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Germination Strategies of Some Accessions of *Allium roseum* L. Collected from Different Bioclimatic Areas of Southern Tunisia

Jamila Zammouri, Azaiez Ouled Belgacem and Mohamed Neffati
Laboratory of Range Ecology, Institut des Régions Arides, 4119 Médenine, Tunisia

Abstract: This study deals with the germination and emergence behaviours of seeds and bulbs of four different accessions of *Allium roseum* L., a typically Mediterranean species considered as potential condiment with good flavour. Experiments were conducted both in laboratory and in field and several parameters were monitored in order to assess and compare the germination behaviour of four accessions of *Allium roseum* collected from four sites located along an aridity gradient in Southern Tunisia. The main achieved results show that the germination capacity of bulbs of the species is very low and does not exceed 30% for all studied accessions. The variation of seedlings emergence in the field constitutes an adaptation indicator of these accessions to aridity and drought confirmed by the presence of a high interaction between the reproduction mode and the environmental conditions. The accessions of the septentrional zones tend to be regenerated by an asexual multiplication (bulbs) whereas, those coming from the meridional (drier) zones tend to be regenerated by the sexual way (seeds). Survival of *Allium roseum* seedlings in Tunisian arid regions counts, therefore, on a sexual propagation much more than on a vegetative propagation.

Key words: *Allium roseum*, germination, adaptation, mode of reproduction

INTRODUCTION

The genus *Allium* comprising about 780 species (Fritsch and Friesen, 2002; Freisen *et al.*, 2005) exhibits a great diversity in various morphological characters, particularly in life form (rhizomes or bulbs) (Fritsch and Keusgen, 2006). Many species have been used as vegetables crops, spices herbal and in medicine for curing various diseases (Haciseferogullari *et al.*, 2005). This genus is primarily found in humid, semi-arid and arid environments (Mes *et al.*, 1997). They have acquired many morphological and ecological adaptations to seasonal rainfall variations and different temperature regimes (Gutterman *et al.*, 1995).

Allium roseum L. is a typically Mediterranean species. It belongs to section *Molium* (Specht and Keller, 1997). In Tunisia, this species comprises different intraspecific taxa (Jendoubi *et al.*, 2001), it may be considered as potential condiment with good flavour (Najjaa *et al.*, 2007) and as therapeutic virtues. It can be found in grassy and bushy places, cultivated fields and fallows and roadsides (Jendoubi *et al.*, 2001). Underground storage organ of *Allium roseum* is oblong bulb about 30-60 cm tall (Quezel and Santa, 1963). Plants flower in March and April and seeds mature in May (Jendoubi *et al.*, 2001).

Germination is a crucial stage in the life cycle of plants (El-Keblawy and Al-Rawai, 2005). Only few studies have been done on seed and bulbs germination of *Allium* species (Gutterman *et al.*, 1995; Kamenetsky and Gutterman, 2000). For instance, germination of some rhizomatous species, mainly inhabitants of moderate climatic zones, which germinated after 4 days of wetting at 20°C and bulbous species from the subgenus *Melanocrommyum* inhabitants of the semi-desert conditions of central Asia, that germinated only after 4 to 7 months of wetting at 0 to 3°C. However, the germination of two accessions of *Allium suworowii* showed differences may be related to after-ripening (Kamenetsky and Gutterman, 2000).

The species from section *Cepa* are well adapted to arid environments of the mountains of the middle Asian Turkistanie region, while species from section *Molium* are more widely distributed from Central Asia to the Mediterranean region (Hanelt *et al.*, 1992).

Thus, *Allium* species from different taxonomical groups and habitats have developed variable germination mechanisms (Kamenetsky and Rabinoswitch, 2006). In two species of this genus and under constant temperature of 5, 10 and 15°C, germination of *A. rotthii* in light and dark is almost the same. In the same experiment, germination of *A. truncatum* seeds in dark was much higher than in light.

At temperature higher than 20°C, no germination of *Allium truncatum* seeds occurred in light, even after 28 days. The opposite was found at 20°C in germination of *A. rotthii* (Guterman *et al.*, 1995).

According to many authors (Lopez *et al.*, 2003; Pico *et al.*, 2003; Bischoff *et al.*, 2006) differences among provenances, particularly in germination, can be inflated by heterogeneous environmental condition sites at which seeds were collected (environment maternal effects) and since no information is available on the germination of seeds and bulbs of *Allium roseum*, we conducted experiments in the laboratory and in the field aiming at studying the response of germination and emergence of seeds and bulbs of four different accessions of *Allium roseum* collected from different bioclimatic areas of southern Tunisia and testing whether differences in behaviours may be considered as a mechanism of adaptation to the environmental conditions in the natural habitats.

MATERIALS AND METHODS

Seeds of four accessions of *Allium roseum* were manually collected from four different sites located along an aridity and continentality gradient of the Center and the south of Tunisia. The main characteristics of these sites are the followings:

- Nasrallah (NAS) (09° 52 554 longitude, 35° 26 143 latitude), characterized by a semi-arid climate with a mean annual rainfall of 290 mm.
- El Fjé (FJ) (10° 38 530 longitude, 33° 30 005 latitude) characterized by an arid climate with a mean annual rainfall of 155 mm.
- El Frid (FR) (09° 04 360 longitude, 34° 29 010 latitude), located at the limit of the arid and the beginning of the Saharan climate with a mean annual rainfall of 100 mm.
- Kamour El Garâa (KG) (09° 33 120 longitude, 32° 32 240 latitude) characterized by a Saharan climate with a mean annual rainfall of 88 mm.

In laboratory: Germination experiments were carried out at the Laboratory of Range Ecology of the Institute of the Arid Regions Médenine during the month of September 2005 in order to test differences in germination between the different dispersion units produced from four accessions of *Allium roseum*.

For all germination experiments four replicates of 25 seeds and bulbs were placed on filter paper in 9 cm petri dish, wetted with 5 mL of distilled water and placed in a germinator (Luminincube II-M.C.A.S, Belgium) in dark at 15°C. This temperature was chosen as the

optimal for germination of seeds of *Allium roseum* (Zammouri, 2007). Similarly, bulbs were incubated in the same conditions. Germinated seeds and bulbs were daily counted and removed during 16 days. We considered seeds to be germinated after radicle appearance (Redondo-Gomez *et al.*, 2007).

Four characteristics of germination were determined: final germination percentage, time to first germination, time to final germination, Mean Time to Germination (MTG). MTG was calculated as:

$$MTG = \sum_i \frac{(n_i \times d_i)}{N}$$

Where:

n = No. of seeds germinated at day

i, d = Incubation period in days

N = Total number of seeds germinating (Brenchely and Probert, 1998; Redondo-Gómez *et al.*, 2007)

In fields: Before seeding, the collected seeds and bulbs were stored in the ambient conditions of the laboratory. The two factors were arranged in split plot design with four replications (seeds/bulbs and accessions) were done on 13 October 2005 in board (2.50 m long and 1.50 m wide) which was filled up with sand coming from arid steppe (1/3 fine sand, 2/3 organic matter). For each treatment, combination 10 seeds at 1 cm depth were sown resulting in 200 seeds per accession.

Bulbs for each accession were according to size medium ($4 < \varnothing < 7$ mm) at percentage of 100 bulbs, it has been planted at depth of 2 cm which position was indicated by the base of the bulb. Frequents counts were made every day.

Statistical analysis: Statistical analysis was carried out using analysis of variance by using SPSS (11.5) (SPSS, 2002). In case of significant effect of the accession and the dispersion unit on tested parameters, the Duncan test was used to compare the means at the 0.05 level of significance (Snedecor and Cochran, 1980).

Linear regression analysis was used to determine the relationships between percentage of germination of seeds and bulbs.

RESULTS

Study of the germination of the units of dispersion of the four sources of *Allium roseum* in the controlled conditions: The analysis of variance (ANOVA) shows a high significant effect of the provenance on the germination capacity of the bulbs ($p < 0.004$) and significant effect on the time of germination ($p < 0.011$).

Table 1: Final germination (%), time to first germination and mean time to germination (MTG) of different accession seeds of *Allium roseum* without treatment and in optimal temperature of germination

Accessions	Final germination (%)		Time to first germination (GD (days)		MGT (days)	
	Bulbs	Seeds	Bulbs	Seeds	Bulbs	Seeds
NAS	28±1.2 ^b	82±1.0 ^b	2.5±0.9 ^b	3.00±0.0 ^a	7.21±2.5 ^b	4.87±0.4 ^b
FR	17±1.1 ^a	98±0.9 ^a	5.0±1.0 ^a	3.00±0.0 ^a	8.23±1.6 ^a	3.78±0.5 ^a
FJ	20±0.7 ^a	88±1.7 ^b	5.5±1.7 ^a	3.00±0.0 ^a	7.46±0.9 ^a	4.98±0.2 ^a
KG	13±0.8 ^a	100±0.0 ^a	8.0±2.4 ^c	2.25±1.3 ^a	11.69±1.5 ^a	4.23±0.3 ^a

Means with the same letter(s) are not significantly different at the level of 5%, for each of the two parameters

Table 2: Emergence percentage of units of scattering of different accession *Allium roseum* in fields

Accessions	Mean emergence percentage (%)	
	Bulbs	Seeds
NAS	13.5±4.00 ^b	38.0±5.7 ^a
FR	4.0±2.90 ^a	60.0±7.7 ^b
FJ	9.5±3.70 ^{ab}	40.0±5.3 ^a
KG	5.0±17.2 ^a	55.5±6.0 ^b

Means with the same letter(s) are not significantly different at the level of 5%, for each of the two parameters

The results about to the germinativa behavior of the bulbs of the various accessions according to the time of incubation are shown in Table 1.

The exam of Table 1 shows that all the studied accessions expressed different responses. The germination capacity of the bulbs varies from 13% at KG to 28% at NAS. The time of germination detected, shows that except for the bulbs of the accession NAS presenting a time of two days germination, all the accessions collected from the aridest sites presented the highest times of germination, they vary between 5 at FR and FJ and 8 days at KG. This suggests the existence of a correlation between the origin of the plant material and its germinativa behavior.

Table 2 shows that the percentage of emergence which remains lower than 50% at all the selected accessions is highest (13.5%) at the accession NAS and is weakest (4%) at the accession FR.

Contrary to the average percentage of emergence of the bulbs of the four accessions, the average percentage of emergence of the seeds is higher, the analysis of the variance shows that the accession does not have a significant effect on emergence of the seedlings resulting from seeds ($p < 0.062$). According to Table 2, this percentage is 60% at FR and the weakest percentage is recorded at NAS (38%). Nevertheless, this percentage remains high at KG (55%) and 40% at FJ.

The percentages of emergence of the seedlings resulting from seeds most significant are recorded at the accessions coming from the arid stations (FD, FJ, KG). Indeed, the highest percentage of emergence was recorded at NAS coming from the most septentrional zone.

The results of the analysis of the variance relating to the emergence of the seedlings resulting from bulbs and

seeds of the four accessions of studied *Allium roseum* showed a highly significant effect ($p < 0.001$) of the unit of dispersion on the percentage of emergence of the seedlings. This percentage seems to be not influenced by the origin of the accession ($p < 0.245$).

The search for a relation between the percentages of emergence of the seedlings resulting from seeds and those resulting from bulbs in relation to the aridity gradient of the accessions has emphasized the existence of a negative correlation expressed by a linear regression (Fig. 2). Thus the most arid accession (KG) produced the highest percentage of emergence of seed and the lowest percentage of the emergence of the bulbs and conversely varies the percentages of emergence of the two units of dispersion for the most septentrional and least arid accession (NAS).

By putting the relation between the germination capacity and the mean time of germination (MTG) of the four accessions, Table 1 shows that the low speed of germination (7.21 days) is recorded at the accession of the most septentrional zone (NAS). Nevertheless, this speed remains more or less high at the accessions of the aridest zones.

The variation of the germination capacity of the seeds of the four studied accessions a is more significant than that of the bulbs (Table 1); it varies from 83 to 100% for NAS and FR, respectively.

In the same way, the times of germination of the seeds are three days at all studied accessions except for KG which is only one day after incubation. Such result could be confirmed by the analysis of the variance (ANOVA) which shows a non significant accession effect ($p < 0.521$) on the time of germination and a high significant effect ($p < 0.001$) of this variable on the germination capacity what shows that the studied accessions react differently towards the temperature of incubation.

The variation of the germination capacity in relation to the provenance of the accessions shows that the aridest ones (FR and KG) with higher germination capacities, presented the lowest speed of germination (3.78 and 4.23 days, respectively). On the other hand, FJ and NAS presented more or less high mean times of germination (4.98 and 4.87 days, respectively).

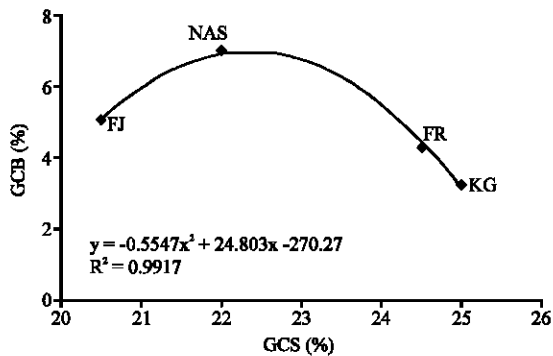


Fig. 1: Variation of the germination capacities of bulbs (GCB) according to that of seeds (GCS) in relation the origin of the accessions of *Allium roseum*

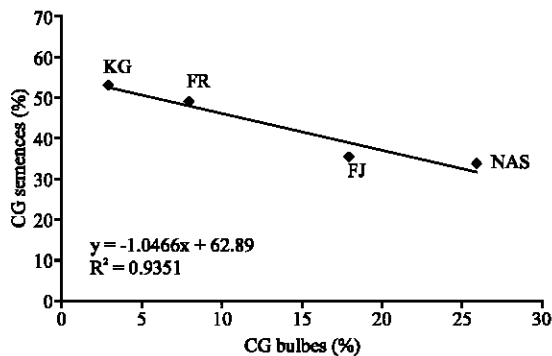


Fig. 2: Variation trend of the percentage of emergence of seedlings issued from bulbs and seeds in relation to the origin of the studied accessions of *Allium roseum*

Following a parabolic trend, Fig. 1 emphasizes a negative correlation between germination capacities of the both dispersion units (bulbs and seeds) of the four studied accessions.

The analysis of the variance highlighted a very high significant interaction ($p < 0.001$) between the accession and the dispersion unit at the level of the germination capacity of the seeds and of the bulbs. The unit of dispersion has a high significant effect ($p < 0.000$) on the germination capacity. Thus the accession the germinative capacity of the studied accessions influences significantly ($p < 0.025$), which shows that each accession has a given germinative capacity as well for the seeds for the bulbs.

Study of the emergence of the units of dispersion of the four accessions of *Allium roseum* cultivated in seedbed:

The analysis of variance (ANOVA) produces a high significant effect of the variable accession on the mean

percentage of emergence of the bulbs ($p < 0.000$). The variation of the mean percentage of emergence of the seedlings issued from the seeds of the four sources retained for this study is illustrated in Table 2.

The highest percentage of emergence, which remains lower than 50% at all studied accessions, is recorded for NAS (13.5%) while the weakest one (4%) is recorded for FR.

Contrary to the mean percentage of emergence of the bulbs of the four accessions, the mean percentage of emergence of the seeds seems to be very high. The analysis of the variance shows that the accession does not have a significant effect on emergence of the seedlings resulting from seeds ($p < 0.062$). According to Table 2, this percentage is 60% at FR and the lowest percentage is recorded at accession NAS (38%). Nevertheless, this percentage remains high at KG (55%) and FJ (40%).

The highest percentages of emergence of the seedlings issued from seeds are recorded at the accessions coming from the arid stations (FD, FJ, KG). However, the highest percentage of emergence of seedlings issued from bulbs, was recorded at NAS coming from the most septentrional zone.

The results of the analysis of the variance about the emergence of seedlings issued from bulbs and seeds of the four studied accessions of *Allium roseum* showed a high significant effect ($p < 0.001$) of the dispersion unit on the percentage of emergence of the seedlings. However this percentage seems to be not influenced by the origin of the accession ($p < 0.245$).

The establishment of a relationship between the percentages of emergence of the seedlings issued from seeds as those issued from bulbs in relation to the aridity gradient of the origin of the accessions highlights the existence of a high negative correlation fitted by a linear regression (Fig. 2). Thus the most arid accession (KG) produced the highest percentage of emergence of seed and the lowest percentage of the emergence of the bulbs. Conversely vary the percentages of emergence of both dispersion units for the most septentrional and least arid accession (NAS).

DISCUSSION

The results of this study show that the germination capacity of the bulbs of *Allium roseum* is very low and does not exceed 30% and this whatever the accession and the conditions of the experimentation. In spite of this weakness, there is a variation which can be in relation to the environmental conditions of the origin of each accession. This could be explained according to

Kamenetsky (1994) by the ecological amplitude of distribution of the species belonging to the *Allium* genus, since the moderate to the arid regions. This low capacity can be probably due to a particular dormancy characterizing the bulbs of this species. This dormancy is very common in the genus of *Allium*. Moreover Kamenetsky and Japarova (1997) classified the species of this genus in the group of the species presenting one period of relatively long dormancy. Such a result corroborates the work of Kamenetsky and Cutterman (2000) who showed that periods of dormancy vary between 3.5 to 4 months characterize *Allium altissimum* species of the relatively wet mountainous zones and it is more than 4 to 5 months at *Allium karataviense* species of the semi-desert zones. Moreover, Kamenetsky and Japarova (1997) showed that *Allium rothii* originating in Negev desert presents one period of dormancy which varies from 6 to 7 months.

Nevertheless, the more or less high germination capacity recorded at the bulbs of the *Nasrallah* accession both in controlled conditions and under environmental conditions is due to rainfall and moisture which characterize the original area of this accession (Kairouan). This is in agreement with the work of Boeken (1991) who showed the existence of a positive correlation between the rainfall and the soil moisture and the percentage of emergence of a desert geophyte (*Tulipa systola*).

The low percentages of emergence of the seedlings issued from the bulbs of the accessions coming from the meridional (Southern) zones seem to be due to a blocking of germination under the effect of an incompatible temperature. These results corroborate what find several authors (Nikolaeva, 1969; Baskin and Baskin, 1998; Donohue, 2002; Mayer *et al.*, 2007) who mentioned that the severity of the environmental conditions (high temperature, low moisture) and the interaction between these factors are able to perpetuate or to induce dormancy. This dormancy can be explained according to Thompson (1970) and Qaderi *et al.* (2005) by the exchange of the ranges of temperatures. It is thus, which Vasseur and Gagnon (1994) announced that a vegetative propagation by bulb of *Allium tricoccum* can be produced only after the severe season of growth. In the same way, the bulbs of several species belonging to under *Melanocrommyum* genus of the semi-desert of the Central Asia cannot germinate according to Kamenetsky and Gutterman (2000) only after an imbibition from 4 to 7 months and at a temperature varying from 0 to 3°C. These requirements make that the development of *Tulipa systol* (species of the family of liliaceae) requires a humidified soil and a temperature lower than 25°C (Gutterman, 1981; Boeken, 1991).

The results of the study of the effect of the temperature on the germination behavior of the seeds of the four accessions, make it possible to regard that a temperature of 15°C as being optimal for the germination of seeds. This corroborates the results of Jendoubi (1999) and Zammouri (2007). This temperature is close to that recommended (16°C) by Specht and Keller (1997) for the germination of the species belonging to the *Allium* genus. The optimum temperature varies from 5 to 15°C for *Allium rothii* (Kamenetsky, 1994). It varies from 15 to 20°C for *Allium ursinum* (Ernst, 1979) and of 15°C for *Allium altissimum* (Kamenetsky and Gutterman, 2000).

The statistical analyses evaluating the effect of the origin of the seeds (accession) on the germination behavior of the four accessions under the controlled conditions confirm the relation which exists between the germination and the temperature of incubation. This result corroborates that obtained by Donohue (2002) who mentioned that the prolonged dry conditions of summer can generate a differed germination until late autumn for the species with typical germination in early autumn.

Variation of the percentage of emergence of the seedlings resulting from seeds both under controlled conditions and under environmental conditions is probably explained by the difference at the level of the adaptation of these accessions to the aridity and drought. This corroborates the results obtained by Thompson (1971) who showed that the seeds of *Silene dioica*, a caryophyllaceae of a population originating from the north, is more dormant than the one from the south.

The variation of the germination behavior is well correlated according to Thompson (1969) with the geographical distribution of the individuals of six species belonging to the family of caryophyllaceae. Analysis of the results of the germination behavior of the units of dispersion of the four accessions shows that the adaptation of the studied accessions of *Allium roseum* is expressed by a low speed of their germination. This can present an advantageous situation for the establishment of this species. According to Krochmal (1960) and Jordan and Haferkamp (1989), more a species is ready to germinate at low temperatures more are the chances of its establishment success. These chances are moreover higher as the germination of the species is faster. The results of this study, confirm the great answer seems to be variable of the germination behavior of the units of dispersion according to the accessions of this species. Indeed, Specht and Keller (1997) underline the close relation which can exist between the geographical origin and the temperature at all the species of the *Allium* genus. On the basis of this study, it appears, that there is a

combination between the mode of reproduction and the environmental conditions which ensure that even under the extreme conditions of aridity, *Allium roseum* ensures its renewal of these units in good time and the good space. In the same way, accessions of the septentrional zones (Nasrallah) account to regenerate itself more by an asexual multiplication (by bulb) whereas, those coming from the drier zones (El Fjé, El Frid and Kamour El Garâa) count more on the sexual multiplication (by seeds) and consequently, survival of *Allium roseum* seedlings in Tunisian arid regions counts on a sexual propagation much more than on a vegetative propagation.

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