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## Seed germination of the endangered *Viola kitaibeliana* and other Italian annual pansies (*Viola* Section *Melanium*, *Violaceae*)

### Abstract

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Here, successful germination protocols for the following four annual pansies are presented: *Viola arvensis* subsp. *arvensis*, *V. hymettia*, *V. kitaibeliana*, and *V. tricolor* subsp. *tricolor*. Seeds were collected mainly in Central Italy (Tuscany, Umbria, and Latium) and in Sicily. The germination ability was tested at the Tuscia Germplasm Bank (BGT) using several methods for dormancy-breaking. Our results show high germination percentages and germination rate for the tested species.

*Key words:* cold stratification, dormancy, priming, *Viola arvensis*, *Viola hymettia*, *Viola tricolor*.

### Introduction

The Sect. *Melanium* DC. ex Ging. of the genus *Viola* (the so-called pansies) is a derived, monophyletic and morphologically well-defined group comprising 125 species, both perennials and annuals (Marcussen & al. 2015). The genus is basically myrmecochorous but different selective pressures, especially seed predation, have produced a clear divergence in dispersal systems. Particularly, in the Sect. *Melanium* diplochory is exhibited by most species which have evolved a system combining ballistic and ant seed dispersal (Beattie & Lyons 1975; Beattie 1985). Seeds are brown to dark brown (only immature ones are pale brown), ovoid to obovoid in shape, all with an elaiosome in the micropylar area and a conspicuous raphe (Scoppola & Magrini 2019).

Pansy seeds have a physiological dormancy (Baskin & Baskin 1995; 2014), so pre-treatments for dormancy-breaking like cold stratification and/or hormones are required for germination. Elaiosome removal from seeds functions as a germination cue in many ant-dispersed plant species (e.g., Horvitz 1981; Blois & Rockwood 1985; Lobstein & Rockwood 1993; Ohkawara 2005). Elaiosome is also known to inhibit seed germination due to the presence of water-soluble inhibitory substances (Sasidharan & Venkatesan 2019). On the other hand, its removal did not improve germination success in *Viola striata* Aiton (Lobstein & Rockwood 1993), *Viola pedata* L. (Gehring & al. 2013), and *Viola pedunculata* Torr. & A. Gray. (Franklin & al. 2017), so in our research elaiosomes were not removed from seeds.

This paper is part of a larger research on the Sect. *Melanium* in Europe (e.g. Magrini & Scoppola 2015a,b,c; Magrini 2019; Scoppola & Magrini 2019). Particularly, research focused on germination ecology of annual pansies are carried out at the Tuscia Germplasm Bank (BGT) where seed accessions collected mainly in Central Italy are long term preserved. Here we present successful germination protocols of four closely related European pansies occurring in Italy: first data for *V. kitaibeliana* Schult. and *V. hymettia* Boiss. & Heldr., and new data for Italy for *Viola arvensis* Murray subsp. *arvensis* and *V. tricolor* L. subsp. *tricolor*.

### 38. *Viola arvensis* Murray subsp. *arvensis* (*Violaceae*) (Fig. 1a)

#### Accession data

- It:** Tuscany. Sarteano (Siena), Mt. Cetona (WGS84: 42.931109°N, 11.875547°E), edge of stony path on calcareous substrate, 1100 m a.s.l., 17 May 2012, *A. Scoppola* (BGT-A-29512, Tuscia Germplasm Bank).
- It:** Umbria. Norcia (Perugia), Forca d'Ancarano (WGS84: 42.819999°N, 13.117497°E), margin of cultivated field, 1013 m a.s.l., 25 May 2012, *A. Scoppola* (BGT-A-29612, Tuscia Germplasm Bank).
- It:** Latium. Monte San Giovanni in Sabina (Rieti), Mt. Tancia (WGS84: 42.315850°N 12.744658°E), stony grassland on calcareous soil, 1180 m a.s.l., 20 May 2014, *A. Scoppola* (BGT-A-28614, Tuscia Germplasm Bank).
- It:** Latium. Tivoli (Roma), Acque Albule (WGS84: 41.963056°N, 12.714722°E), dry grassland on a travertine outcrop, 66 m a.s.l., 20 May 2014, *S. Magrini* (BGT-A-28913, Tuscia Germplasm Bank).

#### Germination data

*Pre-treatments:* sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes, followed by 3 rinses in sterile distilled water. Cold-moist stratification for 1 month at 5°C.

*Germination medium:* 1% agar + 250 mg/l GA<sub>3</sub>.

*Sample size:* 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]	Accession code
100%	constant 20°C	12/12h	6.0	12.2	24.0	12.3	BGT-A-28614
100%	constant 20°C	12/12h	7.0	10.6	30.0	17.3	BGT-A-29512
100%	constant 20°C	12/12h	8.6	13.6	21.3	14.3	BGT-A-29612
95.2%	constant 20°C	12/12h	5.0	9.6	21.7	11.3	BGT-A-28913

#### Observations

*Viola arvensis* subsp. *arvensis* (field pansy) is a Mediterranean-Eurasiatic winter annual pansy widespread throughout Europe and SW Asia as a weed in cultivated

fields, in synanthropic habitats, and open shrublands (Scoppola & Magrini 2019).

Germination tests were carried out with or without cold stratification. The effect of both hydro- and hormo-priming was tested, soaking seeds, respectively, in distilled water and in a solution of gibberellic acid (250 mg/l) for 24 h at 20°C in darkness. Seeds were sown on water-agar with or without the addition of gibberellic acid. No germination was recorded without cold stratification nor for primed seeds, contrarily to results for hydro-priming (ca. 40%) reported by Rajabalipour & al. (2013). High germination (> 95%) was obtained at 20°C only with the combination of both cold stratification and the addition of GA<sub>3</sub> to the substrate, in accordance with results reported by Royal Botanic Gardens Kew (2020). Moreover, seeds were sorted into two colour fractions, pale (immature) and dark brown (mature), to assess seed maturity effect on germination. Both germination percentage and rate were higher for dark than pale brown seeds (100% vs 6.7% and T<sub>50</sub>: 9.9 vs 20.0, respectively), confirming that seed maturity also affects *V. arvensis* seed germination, as reported for *V. wittrockiana* Gams (Agnieszka & Holubowicz 2008).

### 39. *Viola hymettia* Boiss. & Heldr. (*Violaceae*)

#### Accession data

- It:** Latium. Viterbo (Viterbo), loc. Asinello (WGS84: 42.401150°N, 12.072031°E), dry grassland on volcanic soil, 276 m a.s.l., 18 Apr 2018, *L. Cracco* (BGT-A-48318, Tuscia Germplasm Bank).
- Si:** Piazza Armerina (Enna), Cannarozzo (WGS84: 37.409167°N, 14.380556°E), clearing in a pine forest, 730 m a.s.l., 21 Mar 2019, *R. Galesi* (BGT-A-68619, Tuscia Germplasm Bank).

#### Germination data

*Pre-treatments:* sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

*Germination medium:* 1% agar + 250 mg/l GA<sub>3</sub>.

*Sample size:* 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]	Accession code
88.7%	constant 15°C	12/12h	6.8	12.4	22.3	13.1	BGT-A-48318
84.9%	constant 15°C	12/12h	6.0	8.8	24.3	11.7	BGT-A-68619

#### Observations

*Viola hymettia* is an annual pansy widespread in the southern Balkans and the Aegean Islands, quite rare in central and southern Italy and Sicily, linked to stony pas-

tures, dry open habitats and shrub fringes, mostly on sub-acid soils (Scoppola & Magrini 2019).

Here we report the first germination data for *Viola hymettia*. Cold stratification was not required in accordance with results for *V. calaminaria* (Ging.) Lej. (Godefroid & Van de Vyver 2020). The effect of both hydro- and hormo-priming was tested, soaking seeds, respectively, in distilled water and in a solution of gibberellic acid (250 mg/l) for 24 h at 20°C in darkness and sowing them on water-agar medium. Not primed seeds were sown on water-agar with the addition of gibberellic acid (250 mg/l). High germination (> 84%) was obtained at 15°C only for not primed seeds on the substrate with the addition of GA<sub>3</sub>. Very low germination was recorded for both hydro- and hormo-primed seeds of all the tested accessions (0-2% and 8-9%, respectively).

#### 40. *Viola kitaibeliana* Schult. (*Violaceae*) (Fig. 1b)

##### Accession data

- It:** Latium. Nespolo (Rieti), Cerreta (WGS84: 42.162784°N 13.080031E), open grassland on calcareous soil, 1000 m a.s.l., 16 Jun 2010, *A. Scoppola* (BGT-A-14710, Tuscia Germplasm Bank).
- It:** Latium. Varco Sabino (Rieti), Mt. Navegna (WGS84: 42.234799°N 12.992089°E), stony grassland on calcareous soil, 1184 m a.s.l., 2 Jun 2014, *A. Scoppola* (BGT-A-28714, Tuscia Germplasm Bank).
- It:** Umbria. Norcia (Perugia), Forca d’Ancarano (WGS84: 42.819651°N 13.117618°E), stony meadow between fields on calcareous soil, 1010 m a.s.l., 11 May 2012, *A. Scoppola* (BGT-A-29712, Tuscia Germplasm Bank).

##### Germination data

*Pre-treatments:* sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

*Germination medium:* 1% agar + 1) 250 mg/l GA<sub>3</sub> and 2) 500 mg/l GA<sub>3</sub>.

*Sample size:* 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]	Accession code
100% <sup>1</sup>	constant 20°C	12/12h	6.0	12.2	19.0	12.3	BGT-A-28714
100% <sup>2</sup>	constant 20°C	12/12h	8.0	11.7	17.3	12.1	BGT-A-14710
93.3% <sup>1</sup>	constant 20°C	12/12h	7.0	11.6	21.7	12.2	BGT-A-14710
80.3% <sup>1</sup>	constant 20°C	12/12h	6.0	5.8	24.7	9.8	BGT-A-29712

##### Observations

*Viola kitaibeliana* is a winter annual small-flowered pansy extremely rare in Europe where it is a component of early stages of grasslands, stony slopes, and other open places

(Randal 2004; Magrini & Scoppola 2015a, 2015b). In particular, it has a highly fragmented distribution in Italy, where it is endangered. Here we report the first germination data for this species.

Germination tests were carried out after 0 and 1 month of cold stratification at 5°C. Seeds were sown on water-agar with the addition of gibberellic acid. No germination was recorded without cold stratification. High germination (> 80%) was obtained at 20°C only with the combination of both cold stratification and the addition of GA<sub>3</sub> to the substrate.

#### 41. *Viola tricolor* L. subsp. *tricolor* (Violaceae)

##### Accession data

**It:** Latium. Vejano (Viterbo), loc. Acquaforte (WGS84: 42.218919°N 12.110024°E), shrubby grassland, 353 m a.s.l., 14 May 2017, S. Buono (BGT-A-41517, Tuscia Germplasm Bank).

##### Germination data

*Pre-treatments:* sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

*Germination medium:* 1% agar + 250 mg/l GA<sub>3</sub>.

*Sample size:* 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
83.1%	constant 15°C	12/12h	7.0	13.3	24.5	13.6

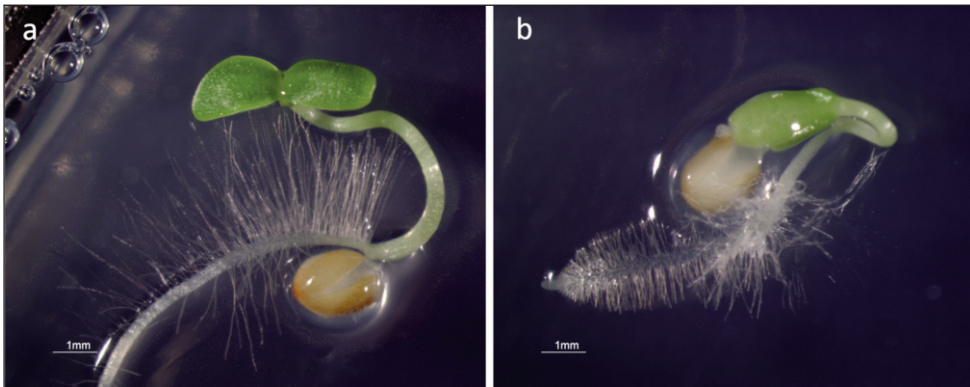


Fig. 1. Germinated seeds of: **a**, *Viola arvensis* subsp. *arvensis* and **b**, *V. kitaibeliana*.

## Observations

*Viola tricolor* subsp. *tricolor* is a pansy occurring in habitats linked to woodlands and semi-natural lands in south Europe (Scoppola & Magrini 2019).

Following Godefroid & Van de Vyver (2020), cold stratification was not required. The effect of both hydro- and hormo-priming was tested, soaking seeds, respectively, in distilled water and in a solution of gibberellic acid (250 mg/l) for 24 h at 20°C in darkness and sowing them on water-agar medium. Not primed seeds were sown on water-agar with the addition of gibberellic acid. High germination (> 83%) was obtained at 15°C only for not primed seeds on the substrate with the addition of GA<sub>3</sub>, in accordance with results reported by Royal Botanic Gardens Kew (2020). Lower germination was recorded for both hydro- and hormo-primed seeds (4% and 16%, respectively).

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