Site effect on seed germination of two species of *Cladanthus* in Morocco

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Abstract

Seed germination of two species of the genus *Cladanthus*: *Cladanthus mixtus* (annual plant) and *Cladanthus scariosus* (perennial plant) was studied. The aim of this work was to determine germination differences at the inter-site level for both species (7 sites for each species) and at the intra-site level (Three accessions for each site) for *C. mixtus*. The germination test was carried out in a laboratory room at an average temperature of 18 °C. Six parameters were determined: Final germination percentage (FGP), mean germination time (MGT), coefficient of the variation of germination time (CVt), germination index (GI), coefficient of velocity of germination (CVG), and median germination time (T_{50}). The average germination percentages were 95.2 and 83.8%, respectively for *C. mixtus* and *C. scariosus*. Germination time and speed were highly variable at the inter-site level for both species. The provenance of seeds significantly affects the germination capacity. Germination was better in the sites: Larache, Sidi Chafi and Sidi Taibi for *C. mixtus* and in Ijoukak 1 and Ijoukak 2 for *C. scariosus.* The mean values of *C. mixtus* and *C. scariosus* are respectively: 92.2-98.7% and 70.0-93.0%. The accession effect in *C. mixtus* was significant for the final germination percent at four sites (Larache, Boghadi, Harcha and Ain El Aouda). For other parameters (MGT, GI and CVG), significant differences were observed mainly in Tiddas and Boghadi. This inter-site variability must be considered for the ex-situ conservation and domestication initiation of *C. mixtus* and *C. scariosus*.

Keywords: *Cladanthus mixtus*, *Cladanthus scariosus*, Germination, Site, Variability, Morocco.

Effet du site sur la germination des graines de deux espèces de *Cladanthus* au Maroc

Résumé

La germination des graines de deux espèces du genre *Cladanthus* : *Cladanthus mixtus* (plante annuelle) et *Cladanthus scariosus* (plante pérenne) a été étudié. L'objectif de ce travail est de déterminer les différences de germination au niveau inter-sites pour les deux espèces (7 sites par *espèce*) et au niveau intra-site (effet accession) pour *C. mixtus*. Le test de germination est réalisé au laboratoire à une température moyenne de 18°C. Les paramètres déterminés sont : pourcentage de germination final (FGP), temps de germination moyen (MGT), coefficient de variation du temps de germination (CV_t), indice de germination (GI), coefficient de vitesse de germination (CVG) et temps médian de germination (T_{50}). Les pourcentages moyens de germination sont de 95,2 et 83,8% respectivement chez *C. mixtus* et *C. scariosus*. Le temps et la vitesse de germination ont montré des différences significatives au niveau inter-sites pour les deux espèces. La provenance des graines affecte significativement la capacité de germination. La germination était meilleure dans les sites : Larache, Sidi Chafi et Sidi Taibi pour *C. mixtus* et Ijoukak 1 et Ijoukak 2 pour *C. scariosus.* Les valeurs moyennes de *C. mixtus* et *C. scariosus* sont respectivement : 92,2-98,7 % et 70,0-93,0 %. L'effet accession chez *C. mixtus* s'est avéré significatif pour le pourcentage de germination dans quatre sites (Larache, Boghadi, Harcha and Ain El Aouda). Pour d'autres paramètres (MGT, GI et CVG), des différences significatives ont été observées principalement à Tiddas et Boghadi. Cette variabilité inter-sites doit être prise en considération pour la conservation ex-situ et l'initiation de la domestication de *C. mixtus* et *C. scariosus*.

Mots-clés : Cladanthus *mixtus*, Cladanthus *scariosus*, Germination, Site, Variabilité, Maroc.

تأثير الموقع على إنبات البذور لنوعين بريان من جنس البابونج (*Cladanthus*)في المغرب

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الملخص

تمت دراسة سلوك الإنبات لنوعين بريان من جنس البابونج *Cladanthus* : *حلالة Cladanthus mixtus* (نبات سنوي) و إرزغي *Cladanthus scariosus* (نبات معمر). الهدف من هذا العمل هو مقارنة السلوك الإنباتي بين المواقع (7 مواقع لكل نوع) وعلى مستوى داخل الموقع لـ *C. mixtus* . تم إجراء اختبار الإنبات في المختبر بمتوسط درجة حرارة تبلغ 18 درجة مئوية؛ و تم قياس المعايير التالية : نسبة الإنبات النهائي (FGP) ، متوسط وقت الإنبات (MGT) ، معامل التغير في زمن الإنبات (CVt)، مؤشر الإنبات (GI) ، معامل سرعة الإنبات (CVG) ، ووقت الإنبات المتوسط (T_{50}) . بلغت نسبة الإنبات 95,2 و 83,8٪ على التوالي في حلالة و إرزغي وقت الإنبات وسرعته متغيرين بدرجة عالية حسب الموقع لكلا النوعين (98,7-92,2) ٪ حلالة و(93,0-70,0) ٪. إرزغي. كان الإنبات أفضل في المواقع التالية: العرائش وسيدي الشافي وسيدي الطيبي لنبات *C. mixtus* وفي موقع إجوكاك 1 و إجوكاك 2 لـ *C. scariosus*. يؤثر أصل البذور بشكل كبير على قدرة الإنبات. متوسط قيم *C. mixtus* و *C. scariosus* على التوالي (92.2-98.7٪ و70.0-93.0٪). تم العثور على تأثير المدخلات في *C. mixtus* ليكون معنويا بالنسبة المئوية للإنبات في معظم المواقع. بالنسبة للمعلمات الأخرى (MGT، GI، CVG)، لوحظت فروق ذات دلالة إحصائية بشكل رئيسي في تداس و بوغادي. يجب أن يؤخذ في الاعتبار هذا التباين بين المواقع وداخلهم لحفظ حلالة و إرزغي خارج الموقع الطبيعي وبدء زراعتهما.

الكلمات المفتاحية :*البابونج*، *Cladanthus mixtus*، *Cladanthus scariosus*، الإنبات، الموقع، المغرب.

Introduction

Asteraceae family is one of the most widely dispersed families of angiosperms and contains at least 1,900 genera with more than 32,000 accepted species (Mandel *et al*., 2019). *Cladanthus* is a small genus containing five species, all native to the Mediterranean region and southwest Europe (Oberprieler *et al*., 2007). The five species are found in Morocco: *Cladanthus mixtus* (L.) Chevall, *Cladanthus arabicus* (L.) Cass, *Cladanthus scariosus* (Ball) Oberpr. & Vogt, *Cladanthus eriolepis* (Maire) Oberpr. & Vogt, and *Cladanthus flahaultii* (Emb). Oberpr. & Vogt. The last three species are endemic to Morocco (Fennane *et al.*, 2014).

Cladanthus scariosus is known under the vernacular names Irezghi or Irezgui. It is a perennial plant with a strong aromatic fragrance. This species is common in many open areas and on sandstone substrates of the Morocco's High, Middle, and Anti Atlas mountains (Ibn Tattou and Fennane, 2008). In traditional medicine, *C. scariosus* is used to treat all disorders where spasm is important. It has tonic, stomachic, analgesic, and antispasmodic properties. Moreover, it is reported to treat diabetes (Katiri *et al.*, 2017).

Cladanthus mixtus is an annual plant. This species grows widely in semi-arid and subhumid bioclimates. It is generally found in open forests, fields, sandy or stony pastures of the plains, and low mountains. In Morocco, this plant is known under the vernacular name "Hellâla" (Bellakhdar, 1997), and grows mainly in the Western Rif and northern Atlantic Morocco (Aafi *et al.*, 2005). Economically, the essential oil of *C. mixtus* is of great interest. Morocco is the only supplier on the international market (Franchomme *et al.*, 1990). In terms of quality, *C. mixtus* was ranked ninth among Morocco's 20 best essential oils (Lawrence, 2009). However, its exploitation at the full flowering stage has drastically reduced the possibilities of seed production and the depletion of natural stocks of the species.

Seed germination, an important characteristic of vegetation regeneration, can be considered the most fundamental event in crop success and one of the easiest ways to save plants. It is also one of the most widely studied areas in plant biology, especially in Asteraceae family, (Brusa *et al*., 2007; Karlsson *et al*., 2008; Baskin and Baskin, 2014, Carasso *et al*., 2020). However, with the exception of one study related to the in vitro germination of *C. mixtus* (Harras *et al.*, 2014), no more studies were found on *Cladanthus* species multiplication.

Species from a wide range of plant families exhibit differences in germination characteristics of seeds collected in different locations. Germination responses vary with latitude, elevation, soil moisture, soil nutrients, temperature, density of plant cover, and degree of habitat disturbance at the sites where the seeds matured, depending on the species (Baskin and Baskin, 1998).

Germination characteristics of seeds collected from several sites can vary in many ways, but one of the most common is the degree of dormancy, as reflected by germination percentages of fresh seeds (Moya *et al.*, 2017).

Apart from the genetic origin, the level of primary dormancy in seeds may be determined by other factors, such as maternal environment during maturation, age of the mother plant during maturation, and the position of the seeds on the plant (Andersson and Milberg, 1998; Fenner and Thompson, 2005). Seed dormancy and germination are complex traits of spermatophytes that are influenced by many genes and environmental factors (Finch-Savage and Leubner-Metzger, 2006; Donohue *et al*., 2010) both in the long term (through ecotypes or clines) and in the short term (through the influence of the seed maturation environment) (Fernández-Pascual and *al*., 2013).

Due to the ecological and economic importance of *Cladanthus* species in Morocco, the present work aims to compare germination behaviour at inter-specific levels of *C. mixtus* and *C. scariosus* and at the intra-site level (accession effect) for *C. mixtus*.

Materials and methods

Plant material

Seeds of *C. mixtus* and *C. scariosus* were collected from natural populations at the mature stage in 2018 and 2019 (Fig. 1). Seeds collection was carried out at different positions of the mother plant. Seeds were considered mature when achenes could be easily detached from the receptacle. They are stored in paper bags at 6°C until used. Seeds from 21 individual plants (accessions) of *C. mixtus* were collected from seven distant sites (Tab. 1). For *C. scariosus,* the seeds of 5–10 adult plants from each site (7 sites in total) were bulked into one population (Tab. 1). In the same location, the minimum distance between each plant was 100 m. Plant specimens were collected and taxonomically identified at the Scientific Institute of Mohammed V University in Morocco.

Figure 1. *Cladanthus mixtus* above, *Cladanthus scariosus* in the below, a: plant, b: flower, c: seeds

Table 1. Location of seed collection sites by species

Species	Site	Site code	elevation (m)	GPS	
				Latitude	Longitude
C. mixtus	Harcha (2018)	CM-H	360	33°29'09.0"N	6°09'30.0"W
	Tiddas (2018)	CM-T	470	33°33'47.21"N	6°15'38.79"W
	Boghadi (2018)	CM-B	1114	33°26'16.4"N	6°03'500"W
	Sidi Chafi (2018)	CM-SC	189	34°00'17.96"N	6°36'36.97"W
	Ain El Aouda (2018)	CM-AE	210	33°48'38.87"N	6°47'56.81"W
	Sidi Taibi (2018)	CM-ST	25	34°11'27.48"N	6°40'14.46"W
	Larache (2018)	CM-L	25	35°11'32.35"N	6°8'27.62"W
C. scariosus	Demnate 1-Ouaoussinet (2019)	CS-D1	1485	31°36'08.4"N	6°56'18.9"W
	Demnate 2–Ait Blal (2019)	CS-D2	1547	31°41'40.2"N	6°43'09.2"W
	Azilal 1 –Ait Bouguemez (2019)	CS-Az1	2400	31°43'43.0"N	6°19'12.4"W
	Azilal 2-Tamda (2019)	CS-Az2	2298	31°48'03.0"N	6°23'01.7"W
	Ijoukak 1-Tagdit Oufla (2019)	CS-IJ1	1959	30°56'28.4"N	8°02'40.1"W
	Ijoukak 2 (2019)	CS-IJ2	1671	31°00'06.5"N	8°09'34.5"W
	Ourika (2018)	CS-OU	875	31°18'40.8"N	7°44'56.0"W

Germination experiment

Germination was performed in November 2020 within the laboratory of aromatic and medicinal plants at INRA. Healthy-looking seeds were carefully selected and randomly incubated in 15 cm diameter Petri dishes on two layers of Whatman No. 1 filter paper. The Petri dishes were moistened with the same quantity of distilled water (10 ml) according to the kinetics of the drying of the filter paper. Four replications of 100 seeds were used for *C. scariosus* and 50 seeds for *C. mixtus*. The experiments were conducted in an environment with an average temperature of 18 °C (ranging between 17-19 °C). Monitoring was done daily using a magnifying glass until germination stopped, for 5 days for *C. mixtus*, and 22 days for *C. scariosus*. When the radicle of a seed exceeds 2 mm in length, it is considered germinated. Viable seeds that had not germinated after monitoring were considered dormant. Seeds covered with fungi were considered dead and were eliminated.

Data treatment

Many parameters of germination were assessed to compare and evaluate the effect of site on the two *Cladanthus*: final germination percentage (FGP), mean germination time (MGT), coefficient of variation of the germination time (CVt), germination index (GI), coefficient of velocity of germination (CVG), and median germination time (T_{50}) using the following formulas:

- *Final Germination Percentage (FGP)*: reflects the final percentage of germination attained but provides no information of the speed or uniformity of germination:

$$FGP(\%) = \frac{Ng}{Nt} x100 \qquad \text{(ISTA, 2015).}$$

Where, N_g is the number of germinated seeds and N_t is the total number of seeds.

- *Mean Germination Time (MGT)*: Measure time-spread of germination as follow:

$$MGT = \frac{\sum_{i=1}^{k} N_i T_i}{\sum_{i=1}^{k} Ni} \qquad \text{(Ranal and Santana, 2006).}$$

Where, T_i is the time from the start of the experiment to the i^{th} interval, N_i is the number of germinated seeds in the i^{th} time interval, and k is the total number of time intervals.

- *Coefficient of variation of the germination time (CVt)*: Measures the variability among seeds in relation to the mean germination time of the sample.

$$CVt = \sqrt{\frac{St^2}{MGT}}, \qquad \text{(Ranal and Santana, 2006).}$$

Where, St^2 is the variance of germination time and MGT is the mean germination time.

- *Germination Index (GI)*: Reflects the percentage of germination per day, so higher GI values indicate higher and faster germination.

$$GI\ (\%/day) = \sum_{i=1}^{k} \frac{Ni}{Ti} \qquad \text{(Ranal and Santana, 2006).}$$

Where, T_i is the time from the start of the experiment to the i^{th} interval, N_i is the number of germinated seeds in the i^{th}, time interval, and k is the total number of time intervals.

- *Coefficient of the velocity of germination (CVG)*: Emphasis on the time required for reaching the final percentage of germination.

$$CVG = \frac{1}{MGT} X\ 100\ , \qquad \text{(Ranal and Santana, 2006).}$$

- *Median germination time* : the part time of germination Tx, where x is the time to reach x% of final germination, for example the formula of *the median germination time* (T50, time to reach 50% of final germination) is as follow:

$$T_{50} = Ti + \frac{(\frac{N}{2} - N_i)(T_j - T_i)}{N_j - N_i}$$ (Farooq *et al*., 2005).

Where N is the final number of germinated seeds, and Ni and Nj are the total number of seeds germinated in adjacent counts at time Ti and Tj respectively, when $Ni < N/2 < Nj$.

Statistical analysis

Data were analysed using SPSS 18 software (IBM Corporation, 2010). The data were processed using a one-way ANOVA analysis of variance to test of inter-site level for both species and the accession (Intra-site) effect inside each site for *C. mixtus*. Means were compared using the Student Newman and Keuls test (SNK), and significance was determined at 95% confidence limits. Excel 2013 was used for some graphs.

Results

Inter-site effect on the germination of C. mixtus

The site or provenance of the seeds has a significant impact on the germination parameters. Harcha had the highest FGP (98.7%) and Ain EL Aouda had the lowest (92.2%) (Fig. 2). MGT was found to be longer in Tiddas followed by Ain EL Aouda, Boghadi and Harcha with 43.2, 40.8, 40.8, and 40.8 hours respectively, which indicates slow germination compared to other sites. Furthermore, the time taken to reach 50% of germination (T_{50}) was significantly longer in Tiddas compared to other sites. The fastest germination indicated by the values of GI and CVG was noted for the sites of Harcha, Larache, Sidi Chafi and Sidi Taibi (Tab. 2). The high value of CVt registered at Ain El Aouda site indicates more irregular germination over time compared to other sites.

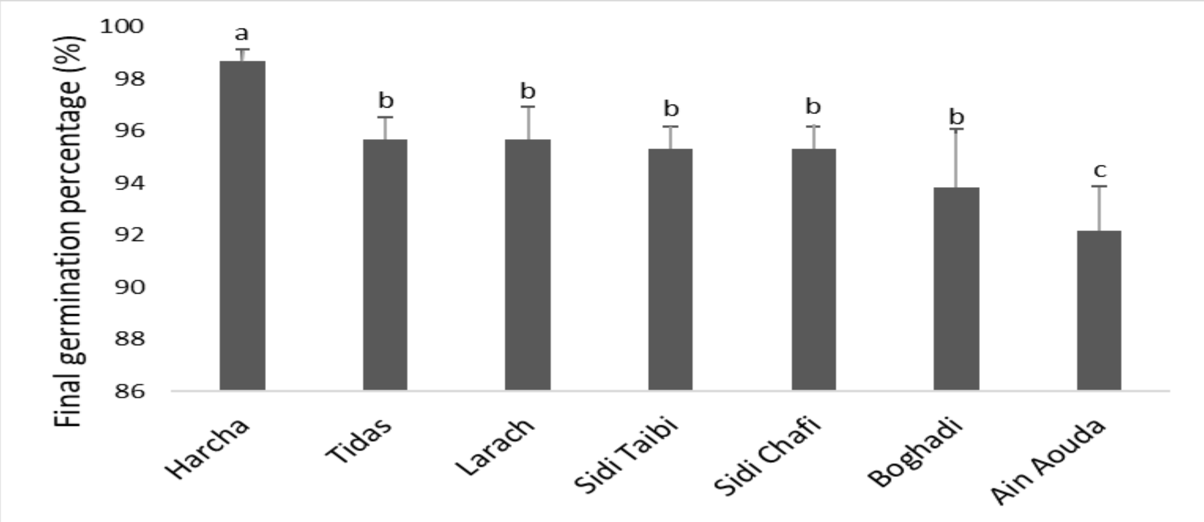

Figure 2. Final germination percentage in *C. mixtus* at the Inter-sites level.

Means with the same letter do not differ significantly according to SNK test at P < 0.05. The error bars represent the standard error of means.

Table 2. Effect of Inter-site level on the germination parameters of *C. mixtus.*

Site	MGT (day)	CVt (%)	GI (seed day^{-1})	CVG (% day^{-1})	T_{50} (day)
Tiddas	1.8 ± 0.1^{a}	37.5 ± 4.7^{bc}	31.4 ± 2.3^{bc}	56.5 ± 3.4^{c}	1.3 ± 0.1^{a}
Ain El Aouda	1.7 ± 0.1^{b}	45.0 ± 4.0^{a}	32.4 ± 1.9^{b}	59.0 ± 2.9^{b}	1.1 ± 0.1^{b}
Boghadi	1.7 ± 0.1^{b}	39.0 ± 3.5^{b}	32.8 ± 3.6^{b}	60.0 ± 4.1^{b}	1.1 ± 0.1^{b}
Harcha	1.7 ± 0.1^{b}	39.4 ± 4.8^{b}	34.1 ± 1.8^{a}	59.4 ± 2.4^{b}	1.2 ± 0.1^{b}
Larache	1.6 ± 0.1^{c}	38.6 ± 4.9^{bc}	34.4 ± 1.3^{a}	62.4 ± 2.2^{a}	1.1 ± 0.0^{b}
Sidi Chafi	1.6 ± 0.1^{c}	39.7 ± 5.4^{b}	34.3 ± 1.0^{a}	61.9 ± 1.8^{a}	1.1 ± 0.0^{b}
Sidi Taibi	1.6 ± 0.0^{c}	34.9 ± 2.9^{c}	34.4 ± 0.9^{a}	63.5 ± 1.4^{a}	1.1 ± 0.0^{b}

FGP: Final germination percentage; MGT: mean germination time; CVt: coefficient of variation of the germination time; GI: germination index; CVG: coefficient of the velocity of germination; T_{50}: median germination time. The means ± standard deviation with different letters are significantly different according to the SNK test at p<0.05.

Accession effect on the germination of C. mixtus

For *C. mixtus,* the results showed that the average FGP was 95.2% at 18 °C. The MGT recorded an average of 1.7 days. The FGP of the studied accessions in *C. mixtus* ranged from 84.5% to 99.0%. The highest FGP was observed in the CM-L14, CM-B10, CM-H1 and CM-H12 and CM-AE4 accessions from Larache, Boghadi, Harcha and Ain El Aouda respectively, while the lowest FGP was recorded in the CM-B8 accession from Boghadi (Tab. 3). The statistical analysis shows a significant difference for this parameter (FGP) between accessions at Tiddas, Boghadi, Ain EL Aouda and Larache (Tab. 3). The germination capacity was not affected by the accession effect on the other sites. For the other studied parameters, generally no significant differences were recorded at many sites except at Tiddas, Boghadi, Sidi Chafi and Ain El Aouda sites where the germination speed indicated by the GI and CVG registered significant differences. In fact, the best accession for Tiddas was CM-T1 with a GI of 33.6 seeds day^{-1} and CVG of 59.9 % day^{-1}. For Boghadi, the accession CM-B10 had the fastest germination process as indicated by GI and CVG with 36.3 seeds day^{-1} and 64.3 % day^{-1} respectively (Tab. 3).

Table 3. Effect of accession on germination parameters studied in *C. mixtus*

Site	Accession	FPG (%)	MGT (day)	CVt (%)	GI (seed day^{-1})	CVG (% day^{-1})	T_{50} (day)
Harcha	CM-H1	99.0 ± 2.0^a	1.7 ± 0.1^a	42.2 ± 3.4^a	34.9 ± 1.3^a	59.5 ± 2.4^a	1.1 ± 0.1^a
	CM-H12	99.0 ± 1.2^a	1.7 ± 0.1^a	35.5 ± 5.4^a	32.9 ± 2.3^a	58.1 ± 2.5^a	1.2 ± 0.1^a
	CM-H3	98.0 ± 1.6^a	1.7 ± 0.1^a	40.4 ± 3.2^a	34.6 ± 1.1^a	60.4 ± 2.6^a	1.1 ± 0.1^a
Tiddas	CM-T1	98.5 ± 1.9^a	1.7 ± 0.1^b	34.1 ± 2.1^a	33.6 ± 2.2^a	59.9 ± 2.6^a	1.2 ± 0.1^a
	CM-T2	96.0 ± 2.3^a	1.8 ± 0.1^a	40.4 ± 4.7^a	31.3 ± 1.4^{ab}	55.1 ± 2.9^b	1.3 ± 0.1^a
	CM-T6	92.5 ± 1.0^b	1.8 ± 0.1^a	38.0 ± 5.4^a	29.4 ± 1.3^b	54.5 ± 2.1^b	1.3 ± 0.1^a
Boghdadi	CM-B10	99.0 ± 1.2^a	1.6 ± 0.0^b	36.0 ± 1.4^a	36.3 ± 0.3^a	64.3 ± 0.2^a	1.1 ± 0.0^a
	CM-B12	98.0 ± 1.6^a	1.7 ± 0.1^a	40.1 ± 4.3^a	33.8 ± 1.8^b	58.8 ± 2.9^b	1.2 ± 0.1^a
	CM-B8	84.5 ± 6.8^b	1.8 ± 0.1^a	41.0 ± 2.4^a	28.4 ± 0.6^c	56.9 ± 3.6^b	1.2 ± 0.2^a
Sidi Chafi	CM-SC13	97.5 ± 1.9^a	1.6 ± 0.0^b	33.6 ± 2.9^b	35.1 ± 0.6^a	63.8 ± 1.0^a	1.1 ± 0.0^a
	CM-SC6	94.5 ± 3.4^a	1.6 ± 0.0^a	41.8 ± 4.4^a	34.1 ± 1.1^a	61.8 ± 1.7^b	1.1 ± 0.0^a
	CM-SC2	94.0 ± 2.3^a	1.6 ± 0.0^a	43.5 ± 2.1^a	33.6 ± 0.7^a	60.3 ± 0.5^b	1.1 ± 0.0^a
Ain El Aouda	CM-AE4	99.0 ± 1.2^a	1.7 ± 0.1^a	44.3 ± 3.2^a	34.1 ± 1.4^a	57.7 ± 3.2^a	1.2 ± 0.1^a
	CM-AE24	90.5 ± 3.4^b	1.7 ± 0.1^a	42.2 ± 2.9^a	32.1 ± 1.4^b	60.4 ± 3.5^a	1.1 ± 0.1^a
	CM-AE22	87.0 ± 3.5^b	1.7 ± 0.0^a	48.4 ± 3.6^a	31.0 ± 1.4^b	59.0 ± 1.4^a	1.1 ± 0.0^a
Sidi Taibi	CM-ST12	98.0 ± 0.0^a	1.6 ± 0.0^a	34.4 ± 2.4^a	35.0 ± 0.2^a	63.0 ± 0.8^a	1.1 ± 0.0^a
	CM-ST18	94.0 ± 1.6^a	1.6 ± 0.0^a	35.3 ± 2.3^a	33.9 ± 0.8^a	63.3 ± 1.3^a	1.1 ± 0.0^a
	CM-ST23	94.0 ± 3.7^a	1.6 ± 0.1^a	35.0 ± 4.4^a	34.2 ± 1.2^a	64.2 ± 2.1^a	1.1 ± 0.0^a
Larache	CM-L14	99.0 ± 1.2^a	1.6 ± 0.0^a	40.0 ± 4.1^a	35.3 ± 1.0^a	61.3 ± 1.8^a	1.1 ± 0.0^a
	CM-L26	97.0 ± 2.6^a	1.6 ± 0.1^a	39.5 ± 6.5^a	34.4 ± 0.7^a	61.3 ± 2.1^a	1.1 ± 0.0^a
	CM-L21	91.0 ± 4.2^b	1.6 ± 0.0^a	36.3 ± 4.2^a	33.4 ± 1.6^a	64.5 ± 1.2^a	1.0 ± 0.0^b

FGP: Final germination percentage; MGT: mean germination time; CVt: coefficient of variation of the germination time; GI: germination index; CVG: coefficient of the velocity of germination; T_{50}: median germination time. The means ± standard deviation with different letters are significantly different according to the SNK test at p<0.05.

Inter-site effect on the germination of C. scariosus

The site effect was significant for FGP and other germination parameters in *C. scariosus* according to the SNK test, except for CVt, which was high for all sites, indicating non-uniform germination over time (Fig. 3 and Tab. 4).

The FGP ranged from 70 to 93% and the MGT from 4.2 to 5.9 days at 18 °C with respective means of 83.8% and 5.1 days (Fig. 3). According to the results, seeds collected at Demnate 1 and Demnate 2, Ijoukak 1 and Ijoukak 2 and Ourika showed the highest FGP, followed by Azilal 1 and Azilal 2. The highest FGP was observed in Ijoukak 1 (93.0%), and the lowest in Azilal 2 (70.0%). The longest MGT and T_{50} were observed in seeds from Azilal 2 and Ourika sites, with 5.9 and 5.7 days, 5.2 and 4.8 days respectively. Germination occurs at a faster rate in the Ijoukak 1, Ijoukak 2, and Demnate 2 sites. This is also indicated by the mixed parameter of germination capacity and speed GI that was 24.9, 25.3 and 24.3 seed day^{-1} respectively. However, Azilal 2 had the lowest value with 14.6 seed day^{-1} (Tab. 4).

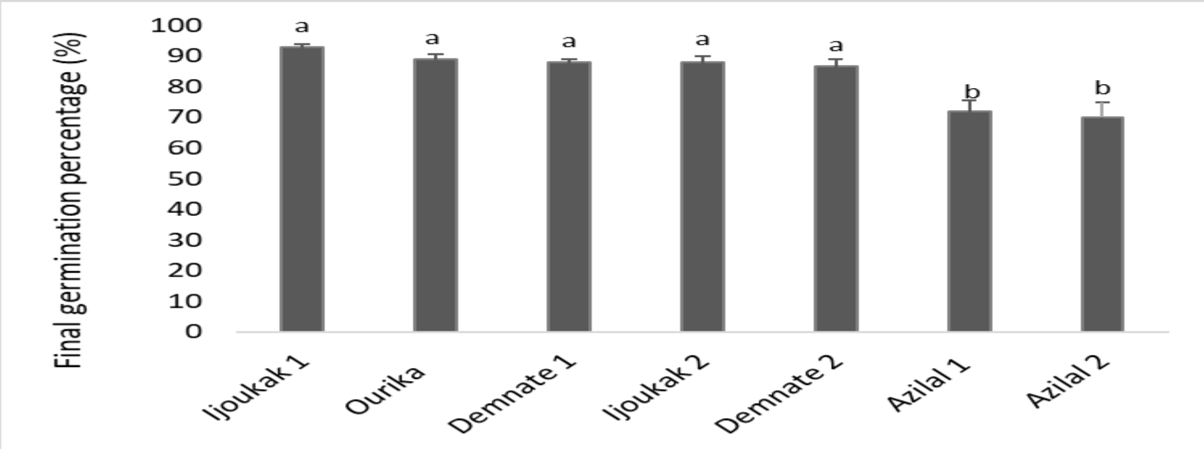

Figure 3. Final germination percentage in *C. scariosus* provenances

Means with the same letter do not differ significantly according to SNK test at P < 0.05. The error bars represent the standard error of means.

Table 4. Effect of Inter-site on germination parameters for *C. scariosus.*

Site	MGT (day)	CVt (%)	GI (seed day^{-1})	CVG (% day^{-1})	T_{50} (day)
Ijoukak 1	4.9 ± 0.7^{ab}	65.0 ± 9.0^{a}	24.9 ± 1.7^{a}	20.7 ± 3.0^{ab}	3.6 ± 0.2^{c}
Ourika	5.7 ± 0.4^{a}	57.3 ± 5.3^{a}	20.2 ± 0.0^{b}	17.5 ± 1.1^{b}	4.8 ± 0.2^{ab}
Demnate 1	5.6 ± 0.7^{a}	47.0 ± 7.8^{a}	20.0 ± 1.8^{b}	18.2 ± 2.3^{b}	4.6 ± 0.7^{ab}
Demnate 2	4.3 ± 0.2^{b}	51.2 ± 16.5^{a}	24.3 ± 1.5^{a}	23.2 ± 0.8^{a}	3.4 ± 0.2^{c}
Ijoukak 2	4.2 ± 0.3^{b}	52.0 ± 6.2^{a}	25.3 ± 1.7^{a}	23.7 ± 1.6^{a}	3.4 ± 0.3^{c}
Azilal 1	5.2 ± 0.3^{ab}	50.6 ± 6.4^{a}	17.3 ± 2.0^{b}	19.3 ± 1.2^{b}	4.3 ± 0.4^{b}
Azilal 2	5.9 ± 0.6^{a}	48.7 ± 10.5^{a}	14.6 ± 1.8^{c}	17.0 ± 1.7^{b}	5.2 ± 0.3^{a}

MGT: mean germination time; CVt: coefficient of variation of the germination time; GI: germination index; CVG: coefficient of the velocity of germination; T_{50}: median germination time. The means ± standard deviation with different letters are significantly different according to the SNK test at p<0.05.

Means with the same letter do not differ significantly according to SNK test at P < 0.05; The error bars represent the standard deviation of means).

Discussion

The average germination percentage recorded at 18 °C is 95.2% for *C. mixtus* and 83.8% for *C. scariosus*. Indeed, germination is a complex physiological process where the germination power of seeds is strongly affected by the species (Cury *et al*., 2010) and by environmental factors (Belmehdi *et al*., 2018; Nedjimi *et al*., 2018). Studies have been carried out on other genera of the family Asteraceae, showing different results depending on the genus and species. The percentage of seed germination of species of the genus *Flourensia* (F) at 25 °C is higher than 60% for *F. oolepis* and *F. campestris.* On the other hand, seeds of *F. hirta, F. leptopoda, F. niederleinii*, *and F. tortuosa* do not exceed 30% (Delbón *et al*., 2017). According to Zarghani *et al*. (2015), germination percentage of some Asteraceae species varied from 56.0–86.6% in *Matricaria aurea (Loefl.) Schultz-Bip* (annual plant), 96.0–97.3% in *Cynara scolymus L.* (annual and perennial), 89.3–100% in *Achillea millefolium L*. (perennial), 50.6–78.6% in *Cichorium intybus L*. (perennial) and 73.3- 90.6% in *Echinacea purpurea L*. (perennial plant). The optimal temperatures for germination were specified at 20 °C and 25 °C.

Temperature, dry season, light, habitat and elevation greatly influence germination. *C. mixtus* and *C. scariosus* showed high germination capacities. This indicates that the temperature conditions of 18 °C used in this study could be one of the ideal regimes for these species, but this should be investigated in depth. Note that temperature has a significant effect on germination (Belmehdi *et al*., 2018; Fernández-Pascual *et al*., 2013).

Geo-climatic data can also significantly affect germination of species, hence the importance of provenance. In this study, analysis of variance showed significant differences for the percentage and other parameters of germination at the inter-site level in *C. mixtus* and *C. scariosus*.

For *C. mixtus*, the results showed that the FGP varied significantly between the sites, with a delayed germination observed in the Tiddas, Boghadi and Harcha sites. This result could be explained by ecological differences between sites. For example, elevation at Tiddas, Boghadi and Harcha is quit higher compared to other sites. In fact, the site effect on seed germination was affirmed by several authors (Debi and Parkash, 2015; Ndihokubwayo *et al*., 2017; Moya *et al*., 2017; Akaffou *et al*., 2021). Further works by Xu *et al*. (2017) and Wenjing *et al.* (2020) showed that both habitat and elevation have a marked effect on germination.

The site effect on germination parameters of *C. scariosus* revealed that Azilal sites (Azilal 1 and Azilal 2) recorded the lowest germination percentages with 72.0% and 70.0%, respectively, in comparison to other sites. The GI and CVG parameters indicating the germination velocity were found to be low at Azilal and Ourika sites. The high elevation that characterizes Azilal region (>2200 m) could explain this behaviour. Indeed, Wenjing *et al.* (2020) confirm that germination percentages among species decrease significantly as elevational gradients increase. Marginal climates for seed development at higher elevations can lead to incomplete seed maturation, which is sometimes hard to detect visually in the seed but can be revealed during germination (Edwards, 1980). In fact, *C. scariosus* occupies a wide geographic and ecological range, which explains why populations from high elevations and different latitudes tend to have different germination capacities. It should also be noted that the rainfall during seed formation significantly influenced the germination performance (Acharya *et al*., 1992). By comparing the duration of the dry season (low rainfall) in four origin sites (Fig. 4), we see that this duration is longer in Ijoukak and Ourika (7 months), followed by Demnate (6 months) and Azilal has the smallest dry season (Fig. 4, climatic data analysed from the site: https://power.larc.nasa.gov (since 1981 to 2021)). Likewise, the temperatures recorded in this period are higher, and the precipitation is greater in Azilal region with 428 mm as the mean annual precipitation and low in Ourika with 245 mm. Furthermore, the mean annual temperature in Azilal ranges between 13.5 °C and 15.8 °C. Therefore, we can say that bioclimatic conditions were probably not favourable for the germination of *C. scariosus* seeds collected at Azilal sites because some seeds probably did not complete their maturation.

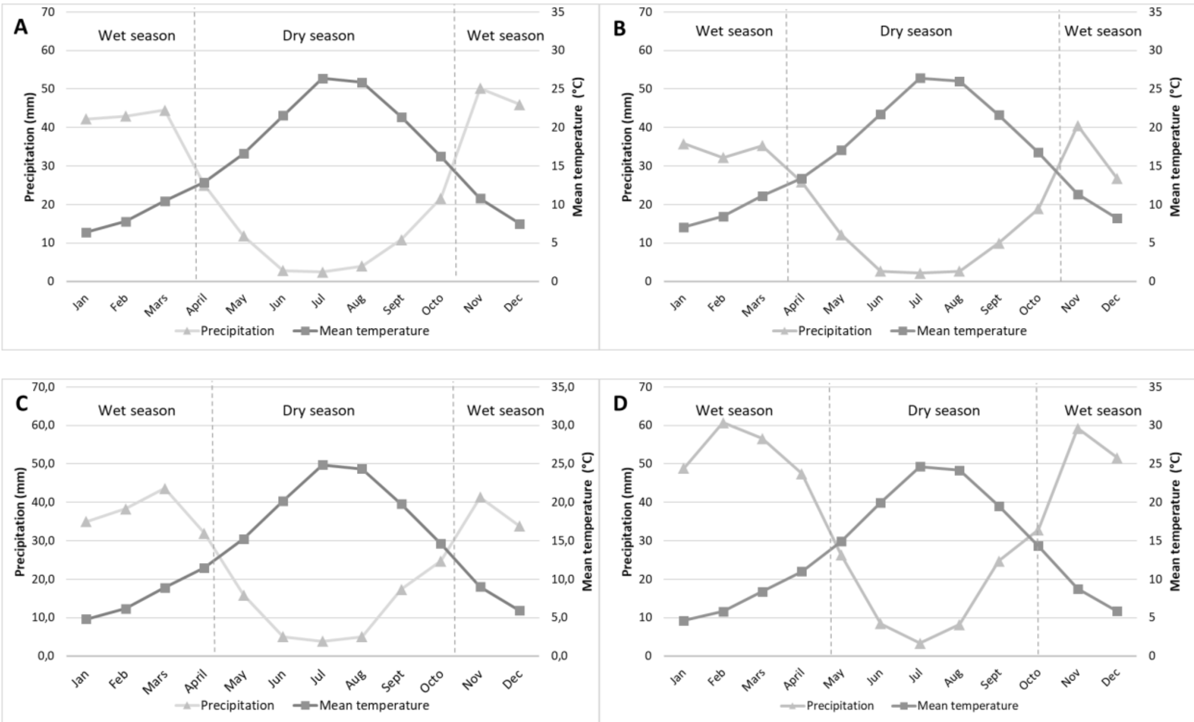

Figure 4. Ombrothermic diagrams for Ijoukak (A), Ourika (B), Demnate (C) and Azilal (D)

The accession effect on germination parameters of *C. mixtus* accessions was shown to be significant for the studied germination parameters. This effect could have originated from a possible genetic diversity between accessions, the environmental conditions or the development position of the seeds on the mother plant, which may not have the same germination requirements. Day length and other environmental factors like water stress and temperatures during maturation may also contribute to the phenotypic plasticity and diversity of seed germination in many plant species (Baskin and Baskin, 1998).

It is also important to consider the age of the mother plant, which can influence the germination capacity. Do and Attims (1978) showed that young plants produce far fewer dormant seeds than older plants. For *Carex secalina* plants, the age of the mother plant significantly influenced the germination capacity (Lembicz *et al*., 2011).

This is not the case for *C. mixtus* which is an annual plant, but life cycles may differ between regions. However, for *C. scariosus* the age of the mother plant could be a source of variability, but it was not easy to estimate the age of adult plants in the field on natural populations. It is also important to point out that *C. scariosus* is a biseasonal-flowering perennial shrub and differences in seed germinability according to the season of seed maturation could be registered.

Conclusion

In conclusion, this study was carried out to evaluate the inter-site effect on the germination of *C. mixtus* and *C. scariosus* and the intra-site (accession) of *C. mixtus.* This study showed that the germination of *C. mixtus* and *C. scariosus* seeds at a temperature of 18 °C is very encouraging, with germination percentages of 95.2% and 83.8%, respectively. The inter-site effects on the germination parameters of *C. mixtus* and *C. scariosus* were statistically significant. We found that germination was influenced by seed provenance. The seeds collected in Harsha region were the best in terms of germination percentage for *C. mixtus*, while for *C. scariosus*, seed collected from several sites germinated very well except Azilal. These results may indicate plasticity within the two species, especially for *C. scariosus* and the potential capacity to adapt to different types of bio-climate. Conservation and sustainable use of genetic resources of *Cladanthus* species depend on knowledge of the extent and pattern of interspecific variation. Thus, we propose: to pursue studying other factors that may influence germination, such as temperature, age of seeds, position of the achenes in the capitulum, position of flowers in the mother plant's inflorescence, water and salt stress during seed maturation and during germination, seedlings growth, and their characterization under different biotopes.

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