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Effect of Temperature on Seed Germination of Korean Native *Viola* Species

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Abstract - Present studies were performed to determine the physiology of seed germination in *Viola* species native to Korea. Twelve species, 1 variety and 1 form were collected, classified and used as materials: *V. collina*, *V. blandaefomis*, *V. rosii*, *V. chaerophylloides*, *V. phalacrocarpa*, *V. patrinii*, *V. mandshurica*, *V. mandshurica* for. *albescence*, *V. seoulensis*, *V. yedoensis*, *V. keiskei*, *V. variegata*, *V. variegata* var. *chinensis*, and *V. verecunda*. *V. tricolor* 'Helen Mount' was also used to compare wild with cultivated species. In order to investigate the effect of temperature on seed germination, seeds stored at 4±2℃ for 10 months or 4 years were incubated at 10, 15, 20, 25℃ under 16h illumination with 4 replicates per treatment. Seeds which had not germinated at 10℃ were transferred to 30℃ to assess the effect of temperature change in germination. Germination percent and the days of first, 40% and 80% germination were assessed. Capability of seed germination varied with taxon; Species belonging to subsection Patellares had high ability of germination, compared to species in the other subsections, and series Chinensis was the best among subsection Patellares. Species capable of high germination germinated in all temperatures with reasonably high germination rate, but the other species responded sensitively to temperature with different germination patterns. Higher the temperature, shorter the incubation time required for first, 40% and 80% of germination. Therefore, high temperature was effective in almost all species, not only for inducing high rate of germination but also the uniformity of germination. Temperature change from 10℃ to 30℃ had a positive effect on seed germination.

Key words - Seed germination, *Viola*, Temperature, Taxon

Introduction

The common pansy (*Viola tricolor* L. var. *hortensis* DC), most widely grown *Viola* species in the world, is a horticultural hybrid involving 5 species - European *V. tricolor*, *V. lulea*, *V. cornuta*, *V. attaica* and *V. calearata* (Lee, 1998). It was originally a perennial with winter hardiness, but shows depressed summer growth caused by high temperature above 30℃ in Korea.

The genus *Viola* in Korea consists of three sections, Dischidium, Chamaemelanium and Nominium, based on morphological characters (Lee, 1993). Most *Viola* species belonged to the section Nominium which consists of six subsections (Ko *et al.*, 1998). The subsection Patellares is the largest and contains three quarters of *Viola* species in Nominium (Lee, 1993).

Korean native *Viola* species are useful genetic resources for Pansy breeding, because they have strong heat tolerance (Oh *et al.*, 1998). Therefore, native *Viola* species can be used not only for improving summer depression of pansy that is grown as spring bedding plants, but also for enhancing many characters of pansy such as color, aroma, plant shape, hardiness and so on (Lee, 1998). Native species can be also developed to become materials for indoor planting, ground covers and hanging baskets (Lee, 1993). Therefore we need to know more about physiology and cultivation methods for masspropagation and plant breeding of *Viola* species (Lee *et. al.*, 2003). Especially, information on the physiology concerning seed germination is absolutely necessary for basic research as well as for propagation of species in nature.

However, reports on the seed germination is very scarce (Lee and Han, 1994). The objective of present experiment is to study the effects of different temperature on the seed germination in the genus *Viola*.

Materials and Methods

Plants of many *Viola* species were collected from native habitat from March to September and transplanted to the Chungbuk National University farm. They were grown under 70% shade for one or two more years, identified and classified for further experiment. Some of the characters used for species classification are plant vigor, presence of stem, leaf shape, leaf margin, leaf top, leaf base, leaf vein, stipule, stigma, spur, capsules, petals, shape of rhizome and root, presence of flower color and hair, and shape of bracts. The information on the natural habitat were also collected and used for the analysis of the experiment.

Table 1 lists the name of *Viola* species employed in this

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experiments. Seeds of all *Viola* species were maintained in refrigerator (4±2℃) for 10 months, except in 2 species, *V. collina* and *V. verecunda* for 4 years. After inspecting the seed purity, 100 seeds were placed on 2 moist Whatman filter paper in 9cm petri-dish and kept in incubators of 10, 15, 20, 25℃ under 16 hour light periods. Each treatment consists of 4 replicates.

Petri-dishes were examined every day for seed germination pattern: days of first germination, 40% and 80% germination, and overall germination percentage were recorded. Not germinated seeds at 10℃ after 50 days were transferred to 30℃ and same germination characters were investigated.

Results and Discussion

Viola species are usually propagated by seeds and grow well on soil containing granite, gneiss and porphyry. During the cultivation of *Viola* species for this experiment, seeds of some species were observed to germinate immediately after separation from capsules, while some germinated during following spring. There was a great deal of variation in the number of seedling emergence after natural sowing. The cause of this variation, whether it is genetic or physiological, should be elucidated. If it is caused by physiological factors, temperature is thought to be one of the most important environmental

Table 1. Taxon, scientific and Korean name of *Viola* species used in this study

Taxon		Scientific name	Korean name
Subsection	Series		
Hypocarpae	-	*V. collina*	둥근털제비꽃
Plagiostigma	-	*V. blandaefomis*	엷은잎제비꽃
Vaginatae	-	*V. rosii*	고깔제비꽃
Patellares	Pinnatae	*V. chaerophylloides*	남산제비꽃
	Chinensis	*V. phalacrocarpa*	털제비꽃
		V. patrinii	흰제비꽃
		V. mandshurica	제비꽃
		V. mandshurica for. *albescence*	흰꽃제비꽃
		V. seoulensis	서울제비꽃
		V. yedoensis	호제비꽃
	Variegatae	*V. keiskei*	잔털제비꽃
		V. variegata	알록제비꽃
		V. variegata var. *chinensis*	자주알록제비꽃
Bilobatae	Verecundae	*V. verecunda*	콩제비꽃

Table 2. Effect of temperature on germination percent in *Viola* species

Plant	Germination (%)			
	10℃	15℃	20℃	25℃
*V. collina**	3.5± 1.5	3.3± 0.6	3.3± 0.6	8.8± 3.1
V. rossii	0	0	0	0
V. verecunda	7.3± 4.7	15.0± 7.9	6.8± 4.0	5.3± 5.1
*V. verecunda**	10.3± 1.2	10.3± 1.5	5.3± 1.5	5.3± 2.5
V. blandaeformis	8.5±12.0	59.3±10.5	52.5±13.4	25.0±10.3
V. chaerophylloides	45.0± 1.7	47.3± 1.2	20.0± 3.6	19.3±12.5
V. variegata	72.0±18.1	72.3±28.0	52.0± 6.9	55.8±22.5
V. variegata var. *chinensis*	50.0± 9.5	57.0±11.5	60.8± 4.7	54.8± 9.9
V. keiskei	34.0± 9.8	39.0±21.7	48.8± 7.5	47.8±13.9
V. phalacrocarpa	67.3±23.9	80.8±12.1	83.3±18.9	60.0± 9.8
V. seoulensis	92.0± 3.0	95.0± 3.6	97.0± 3.0	78.3± 1.5
V. patrinii	25.3±11.6	17.3± 7.1	56.3±16.9	65.8±20.0
V. mandshurica	87.0± 5.3	96.3± 1.5	93.7± 4.5	95.3± 4.5
V. mandshurica for. *albescence*	48.0±18.7	65.5±16.3	72.0±28.6	64.3±11.0
V. yedoensis	84.0±14.7	93.0± 1.0	88.8±12.7	90.3± 8.7
V. tricolor 'Helen Mount'	88.0± 3.6	93.3± 2.5	88.0± 4.4	89.3± 3.2

*4 years cold (4℃) pretreatment.

conditions affecting seed germination. So 4 different temperature regimes were employed to see the effects of temperature on seed germination in *Viola* species.

Table 2 shows that percent germination varied greatly according to species, especially to taxon. Regardless of storage temperature, species belonging to subsection Patellares had higher germination rate than species in other subsections. The decreasing order of percent germination is *V. blandaefomis* in subsection Plagiostigma, *V. verecunda* in Bilobatae, *V. collina* in Hypocarpae and *V. rosii* in Vaginatae. In Patellares, series Chinensis had the highest germination rate, followed by series Variegatae and series Pinnatae. The species with higher percent germination generally required less number of days for the first germination, 40% and 80% germination, and they also showed more uniform germination than other species (Table 3-5). Lee and Han (1994), reporting similar results, said that germinability is controlled by genetical factors as well as by the density of species in nature.

Table 3. Effect of temperature on first germination days in *Viola* species

Plant	First germination days			
	10℃	15℃	20℃	25℃
*V. collina**	22.0± 6.9	29.5±14.6	32.0±22.5	23.3± 5.5
V. rossii	-	-	-	-
V. verecunda	24.3± 7.8	16.0± 4.6	19.0± 1.2	10.0± 3.5
*V. verecunda**	21.0±10.4	10.3± 1.5	35.0±20.8	27.0± 9.6
V. blandaeformis	18.3± 2.6	4.0± 0.6	7.5± 0.7	6.0± 0.6
V. chaerophylloides	15.0± 0.0	8.8± 1.2	8.0± 1.0	5.0± 0.0
V. variegata	12.0± 2.0	6.0± 0.0	4.3± 0.6	3.0± 0.0
V. variegata var. *chinensis*	15.0± 0.0	9.3± 0.6	5.0± 0.0	4.3± 0.6
V. keiskei	10.0± 2.6	6.8± 0.6	3.8± 0.6	3.0± 0.0
V. phalacrocarpa	15.0± 0.0	8.0± 0.0	4.0± 0.0	4.0± 0.0
V. seoulensis	8.0± 0.0	5.0± 0.0	3.0± 0.0	3.0± 0.0
V. patrinii	21.0± 5.6	6.3± 2.3	4.0± 0.0	3.0± 0.0
V. mandshurica	13.0± 1.7	8.0± 0.0	4.0± 0.0	3.0± 0.0
V. mandshurica for. *albescence*	17.0± 1.4	7.8± 0.4	3.3± 0.5	3.3± 0.5
V. yedoensis	11.0± 0.0	5.3± 0.6	4.0± 0.0	3.0± 0.0
V. tricolor 'Helen Mount'	8.0± 0.0	5.0± 0.0	2.0± 0.0	2.0± 0.0

*4 years cold (4℃) pretreatment.

Table 4. Effect of temperature on 40% germination days in *Viola* species

Plant	40% germination days			
	10℃	15℃	20℃	25℃
*V. collina**	-	-	-	-
V. rossii	-	-	-	-
V. verecunda	-	-	-	-
*V. verecunda**	-	-	-	-
V. blandaeformis	-	15.0± 1.0	11.5± 0.6	-
V. chaerophylloides	36.0± 5.2	63.0± 2.0	-	-
V. variegata	16.0± 1.7	26.7±32.3	9.0± 4.4	4.0± 0.0
V. variegata var. *chinensis*	27.5± 0.7	28.7±28.0	7.7± 0.6	6.7± 0.6
V. keiskei	-	11.0± 1.0	7.5± 0.6	5.0± 0.0
V. phalacrocarpa	31.7±14.4	11.0± 1.0	6.3± 1.5	5.7± 1.5
V. seoulensis	17.0± 0.0	12.0± 1.0	13.3± 0.6	11.7± 5.8
V. patrinii	-	-	7.0± 0.0	5.0± 0.6
V. mandshurica	19.7± 1.2	10.3± 1.2	5.3± 0.6	4.0± 0.0
V. mandshurica for. *albescence*	34.0±12.1	24.8±11.9	5.5± 1.0	5.0± 1.4
V. yedoensis	16.3± 3.1	7.7± 0.6	5.7± 2.1	3.7± 0.6
V. tricolor 'Helen Mount'	9.0± 0.0	5.0± 0.0	4.0± 0.0	2.0± 0.0

*4 years cold (4℃) pretreatment.

Table 5. Effect of temperature on 80% germination days in *Viola* species

Plant	80% germination days			
	10℃	15℃	20℃	25℃
V. collina[*]	-	-	-	-
V. rossii	-	-	-	-
V. verecunda	-	-	-	-
V. verecunda[*]	-	-	-	-
V. blandaeformis	-	-	-	-
V. chaerophylloides	-	-	-	-
V. variegata	-	-	-	-
V. variegata var. *chinensis*	-	-	-	-
V. keiskei	-	-	-	-
V. phalacrocarpa	-	49.5±16.3	28.5±12.0	-
V. seoulensis	28.0± 2.6	20.0± 5.0	40.3± 5.1	-
V. patrinii	-	-	-	-
V. mandshurica	33.3± 5.5	13.0± 0.0	8.3± 1.5	5.8± 2.1
V. mandshurica for. *albescence*	-	-	-	-
V. yedoensis	33.0±21.2	16.8± 9.1	7.0± 0.0	18.3±10.0
V. tricolor 'Helen Mount'	11.0± 1.0	7.0± 1.7	6.0± 1.0	4.0± 1.7

[*]4 years cold (4℃) pretreatment.

Species with high percent germination, such as *V. seoulensis* (97%) and *V. patrinii* (65.8%), showed more than 50% germination, regardless of temperature level, indicating good germination capacity from spring season to fall. Optimum germination temperature was relatively high with 20~25℃.

The results of present experiments showed that Korean *Viola* species had no seed dormancy, so seeds germinated right away if adequate environmental condition, such as moisture, existed. And many seedlings were observed to be growing on the field during the seeding period. Similar results were reported by Lee and Han (1994). It is likely that the ease of germination makes the species with high germinability dominant in natural habitats. Among 6 species of series Chinensis in Patellares, *V. patrinii* showed 25.3~17.3% germination by 10~15℃ treatments. *V. mandshurica* for. *albescence*, a variant of *V. mandshurica*, had lower percent germination than original species, which is commonly observed phenomena.

Some taxonomists classified *V. phalacrocarpa* and *V. seoulensis* as same species, but in this experiment they showed different pattern of germination with latter having higher rate. In *V. seoulensis*, good and uniform germination was observed with all temperature level, while in *V. phalacrocarpa* generally lower germination percentage was obtained with all treatments, which makes 2 species improbable as same species.

V. yedoensis also demonstrated more than 80% germination at all temperatures. The species belonging to series Variegatae in Patellares and *V. chaerophylheides* showed lower germination percentage than species of Chinensis. Highest percentage was 47.3% in *V. chaerophylloides* and 72.3% in *V. variegata*. Optimum germination temperature was 10-15℃ in *V. chaerophylloides* and *V. variegata* while it was 20-25℃ in *V. keiskei*.

Low percentage less than 15% were observed with *V. verecunda* in Bilobatae, *V. collina* in Hypocarpae and *V. rosii* in Varginatae, except 59% in *V. blandeformis*. *V. rosii* did not germinate at all treatments, which might be caused by the loss of germination ability by the 10 month cold treatment. Optimum temperature for germination varied - *V. collina* at 25℃, *V. verecunda* 10-15℃ and *V. blandaeformis* 15-20℃. To assess seed longevity, the seeds of *V. verecunda* were cold treated for 10 months and 4 years and subjected to germination tests. There was no differences in germination ability, indicating long life of *V. verecunda* seeds.

To compare Korean native *Viola* species with European small pansy species, the seeds of *V. tricolor* 'Helen mount' was employed for gemination tests. Pansy showed more than 88% uniform germination at all temperature, which is similar pattern of germination in Patellares.

To assess the germination speed and uniformity, days required for the first germination and 40% and 80% germination were shown in Table 3-5. In general, species with high percentage showed high germination speed and rapid termination of seed germination. Initiation, 40% and end of germination were hastened by higher temperature, especially most evident at 25℃. The species with high germination rate, Patellares and small pansy, showed rapid germina-

tion initiation (2-5 days) and *V. blandaeformis* was in next order with 6 days. *V. collina* which showed the lowest germination rate needed 22 days for the start of germination. Same trend was observed with 40% germination and termination of germination measured by 80% germination was observed only in Patellares and small pansy. Seeds of Patellares and small pansy that had high germination rate germinated rapidly at 25℃. Especially in pansy, initiation and 40% germination occurred within 2 days and germination ending within 4 days. All tested species germinated completely within 3 days, demonstrating uniformity in germination. Germination was delayed at low temperature, but even at 10℃ germination was terminated within 11 days.

V. chaerophylloides showed different germination pattern compared to other Patellares - the species germinated better at low temperature. Under 10℃, final germination percentage was 45%, while under 20~25℃ it was 20%. In other species of Patellares, high temperature treatments generally enhanced seed germination. Eighty percent germination was achieved at 25℃ in *V. mandshurica*, 20℃ in *V. phalacrocarpa* and *V. yedoensis*, and 15℃ in *V. seoulensis*. In *V. variegata*, germination percentage was below 80%. Days required from germination initiation to 80% germination in some species was 5.8 at 25℃ in *V. mandshurica*, 7 at 20℃ in *V. yedoensis*, 20 at 15℃ in *V. seoulensis* and 28.5 at 20℃ in *V. phalacrocarpa*.

In *V. blandaeformis*, seeds started to germinate after 4 days at 15℃ with 59% germination, but 40% germination rate occurred within 11.5 days at 20℃, compared to 15 days at 15℃.

V. collina germinated most slowly among all species, reaching final germination rate of only 8.8%. In *V. verecunda*, 2 different cold treatments, 10 month and 4 years, yielded different results. At 25℃, germination initiated after 10 days by former treatment, but 10.3 days at 15℃ by latter treatment. However, germination percentage was too low to get any information on the effect of pretreatment duration on the seed germination in this species.

Considering the general beneficial effects of high temperature on seed germination, we transferred seeds that were exposed to 10℃ to 30℃ and the results are shown in Table 6. In general, germination was promoted by high temperature. For example, in *V. verecunda*, 10.3~15% germination obtained after 50 days at 10-25℃ was increased to 16.1~16.3% at 30℃ after 30 days, which suggested beneficial effect of change from 10℃ to 30℃ on seed germination. Similar results were obtained in *V. collina* in which 8.8% germination at 25℃ was increased to 15.3% at 30℃. Further studies on the effect of change in temperature on the seed germination in *Viola* are needed.

In conclusion, plants of *Viola* species could be easily propagated by seeds even in hot, humid summer season. Depending on taxon, species have different germination ability. Species of subsection Patellares have higher ability than other subsections. In Patellares, series Chinensis had higher ability compared to other series.

Temperature experiments showed that germination of species with high germination ability were not affected by different treatments, but germination was influenced by treatments in cases of species with low germination ability. It was observed that the days for first, 40%

Table 6. Effect of temperature change from 10℃ to 30℃ on seed germination in *Viola* species

Plant	Germination percent	First germination days	40% germination days	80% germination days
V. collina	15.3± 7.3	2.0±8.0	-	-
V. rossii	-	-	-	-
V. verecunda	16.3±16.0	4.3±1.2	-	-
V. verecunda *	16.1± 7.8	2.0±0.0	-	-
V. blandaeformis	1.2± 1.7	3.0±0.0	-	-
V. chaerophylloides	12.2± 4.9	2.7±1.5	-	-
V. variegata	10.0±10.6	4.0±1.7	-	-
V. variegata var. *chinensis*	5.4± 1.3	2.3±2.3	-	-
V. keiskei	2.7± 2.2	9.7±5.5	-	-
V. phalacrocarpa	4.7± 6.8	2.0±0.0	-	-
V. seoulensis	14.3±20.3	2.0±0.0	-	-
V. patrinii	44.0±33.2	2.0±0.0	5.3±1.2	-
V. mandshurica	44.7± 9.9	2.0±0.0	14.0±7.1	-
V. mandshurica for. *albescence*	16.7±11.7	2.0±0.0	-	-
V. yedoensis	18.0±15.6	2.0±0.0	-	-
V. tricolor 'Helen Mount'	0	-	-	-

*4 years cold (4°C) pretreatment.

and 80% germination were hastened by high temperature, and germination rate and percentage were promoted under higher temperature. Changes from low temperature to high temperature resulted in better germination ability.

It seems that germinability generally poses no problem for the breeding and cultivation of *Viola* species. However, for species with low germination ability, germination tests should be continued to find stable and reliable propagation methods. Some other information on seed germination is ① whether seeds require darkness for germination, ② effect of seed maturity on seed longevity and germination. For the seeds with still low germination rate after extensive studies, various measures to enhance germination rate such as washing seeds with water, mechanical and acid scarification to seed coat, change in moisture in seeds, and use of growth regulators are recommended to be employed for further studies.

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