithophytes.

As a result, the seeds of the studied species are non-dormant or have B1 (a shallow physiological inhibition) according to classification by Nikolaeva (1977). V.I. Voroshilov (1960) notes that freshly germinated seeds in species of tropical origin do not have a dormant period and can germinate immediately after dissemination. Indeed, many of the studied species are characterized by seed germination as a result of self-seeding from June to September, when weather conditions are favorable for this (high temperature and moderate moisture). Species whose freshly harvested seeds did not immediately germinate included endemics of the south of Siberia (*V. czemalensis* and *V. incisa*), one endemic from Western Europe (*V. jooi*), and a North American species (*V. cucullata*). Specific requirements for seed germination have been determined with respect to two species: *V. jooi* and *V. incisa*. The first species has a restricted area and is exotic for Europe; in nature, it occurs in Romania and Ukraine and belongs to calciphiles. The second species is exotic for the Russian area and is an endemic of Southern Siberia. The freshly harvested seeds of these species failed to germinate, since they required cold

stratification or had to be stored in laboratory conditions for 3 months. At the same time, in some years (seed harvesting in 2013), cold stratification was also required after seed storage in laboratory conditions, since a single germination of seeds was found when germination was made in room conditions.

*V. incisa* (Krasnoyarsk krai) is distinguished among the studied species by its highest biological longevity (9 years) and different regimes of germination of freshly harvested seeds and long-term stored seeds. We have made a hypothesis on the hybridogenic nature of this species (Elisafenko, 2009; Elisafenko and Zhmud', 2011). This could be an explanation for the data that was obtained.

### CONCLUSIONS

The seeds of most of the studied species massively germinated at +25...+28°C. The seeds of *V. czemalensis* and *V. cucullata* species and the freshly harvested seeds of *V. incisa* and *V. jooi* germinated after two-stage stratification. The one-stage regime testifies to the tropical origin of the genus. The need for cold stratification is evidence of the species adaptation for conditions of middle latitudes.

In most species the period prior to germination was  $5 \pm 2$  days in 1-year-old seeds, being twice as long in freshly harvested seeds and continuing to extend with the storage period in 3-year-old seeds. The period of seed germination in most of species continued within 30 days. The maximum germinating ability of 1-year-stored seeds reached 90–100% in 10 of the 14 studied species. The lowest value was 40% in *V. jooi*.

In the case of dry storage of seeds in laboratory conditions, the loss of 50% of germinating ability corresponded to 4 years in most species. The highest indicator of biological longevity was found in *V. incisa* (9 years) and the lowest indicator was 4 years in *V. alexandrowiana*, *V. czemalensis*, *V. ircuitiana*, *V. irinae*, and *V. milanae*. For most of the studied species, the profitability of introduction longevity is 4 years, with the lowest longevity being 3 years (*V. cucullata*)

**Table 5.** Correlation of germinating ability, energy, and intensity of germination energy in the case of long-term storage in laboratory conditions

	Sample scop	Germinating ability/germination energy	Germination energy. Intensity of germination energy	Germinating ability/intensity of germination energy
<i>V. alexandrowiana</i>	20	0.64	0.78	0.09
<i>V. dactyloides</i>	28	0.84	0.48	0.02
<i>V. incisa</i>	36	0.98	0.92	0.89
<i>V. selkirkii</i>	31	0.59	0.84	0.15

and the highest longevity being 8 years (*V. incisa* and *V. dissecta*). The intensity of seed germination energy is less dependent on seed storage period than the germinating ability and germination energy are.

The short period prior to seed germination and the short period of germination itself provide the maximum germinating ability of seeds throughout summer.

Cold stratification is favorable for lithophytes and calciphiles, which are endemics.

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