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## Seed germination protocols in the genus *Salsola* (*Amaranthaceae*) in Italy

### Abstract

Magrini, S., Alonso Simón, A., Zucconi, L. & Cristaudo, A.: Seed germination protocols in the genus *Salsola* (*Amaranthaceae*) in Italy [In Magrini, S. & Salmeri, C. (eds), Mediterranean plant germination reports – 3]. Fl. Medit. 31: 299-304. 2021. <http://dx.doi.org/10.7320/FIMedit31.299>

Here, successful germination protocols for the following three annual *Salsola* species are presented: *Salsola soda* (= *Soda inermis*), *S. squarrosa* subsp. *controversa*, and *S. tragus* subsp. *tragus*. It is the first report of germination data for the three species for Italy. Seeds were collected in beaches and dunes along the Tyrrhenian coasts in Sicily and south Tuscany and north Latium (central Italy). The germination ability was tested at the Catania Germplasm Bank (BGS-CT) and the Tuscia Germplasm Bank (BGT) at constant and alternating temperatures (from 5 to 40°C), under both light (with a 12/12h photoperiod) and total dark. Our results show a high germination ability and germination rate for all the tested species.

*Key words:* beach, coastal dunes, halophytes, Latium, psammophytes, Sicily, Tuscany.

### Introduction

Annual *Salsola* L. species (*Amaranthaceae*), generally, have a relatively longer life cycle from seed germination in spring to fruit ripening in late autumn. Like other species of arid areas, they are characterized by germination heterochrony, the germination of seeds dispersed in a single season, which exhibits continuous germination during a long period (Liu & al. 2013). These species are defined as “very fast germinating species” (Parsons 2012) because seeds germinate in a very short period after the substrate is wetted. They produce seeds with a spiral embryo that is completely developed (Parsons 2012), so the cells can simply elongate after imbibition, rapidly breaking the fruit cover in just one to three days (Wallace & al. 1968). This allows a rapid response to favourable environmental conditions (Liu & al. 2013). The germination timing and rate are particularly important for species growing in stressful habitats like saline deserts (Luciani & al. 2001; Gul & al. 2013), where the establishment of a new generation depends on narrow opportunity windows. This kind of germination is the adaptive strategy of desert halophytes because the salinity of the soil is reduced by the rain only for a short duration, so rapid responses, like

imbibition and rapid germination, increase their colonization ability (Luciani & al. 2001; Liu & al. 2013).

Here we present successful germination protocols for three species of *Salsola* occurring along the coasts of Sicily, *Salsola soda* L., and the Italian peninsula, *S. squarrosa* Steven ex Moq. subsp. *controversa* (Tod. ex Lojac.) Mosyakin and *S. tragus* L. subsp. *ragus*.

### 63. *Salsola soda* L. (= *Soda inermis* Fourr.) (*Amaranthaceae*)

#### Accession data

**Si:** Sicily. Paternò (Catania), Salinelle del Fiume (WGS84: 37.567984°N, 14.864458°E), mud volcanoes with emission of salt water, 108 m a.s.l., 13 Nov 1998, *A. Cristaudo* (BGS-CT, Catania Germplasm Bank).

#### Germination data

*Pre-treatments:* no treatment.

*Germination medium:* 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

*Sample size:* 50 seeds for each test.

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>i</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
100%	constant 30°C	12/12h	1.0	3.5	7.0	3.9
100%	constant 30°C	0/24h	1.0	4.4	9.0	4.4
100%	constant 25°C	12/12h	2.0	3.2	7.0	3.3
100%	constant 25°C	0/24h	2.0	4.0	11.0	4.4
100%	constant 15°C	0/24h	2.0	4.6	11.0	5.3
100%	alternating 15/35°C	0/24h	2.0	5.2	11.0	6.0
98%	constant 10°C	0/24h	1.0	4.7	8.0	5.3
98%	constant 20°C	12/12h	2.0	4.6	11.0	5.2
98%	constant 15°C	12/12h	2.0	5.6	11.0	6.1
98%	alternating 10/30°C	12/12h	1.0	2.3	9.0	3.2
98%	alternating 10/30°C	0/24h	1.0	4.0	9.0	3.9
98%	alternating 15/35°C	12/12h	2.0	4.6	11.0	5.5
96%	constant 10°C	12/12h	1.0	5.1	9.0	5.6
96%	constant 20°C	0/24h	2.0	4.5	11.0	5.4
94%	constant 40°C	0/24h	1.0	1.5	8.0	2.8
90%	constant 35°C	12/12h	1.0	2.4	8.0	3.5
90%	constant 35°C	0/24h	1.0	4.6	9.0	4.8
86%	constant 40°C	12/12h	1.0	2.5	9.0	3.7

#### Observations

*Salsola soda* L. is an annual herb that is native to the Mediterranean Basin. It is a succulent halophyte that typically grows in coastal regions. Here we report the first germination data for this species for Italy. Germination tests were carried out at the Catania Germplasm Bank using eight constant (5–40°C) and two alternate (10/30°C and 15/35°C) temperatures both in the light (12/12h) and in full darkness.

High germination percentages ( $\geq 86\%$ ) were recorded at almost all the tested temperatures, with the highest (98-100%) in the range between 10 and 30°C. At 5°C lower germination percentages were recorded in full darkness and especially in the light (66% and 38%, respectively). In particular, the fastest germination was recorded at 40°C in darkness ( $T_1 = 1.0$  day,  $T_{50} = 1.5$  days, and  $T_{max} = 8$  days) with similar values also in the light. The slowest germination was recorded at 5°C under light conditions ( $T_1 = 8.0$  days,  $T_{50} = 11.3$  days) and full dark ( $T_1 = 5.0$  days,  $T_{50} = 8.8$  days).

The results are in accordance with the germination percentages reported for this species (close to 100% at 20°C) by Ferrer-Gallego & al. (2013) for Spain and by Royal Botanic Gardens Kew (2021), while they are higher than those of Royal Botanic Gardens Kew (2021) at 15°C (98% vs 89%).

#### 64. *Salsola squarrosa* subsp. *controversa* (Tod. ex Lojac.) Mosyakin (*Amaranthaceae*)

##### Accession data

**It:** Latium. Santa Marinella (Roma), Santa Severa (WGS84: 42.018317°N 11.955122°E), spiaggia, 2 m a.s.l., 20 Oct 2021, *A. Alonso Simón* & *S. Magrini* (BGT-A-89521, 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.

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

Germination	Thermoperiod	Photoperiod [light/dark]	$T_1$ [d]	$T_{50}$ [d]	$T_{max}$ [d]	MTG [d]
100.0%	constant 30°C	12/12h	2.8	2.8	5.2	3.4
100.0%	constant 25°C	12/12h	3.0	6.3	9.4	6.3
100.0%	constant 25°C	0/24h	3.2	3.5	10.2	4.6
100.0%	constant 20°C	0/24h	10.2	14.9	22.6	16.2
98.9%	constant 30°C	0/24h	2.6	4.1	10.8	2.9
97.8%	constant 15°C	0/24h	14.0	13.9	17.6	15.0
86.9%	constant 15°C	12/12h	14.0	14.2	17.2	14.8

##### Observations

*Salsola squarrosa* subsp. *controversa* [= *S. tragus* subsp. *pontica* (Pall.) Rilke] is an annual species typical of the sand beach drift-lines, on the Italian coasts (Acosta & Ercole 2015). Here we report the first germination data for this species for Italy. Germination tests were carried out at the Tuscia Germplasm Bank using five constant temperature regimes (10-30°C) and both light and dark conditions.

The results showed high germination percentages ( $\geq 79\%$ ) between 15°C and 30°C, with an optimum temperature range for this species at 20-30°C (98.9-100%), while no germina-

tion was recorded at 10°C. Further tests will be carried out to define the maximum temperature for the germination of this species.

The fastest germination was recorded at 30°C in the light ( $T_1 = T_{50} = 2.8$  days and 5.2 days for the maximum germination) and the slowest at 20°C under light conditions ( $T_1 = 14.0$  days,  $T_{50} = 20.3$  days, and  $T_{max} = 27.0$  days). While strong photoinhibition has been reported in other sand beach species, like *Cakile maritima* Scop. (Thanos & al. 1991), it has not been detected in *S. squarrosa*, like recently reported also for *Cyperus capitatus* Vand. (Salmeri & Trubia 2019) and *Matthiola sinuata* (L.) W.T. Aiton living in the same habitat (Magrini & al. 2019).

### 65. *Salsola tragus* L. subsp. *tragus* (Amaranthaceae)

#### Accession data

**It:** Tuscany. Grosseto (Grosseto), Principina a Mare (WGS84: 42,693297°N, 10,996353°E), spiaggia, 1 m a.s.l., 1 Nov 2020, *S. Magrini & A. Caldelli* (BGT-A-69620, 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.

*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]
92.0%	constant 15°C	12/12h	2.8	7.5	20.8	8.4
86.7%	constant 20°C	12/12h	2.2	4.9	11.0	5.6
84.0%	constant 15°C	0/24h	2.6	7.5	14.2	7.8
83.9%	constant 20°C	0/24h	2.0	3.7	8.8	4.7

#### Observations

*Salsola tragus* (= *S. kali* L. subsp. *ruthenica* Soó) is widely spread from the Baltic Sea to the Mediterranean coasts. It is common in the annual communities of sand beaches along the Italian coasts (Acosta & Ercole 2015). Here we report the first germination data for this species for Italy. Germination tests were carried out at the Tuscia Germplasm Bank using nine constant temperature regimes (5–40°C) and both light and dark conditions.

High germination percentages (> 83%) were recorded only at 15–20°C, with slightly higher values in the light. Very lower percentages were recorded at the other temperatures, only 2–40% in the range 35–40°C, with the lowest percentages recorded at the lower temperature, 5°C, with no germination in the light and only 2% in the dark. Our results are not in accordance with Royal Botanic Gardens Kew (2021) which reported 100% germination at 5°C with an 8/16h photoperiod and 67% at 0°C, suggesting that

the thermal germination behaviour may be affected by the maternal environment of seed production (De Vitis & al. 2014, 2018). As reported also for *S. squarrosa*, in a range of temperatures from 5 to 37°C, no difference in germination was observed between light conditions and full dark.

Wallace & al. (1968) reported a very short germination time (29 min) for *S. tragus* (sub *S. kali* var. *tenuifolium*), but in our tests seeds germinated within two to three days, only at 37-40°C, they germinated in one day. The fastest germination was recorded at 20-25°C in darkness ( $T_{50}$  = 3.7 and 3.5 days, respectively) and the slowest at 10°C under both light conditions and full dark ( $T_{50}$  = 21.6 and 16.4 days, respectively).

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