Effect of maternal environmental factors on seed dormancy of *Astragalus nitidiflorus*

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Resumen

La viabilidad y latencia de semillas de *Astragalus nitidiflorus* fue estudiada durante ocho años consecutivos para determinar si la elevada latencia física detectada en los primeros estudios era una característica fija. El efecto de factores maternos como la posición del fruto en la inflorescencia, posición de la semilla en el fruto o edad de la planta madre fueron estudiados. Para evaluar el efecto del estrés hídrico, plantas creciendo en macetas bajo condiciones de invernadero fueron bien irrigadas o estresadas durante la fructificación (mayo a junio) y se observó la germinación de ambos tipos de plantas. A su vez, la precipitación natural entre mayo y junio en la poblaciones de campo fue registrada y relacionada con la latencia de las semillas naturales. Los resultados mostraron que la latencia física no debería considerarse una característica fija en las semillas de *A. nitidiflorus* ya que factores maternos medioambientales como la sequía o edad de la planta madre influyen en la proporción de semillas que entran en latencia.

Palabras clave: Sequía**;** efecto materno; edad de la planta; experimento de riego; banco edáfico.

Abstract

Viability and seed dormancy of *Astragalus nitidiflorus* were tested during eight consecutive years in order to determine if the high physical dormancy detected in the firsts studies was a fixed trait. Effects of maternal factors as fruit position in the inflorescence, seed position in the fruit or age of the mother plant were tested. To study the effect of water stress, plants growing in pots under greenhouse conditions were well irrigated and stressed in the fruiting stage (May to June) and seed germination from both type of plant was tested. Also field rainfall from May to June in field population were recorded during the study period and related with dormancy of wild seeds. Results showed that physical dormancy should not be considered as fixed traits in *A. nitidiflorus* seeds because maternal environmental factor as drought or mater plant age influence the proportion of seeds that enter dormancy.

Keywords: Drought**;** maternal effect; plant age; irrigation experiment; soil seed bank.

1. Introduction

In most plant species, seeds vary in their degree of germination between and within populations and between and within individuals. Some of these variations may be genetic, but much of them are known to be phenotypic [1]. The so-called maternal environmental effects on germination is described as the phenotypic effects caused by the environmental conditions that would produce seeds that reached different germination percentages and/or had different germination requirements [2]. Following [1] these conditions consist of a combination of the microenvironment experienced by the seed due to its position on the mother plant and the abiotic environment of the plant. Seed dormancy allows the long-term burial of seeds and consequently the formation of persistent soil seed bank [3], enhancing seedling survival by delaying germination to avoid competition from established plants or unfavourable weather conditions.

Astragalus nitidiflorus Jiménez Mun. et Pau (Leguminosae) is a perennial herb endemic to the province of Murcia listed as Critically Endangered in accordance with IUCN (2001) criteria. Previous germination studies showed physical dormancy imposed by a hard coat (internal report). This dormancy type is widely represented in Leguminosae, so we considered the physical seed dormancy in *A. nitidiflorus* as a proven fact. However, from 2005 began studies on the biology of this species [4,5,6], and the last one showing that seed have hard coat but the species is unable to form long-term persistent soil seed bank.

The aim of this paper was to analyse the physical dormancy and germination of *A. nitidiflorus* seeds from different yields and study the effects on seed dormancy of some maternal factors such as *i*) fruit position in the inflorescence and seed position in the fruit, *ii*) the age of the mother plant, and *iii*) water stress in the mother plant.

2. Materials and Methods

2.1. Plant material and study site

Astragalus nitidiflorus is a perennial herbaceous legume short-lived that germinates in autumn. In mature plants flowering begins in March and continues until the end of May, with the maximum flowering moment occurring on April. Fruits are indehiscent legumes that take about two months to ripen and dispersion takes place from early July [4]. The study area has a Mediterranean type climate with semiarid conditions. The mean annual rainfall is around 300 mm and annual ETP of 1,319 mm. The annual drought period lasts normally five months.

2.2. Germination experiments

In order to know the variation in the degree of germinability between years, ripe fruits were harvested each July from 2006 to 2013 in the largest known population (37º 40' N, 1º 08' W). Seeds were removed from the fruits by hand and conserved in the Seed Bank of the Technical University of Cartagena at 4ºC. In July 2013 four replicates of 25 seeds each per year were placed on moistened filter paper in Petri dishes and incubated in a growth chamber at 15º and 12-h photoperiod. The number of germinated seeds was registered every two days for one month and recorded as non-dormant viable seeds (N-DVS). Non-germinated seeds were scarified with sandpaper to break physical dormancy and then placed back on moist filter paper. Those scarified seeds that germinated were recorded as dormant viable seeds (DVS), and those that did not germinate as non-viable seeds (N-VS).

2.3. Maternal environmental effect experiments

To evaluate the effects on germinability of position of the fruit positions in the inflorescence and the seed positions inside the fruit, 20 plants two years old were randomly selected and their ripened fruits collected in July 2013. From each plant, fruits were collected from five different inflorescences in two morphological positions: the basal and apical position. From each fruit, seeds were collected separately from the middle and from the apical end. At the same time, fruits from ten plants two years old and from the same number of plants three years old were collected to test the effect of the age of the mother plant on germinability. Germination response was tested as above in seed germination experiments.

Considering that our study on the germination characteristics was always conducted with seeds collected in the same location we focus the study on the only environmental factor that varied significantly between years, the rainfall. To check the effect of rain on seed dormancy a correlation was made between seed germination and the annual amount of rainfall. However no correlation was obtained ($F_{1, 6} = 2.052$, $P = 0.202$, $R^2 = 0.255$). Germination is highly responsive to environmental stress experienced during seed maturation on the maternal plant, and in some species it was shown that the last 5-15 days of seed maturation is the critical time [1]. Taking into account that the maximum flowering moment occurs on April and that the fruits take about two months to ripen, the period of maximum influence of water availability on *A. nitidiflorus* germinability is more likely to occur during the months of May and June. Then, another correlation analysis was made between seed germination and amount of rainfall in May-June.

Also one experiment was carried out in greenhouse to test the effect of maternal drought on seed dormancy. *A. nitidiflorus* plants were grown in a greenhouse. Plants proceeded from seed collected in July 2011 in the field population and seeded in 48 PVC 2.5 L pots filled with a mixture of black peat and soil from the habitat (6:1 vol.) and arranged on metal crop tables in six rows of 8 pots each. All pots were well irrigated until April 2012. So, from April 2012 two irrigation treatments were implemented, called "well irrigated plants" (WIP) and "stressed plants by drought" (SPD). At the fruiting stage (late July) fruits from both treatments were collected separately and seeds were removed by hand in order to test germination behaviour. Then, four replicates of 25 seeds each per irrigation treatment were plated and their germination responses were tested.

2.4. Statistical analysis

Germination data do not fit a normal distribution, so comparisons of non-dormant viable seeds (N-DVS), dormant viable seeds (DVS), and non-viable seeds (N-VS), between years were performed using Kruskal-Wallis non-parametric test at $P < 0.05$. To analyse the effects of fruit and seed position, plant age and water availability a Mann-Whitney U non-parametric test at $P < 0.05$ were performed. Also, in order to analyse the effect that water stress on the germination of seeds collected from 2006 to 2013 in the field regression curve analyses were performed.

All data was analysed using the statistical package SPSS 20.0 for Windows, and the graph-analysis performed by the software Sigmaplot Version 10.0.

3. Results and Discussion

Seed germination of *A. nitidiflorus* varied greatly depending on the year in which they were yielded ($P < 0.001$). In 2007, 2012, and 2013 the proportion of seeds with physical dormancy (DVS) were 97%, 73% an 87% respectively while in 2010 and 2011 only were 13% and 16%.

Results also showed that fruit position on the inflorescence had no effect on the proportion of dormant seed (P=0.095) and the same happened as regard the seed position in the fruit (P=0.663). However, the age of the mother plant had a significant effect in the proportion of non-dormant viable seeds (P = 0.018) and plants at the first fruiting produce seeds with higher germination percentages (19 ± 6.8%) that oldest plants (1 ± 2%). But the most influential effect on seed germination characteristics clearly was the availability of water at the fruiting stage. Seeds from plants well-irrigated showed total absence of dormancy (all viable seeds -87%- were non dormant) while those from stressed plants by drought showed less viability (only 68% of them were viable) and the proportion of non-dormant seed did not reach 20%.

In the natural population, the percentage of germinated seeds was also significantly affected by the amount of rainfall during the May-June period ($P < 0.001$), increasing seed germination with the amount of rainfall according to a quadratic curve ($F_{1,6} = 8.911$, $P = 0.022$, $R^2 = 0.781$). When rainfall is less than 10 mm in the period from May to June ("dry springs" in 2007, 2009, 2012, and 2013) the average germination percentage was 7.4 ± 1, while when rainfall exceeds 25 mm ("wet springs" in 2006, 2008, 2010, 2011) this value increased to 54.5 ± 11.6. However, germination was not affected by total annual rainfall ($F1, 6 = 2.052$, $P = 0.202$, $R^2 = 0.255$).

Our results showed that the age of mother plants has effect on seed germination, so that older plants produce viable seeds with lower germination percentages in comparison with those from younger plants. This fact is known from a large number of annual species and many examples are cited in [1] supporting the idea that senescence influence seed dormancy. But senescence effect is not a good explication in *A. nitidiflorus* because it is a perennial herbaceous species and seed from plant with different age were collected at the same time. *A. nitidiflorus* plants at the first fruiting stage (two years old) produced an average of 1,500-3,000 seed per plant while plants at the second fruiting stage (three years old) produced 8,000-10,500 seed [4], we could explain our result taking into account the likely negative relationship between maternal fecundity and offspring germination fraction because the risk of experiencing sibling competition increases with increase seed family size. However, in semi-arid land there are no relationship between maternal fecundity and germination. Results suggest that the age structure of the population should be an important factor to consider when estimating the annual replenishment of banks soil seed. At the same time, in perennial species, maternal effects have the potential to influence the age structure of populations, which in turn would influence projected population growth rates, probability of population extinction and genetic variation.

In other hand, results showed that seeds produced in dry springs have a high rate of physical dormancy and are possibly that they accumulate in the soil seed bank, while in wet years seeds are more water permeable being able to germinate when environmental conditions are favourable. The type of response to drought conditions during seed development seems dependent on the kind of dormancy involved. Seeds of *A. nitidiflorus* are known to have physical dormancy due to suberized and impermeable to water seed coat, which is commonly known as hardseedness and is typical of many species from families such as Fabaceae, Malvaceae, Chenopodiaceae and Liliaceae. When dormancy is imposed mechanically by a thick seed coat, as in our case, drought usually increases its thickness, thereby contributing to a reduced germinability [3]. Some authors assume that competition is the ultimate reason for the existence of such maternal environment effects and hypothesized that there is a negative relationship between favourable seasons and seeds germination and then the offspring may reduce negative effects of crowding in the following year. However this hypothesis is true in climate with high precipitation regimes but not for semi-arid ecosystem where plant interactions can be neutral or positive. The total annual rainfall, the amount of rain in each rainfall, and the time between rainfalls are highly variable in semi-arid areas [2]. This is the case of our study where rainfall from 2005 to 2013 period was an average of 306 mm and massive offspring germination is observed in some autumns. The ecological implication of these differences is that seeds produced in a dry spring are likely to have slower rate of dormancy release than those produced in a wet spring. Thus, seeds from a dry year may be more likely to become part of the persistent soil seed bank than those produced in a wet year. This mechanism is responsible for long-term seed dormancy but also provide sufficient germinable seeds for infestation when conditions become favourable. Our findings show that drought from May to June could prevent the emergence of most of the offspring with the first autumn rains. Results showed in this paper are consistent with the population dynamics of *A. nitidiflorus*, characterized by highly variable population censuses between years and specific episodes of massive emergency of seedlings as well as the fact that the soil seed bank of this species oscillate greatly depending on the demographic fluctuations in short-term population shown by [6]. The maximum lifetime of a *A. nitidiflorus* plant is four years and in that time the plant is fruitful three times, so the probability that different yields are affected by plant age or for different drought periods is very high. Then, the offspring will have a very big plasticity to closely match the changing environment conditions of these unpredictable environments.

4. Conclusion

Physical dormancy should not be considered as a fixed trait in *A. nitidiflorus* seeds because maternal environmental factor as drought or mater plant age influence the proportion of seeds that enter dormancy and become part of the seed bank. We call the attention of the researchers to not fall into the mistake of considering dormancy as a fixed trait even though some germination tests demonstrate it. So, the age structure of the population should be an important factor to consider when estimating the annual replenishment of soil seed banks. Besides, the amount of rainfall in late spring need to be considered in order to planning strategies for managing the endangered populations of *A. nitidiflorus* and for others species with similar characteristic to this species.

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