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Effect of temperature, salt stress and pH on seed germination of medicinal plant *Origanum compactum*

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ABSTRACT

The aim of this study was to evaluate the impact of temperature, salt stress and pH on seed germination of a Moroccan medicinal plant *Origanum compactum* (Benth). Mature seeds were isolated from *O. compactum* collected from two areas belonging to Ouezzane province at post-flowering stage. The *in vitro* seed germination tests were carried out in the dark in Petri dishes using an aqueous media. The influence of temperature, salt stress and pH was evaluated by following the evolution of germination over time. The results showed an optimal temperature of 15 °C with a maximal germination percentage of 81%, an optimum pH equal to 7 with 71% as a maximal germination percentage and a negative correlation between NaCl concentration and seed germination. The seed germination was null at high temperature (25 °C) and at high NaCl concentration (> 7.5 g/l) as well as at acid pH (pH < 3.5). These data may serve as guidelines for species-specific propagation protocols, and *ex situ* conservation.

1. Introduction

The genus Origanum has a local distribution mainly around the Mediterranean basin, and it's characterized by a large morphological and chemical diversity (Kokkini, 1997). It is a taxonomically complex group of aromatic herbs that are used in several areas over the world for their aromatic, medicinal as well as culinary properties (Aboukhalid et al., 2016). This genus has been divided into 38 species, 6 subspecies, and 17 hybrids, arranged in three groups and 10 sections (Ietswaart, 1980). Thanks to this classification, five others species and one hybrid were identified, up the number of species to 43 and hybrids to 18.

Among this forty-three species included in the Genus Origanum, *O. compactum* is one of the most important medicinal species in term of ethno-botany in Morocco. It is locally known as "Zaatar". In its natural state, *O. compactum* grows on dry, rocky land and calcareous reliefs up to 700 m, it grows on forest land between trees and shrubs and blooms from June to August. It is considered a highly endangered species due to its over-exploitation (Aboukhalid et al., 2016).

It was reported that seed culture is an alternative and easy method of commercial propagation and it's being used widely for the commercial propagation of a large number of plant species, including many medicinal plants. However, the propagation of medicinal plants using seed germination is affected by numerous abiotic factors such as temperature, light, salt stress and pH (Haeussler and Tappeiner, 1993; Vleeshouwers et al., 1995; Prado et al., 2000; Abbad et al., 2011). Salinity is one of the major abiotic factors that limit crop production (Koyro, 2006). Through enhancement of osmotic pressure, salinity leads to the reduction of water absorbency which subsequently affects several other metabolic and physiological processes leading to a prolongation of the duration of seed germination (Kang and Saltveit, 2002; Ramin, 2006). Furthermore, several other studies have shown that osmotic and salt stress can delay, reduce or prevent germination (Liopa-Tsakalidi et al., 2011; Zhou et al., 2005; Gorai et al., 2011; Keshavarzi, 2012; Sharma et al., 2014). Temperature plays a major role in determining the periodicity of seed germination and the distribution of species (Guan et al., 2009). Germination rate usually increases linearly with temperature up to an optimal temperature, after which germination rate declines sharply (Alvarado and Bradford, 2002; Kumar, 2012; Ranjbar et al., 2013; Tolyat et al., 2014; Fallahi et al., 2015). In addition to these factors, germination is also affected by pH (Vleeshouwers et al., 1995).

To our knowledge, no published study has investigated the effect of abiotic factors on *O. compactum* seed germination. In this study, we have evaluated the influence of temperature, salt stress and pH on *O. compactum* seeds germination.

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2. Materials and methods

2.1. Seeds collection

Mature seeds were collected in August 2012 from two sites (Assara and Dar-elgaba) in the Zoumi region, province of Ouazzane, northern Morocco. Seeds were carefully prepared; the aim was to conduct a manual sorting to avoid any injury to the seeds. These seeds have not undergone any special treatment. The conservation was made in tubes until their eventual use.

2.2. Germination experiments

Seeds were surface sterilized in sodium hypochlorite solution for 1 min to prevent fungus attacks. Then, seeds were washed with distilled water and air-dried before use in the germination experiments. Isolated seeds were germinated in Petri dishes containing two disks of Whatman No. 1 filter paper, soaked with 1.5 ml of distilled water. Each essay was about 90 seeds, 3 repetitions of 30 seeds per Petri dish.

To determine the optimal parameters of seed germination, the impact of certain abiotic factors on kinetics and percentage of germination was evaluated. In order to determine the effect of temperature, germination experiments were conducted in dark, at 10, 15, 20 and 25 °C. To test the impact of salt stress, seeds were cultured in 0, 1, 2.5, 5, 7 and 10 g/l of NaCl. Finally, four values of pH were tested (2, 3.5, 5 and 7). For each treatment, the dishes were carefully closed and incubated in the dark at the optimal temperature that was determined in the first test. A seed was considered to have germinated at the emergence of the radicle (radicle > 1 mm) (Bewley and Black, 1994).

The evolution of germination was followed for a month by calculating the cumulative germination percentage at every three days (Ndour et Danthu, 1998). The germination rate is the ratio, expressed as a percentage of the number of seeds germinated on the total number of seeds. Experiments were realized in triplicate.

2.3. Statistical analysis

Data were analyzed using SPSS 20. One-way ANOVA tested for the significance of the main effects of temperature, salt stress and pH. A Tukey test was used to estimate the significant differences between individual treatments. Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Effect of temperature

Analysis of variance comparing the temperatures showed that the highest germination percentage after 30 days of culture was obtained at 15 °C (81% Assara; 68% Dar-elgaba) (Fig. 1). The evolution of the germination curve showed the existence of a lag time between 4 and 10 days. The temperature acted mainly on the germination rate, but affected the maximum germination capacity of *O. compactum* as well. The curves of germination kinetics showed similar behavior of the seeds from both sites towards different temperatures. At temperatures of 10 and 20 °C, the latency period lasted 10 days and the germination percentage oscillated between 13% and 45%. Finally, the lowest results were observed at 25 °C (2%).

These results indicated that the two studied sites have an important germination potential at temperature of 15 °C which is qualified as "optimal temperature of germination". The effect of this temperature on the germination period was similar for the two studied sites. The temperature of 15 °C have reduced latency period, increased the percentage and the speed of germination and blocked the inhibitory effects of factors causing germination of *O. compactum*. In summary, once seeds start to germinate, higher temperatures can stimulate germination up to an optimal temperature, after which the speed of germination declines (Fig. 2).

The study of the effect of temperature on *O. compactum* seed germination indicated that this factor is determinant. This joins the idea of the role of temperature in the kinetic of seeds biochemical reactions. For *O. compactum* species, the minimum temperature of germination was 10 °C, below this temperature, seeds became sensitive to chill. As for the optimum temperature of germination, it ranged between 10 and 15 °C which is similar to the ecological environment where this species was collected (Ouazzane) and which promoted a good germination. This is in accordance with the proposition of Sento (1971) and Wood and Prichard (2003) that the performance of germination at a range of temperature reflects an adaptation to the ecological origin of the species.

To better understand the ecological significance of the germinal behavior of the studied species, we adopted the classification used by Neffati (1994). This classification was based on the relative values of the two main factors of germination; which represent the germination speed in relation to temperature, allowing obtaining the highest germination rate. These phenomena were considered by Neffati to be consistent with what is known about the role of the thermal factor in the activation of metabolic reactions which are a great asset to the adaptation of species to arid conditions. In addition of its crucial role in the activation of catalysts, the temperature plays a role in the accumulation of GA by stimulation of transcription factors *via* different chromatin channels (Neffati, 1994).

Several studies have demonstrated that the optimum temperature of germination may vary between species, and even between seed-lots within the same species; Eberle et al. (2014) showed that the optimal temperature of germination for Calendula was about 15 °C. However, for purple and green basil, the optimal temperature of germination ranged between 25 and 30 °C, respectively (Fallahi et al., 2015). This difference was related to environmental and genetic conditions.

3.2. Effect of salinity

Fig. 3, shows the effect of NaCl on seed germination during 22 days of culture. The mentioned results in this figure illustrate the variation of the percentage of germination with time at increasing concentrations of NaCl. In the absence of salt stress (0 g/l of NaCl), the progress of seed germination was similar at the two studied sites. In fact, the maximal germination percentage at 0 g/l NaCl was around 72% in Assara site and 57% at Dar-elgaba site. These results have revealed evidence that the *O. compactum* seeds from both sites have a good germination potential. Based on the results of variance analysis, salinity stress affected significantly the germination percentage ($P < 0.05$) (Fig. 4).

The increase in salt stress caused not only a reduction in germination percentage, but also an increase in latency period. Seed germination in the absence of salt stress followed a three phase-curve, but in high concentrations of NaCl (5, 7.5, 10 g/l), the germination percentage was almost null. The increase of NaCl concentration caused a prolongation of the germination period of *O. compactum* seeds. In fact, for seeds from Aassara site, this period lasted 7 days at 1 g/l and 13 days at 7 g/l of NaCl. According to Ben Miled et al. (1986), this delay is explained by the time required for seeds to trigger mechanisms that allow them to adjust their osmotic pressure with the surrounding stress.

For the control, the latency period was very short and lasted only four days; the exponential phase of germination lasted six days in both the studied sites before reaching the stationary phase where germination stopped at a maximum germination percentage. Under increasing salinity stress, the shape of the exponential phase changes causing a delay and a slower speed of germination. The decrease in the percentage of seed germination was due to an osmotic dormant process developed in response to the saline stress conditions, thus representing a coping strategy against environmental constraints (Prado et al.,