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Abstract

The aim of the work was to determine the germination and germination energy of 32 clary sage (*Salvia sclarea* L.) accessions collected in the period 1998-2009 and preserved in the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology, University of Zagreb Faculty of Agriculture. The highest germination was observed in accessions which have been preserved since 2009 (90 and 94 %) and 2008 (76, 70, 68 and 62 %). It can be concluded that clary sage seeds have very good germination, that they tolerate storage very well, that they have been stored in good conditions and that regeneration in plant gene banks should not be carried out often.

Keywords: clary sage, germination energy, seed conservation, genebanks

Introduction

Plant productivity in agroecosystems is determined by seeds, which are the most important criterion for plant existence and dispersal, and rapid seed germination, which ist is the main criterion for plant establishment and the most important stage in the plant life cycle (Bentsinka and Koornneef, 2008; Sedghi et al., 2010). For accessions kept in the gene banks and seed collections, it is important to regularly check the germination of the preserved accessions (Government of the Republic of Croatia, 2021). Since many plant species as well as certain local plant populations are threatened with extinction due to various disasters and the replacement of local populations by new, high-yielding cultivars, plant gene banks have been established worldwide (NN 89/02). The Croatian Bank of Plant Genes (HBBG) was established in 1991 as a national project to organise activities related to conservation and sustainable use of plant genetic resources in the Republic of Croatia (Kolak and Šatović, 1996; Kolak et al., 2004). Different climate, relief and soil types in Croatia are the main reason for the richness of Medicinal and Aromatic Plant (MAP) species, whose natural populations have a great biological diversity at the morphological, biochemical and genetic levels, and whose collection and use has a very long tradition in Croatia (Šatović et al., 2016). The basic MAP collection was established in 1998 at the Department of Seed Science and Tecnology, University of Zagreb Faculty of Agriculture while the Working Group for Medicinal and Aromatic Plants was established in 2006 (Šatović et al., 2016; CPGRD, 2022). Within this collection, plant genetic sources of medicinal and aromatic plants are systematically collected, characterised, preserved, evaluated, documented and regenerated for introduction into agricultural production and breeding programmes (Šatović et al., 2016). The procedure for inclusion in the collection includes cleaning, moisture determination, drying, vitality testing, packaging and registration. The accessions are stored in classical ex situ rooms for medium-term seed storage, in a cold chamber at + 4 °C, in three-layer bags or jars. The vitality of the seeds of each accession is regularly monitored and, if necessary, regeneration is carried out (Šatović et al., 2016). In total, more than 4,411 accessions are stored in the HBBG, of which 2,604 are medicinal and aromatic plants (CPGRD, 2022). About 80 % of the collected material is wild material of Croatian origin, taken during collection missions or donated, while foreign samples come from other national gene banks, botanical gardens and research institutes (Brezovec et al., 2006; Šatović et al., 2016)

Clary sage (*Salvia sclarea* L.) is a biennial or perennial aromatic medicinal plant belonging to the Lamiaceae family (Peana and Moretti, 2002). It is cultivated for its high-quality essential oil, which is used in the perfumery and cosmetics industries, as a flavour enhancer, in tobacco products and in the production of liqueurs (Carrubba et al., 2002). Although cultivation in the Mediterranean region has been documented for centuries, the popularity of

this plant has increased in recent years (Yaseen et al., 2014). The growing demand for clary sage partly reflects its therapeutic properties, which include the ability to lower blood pressure, treat anxiety, stress, menstrual disorders and various skin conditions (Džamić et al., 2008; Yang et al., 2014). Numerous studies have found that the essential oil of the aerial part of the plant is rich in linalool and linalyl acetate, which have antioxidant, antimicrobial and cytotoxic effects (Tadtong et al., 2012).

The aim of the work is to determine the germination and germination energy of clary sage (*Salvia sclarea* L.) accessions from the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology, University of Zagreb Faculty of Agriculture.

Material and methods

Seeds of 32 accessions of clary sage from the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology of the University of Zagreb, Faculty of Agriculture, within the framework of the National Program for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture of the Republic of Croatia, were used for this study.

Germination and germination energy testing was conducted under controlled laboratory conditions at the University of Zagreb Faculty of Agriculture, Department of Seed Science and Technology, in 2021 as per the ISTA (1993) Rules. Before the experiment, the seeds were surface-sterilized with 3% Izosan for three minutes and rinsed under running water. They were then rinsed in distilled water and dried to optimum moisture.

Due to the limited amount of seeds each accession was germinated in two replicates, two Petri dishes with 25 seeds each. The seeds were germinated on germination paper (Munktell 21/N, 580x580mm, 80g/qm) in 10-cm-diameter Petri dishes (Steriplan®, DURAN®, DWK Life Sciences GmbH, Germany). Petri dishes with seeds were for germination placed in a germination chamber at a constant temperature (25 °C ± 1 °C) with a photoperiod of 16 h of light and 8 h of darkness. The number of germinated seeds (seeds with a root size ≥ 2 mm) was determined every other day over a period of 21 days.

At the end of the study, the following parameters were calculated:

Germinability (G, %) represents the number of germinated seeds in percentage (Scott et al., 1984.) and was calculated according to the formula:

$$G = \frac{n_k}{n} \times 100$$

where n_k indicates the number of germinated seeds, and n is the total number of seeds in the experiment.

Mean germination time (MGT, day) is the germination time in days and was calculated

according to the formula:

$$MGT = \frac{\sum_{i=1}^{k} n_i \, t_i}{\sum_{i=1}^{k} n_i}$$

where t_i is the time from the beginning of the experiment to the observation time (i^{th}), n_i is

the number of germinated seeds in the ith time, and k is the last day of germination (Ranal et al., 2009.).

Results and discussion

In this study, the germination rate and germination energy of 32 accessions of clary sage from the Collection of Medicinal and Aromatic Plants were tested over 21 days.

At the end of the research, the germinability and the mean germination time were calculated. Table 4 shows that of the 32 accessions, the seeds of 28 accessions germinated, while four accessions did not have a single germinated seed.

Table 1. A list of clary sage accessions with information on the location and year of collection, germinability (G, %) and mean germination time (MGT, day)

No.	Accession	Location of collection	Year of collection	Germinability (G, %)	Mean germination time (MGT, day)
1	MAP00002	Slovakia	1996	22	9.54
2	MAP00141	Czech Republic	1998	0	/
3	MAP00377	Italy	1999	2	7.00
4	MAP02196	Kaštel stari	2008	34	2.97
5	MAP02197	Šolta	2008	8	2.00
6	MAP02198	Šolta	2008	62	2.14
7	MAP02199	Šolta	2008	68	2.15
8	MAP02200	Hvar	2008	36	2.00
9	MAP02201	Korčula	2008	68	2.29
10	MAP02202	Korčula	2008	76	2.06
11	MAP02203	Korčula	2008	34	3.32
12	MAP02204	Brač	2008	52	3.35
13	MAP02205	Brač	2008	70	4.37
14	MAP02206	Vrgorac	2008	0	/
15	MAP02207	Murter	2008	50	2.35
16	MAP02208	Murter	2008	20	2.65
17	MAP02209	Živogošće	2008	38	2.58
18	MAP02210	Trogir	2008	20	4.67
19	MAP02211	Trogir	2008	4	4.00
20	MAP02212	Vrpolje	2008	12	7.84
21	MAP02213	Trogir	2008	0	/
22	MAP02214	Trogir	2008	20	5.80
23	MAP02216	Šibenik	2008	20	8.59
24	MAP02217	Prokljansko jezero	2008	42	2.86
25	MAP02218	Žirje	2008	44	2.09
26	MAP02219	Dugi otok	2008	62	4.08
27	MAP02220	Brač	2008	34	6.90
28	MAP02271	Split	2008	20	9.25
29	MAP02386	Dugi otok	2009	94	3.07
30	MAP02392	Dugi otok	2009	0	/
31	MAP02393	Tribunj	2009	90	2.49
32	MAP02405	Stankovci	2009	50	3.27

Based on the research conducted, clary sage seeds were found to have very good germination considering the past years the seeds were stored.

The highest percentage of germination (Table 1) was observed in accessions 2386 (94 %) from Dugi otok and 2393 (90 %) from Tribunj, stored since 2009, slightly less in accessions 2202 (76 %) from Korčula, 2205 (70 %) from Brač, 2201 (68 %) from Korčula, 2199 (68%) from Šolta, 2219 (62 %) from Dugi otok and 2198 (62 %) from Šolta, stored since 2008. The other samples had a germination rate of about 50 % or less. Although accession 2197 had only eight germinated seeds, it had the fastest average germination time of two days, next to accession 2200 (Table 1). Besides

them, the fastest germination time was observed in accessions 2218 (2.09) from Žirje, 2209 (2.58) from Borovik, 2208 (2.65) from Murter and 2196 (2.97) from Kaštel Stari, which have been kept since 2008. Although accession 2386 had the highest percentage of germination (94 %) compared to the less germinated samples, it had a slightly longer germination time (3.07).

The results of this study suggest that the seeds were stored under good conditions and that regeneration in plant genebanks does not need to be carried out often.

A similar study was conducted by Žutić and Dudai (2008) who, among others, investigated the germination of Dalmatian sage (*Salvia officinalis* L.) cultivar "Moran" at 25 °C with constant lighting, stored at 6 °C and aged from 0 to 15 years. The highest germination and vigour were found in 3 and 8 year old seeds (81 and 73 %) and the authors concluded that ageing of sage seeds is a relatively slow process.

Conclusion

The research revealed that the seeds of clary sage showed very good germination, which means that it tolerates storage well, i.e. that it was stored under good conditions, so that regeneration in plant genebanks does not have to be carried out often. Out of 32 research accessions, nine had a germination level of more than 50%.

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