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The role of breeding system in the reproductive success of *Narcissus cavanillesii* A. Barra & G. López (*Amaryllidaceae*)

Abstract

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The main purpose of this study was to evaluate the influence of the breeding system in the reproductive success of *Narcissus cavanillesii* A. Barra & G. López (*Amaryllidaceae*), a species listed under Annexes II and IV of European Community Habitat and Species Directive. This taxon, which occurs in only two populations in Portugal, is of conservation interest in this country due to its low area of occupancy and population size, and fragmentation. Hand-pollinations were carried out to evaluate the performance of self- and cross-pollination offspring. Results show that xenogamous pollinations produce more fruits and seeds than autogamous ones. However, no significant differences were detected neither in seed weight nor in seed germination percentage between treatments. Germination rate (T_{50}) was significantly higher in autogamous crosses whereas radicle vigour as well as the number of days until the appearance of the first leaf were lower. A fitness coefficient was estimated for each of these parameters by comparing the results obtained from selfing and outcrossing treatments. These results are important for the formulation of effective management strategies in the conservation of this scarce geophyte in Portugal. This study was promoted by EDIA, S. A. and co-financed by EDIA, S. A. and European Regional Development Funds (ERDF).

Introduction

There is a general concern about the conservation of rare and threatened species. In many cases these species have small size or fragmented populations and are endangered by habitat destruction or fragmentation. These populations often have reduction in the performance of progeny due to inbreeding levels (Barret & Kohn 1991; Menges 1991; Given 1994) or pollinator limitation that negatively affects reproductive success (Ellstrand 1992; Kearns & Inouye 1993; Gómez 2000; Lau & Galloway 2004). In this context, breeding system is an important factor that conditions the species reproductive success. Thus, information about reproductive biology is useful in assessing management strategies for rare or endangered species (Albert & Iriondo 1997; Osunkoya 1999; Navarro & Guitián 2002).

N. cavanillesii is an autumnal flowering geophyte included in Annexes II and IV of the European Community Habitat and Species Directive (Council Directive – 92/43/EEC).

The geographical distribution range of this autumnal flowering species extends from Algeria and Morocco (Maire 1959; Valdés & al. 1987) to Portugal and Spain. Populations are mainly distributed in SW Spain, essentially in Andalusia where it is considered a common taxon (Valdés & al. 1987). In Spain this species is also reported from Extremadura (Rivas-Goday & Ladero-Álvarez 1973; Devesa 1995) where it is classified as a taxon of special interest (Decreto 37/2001 del 6 de marzo, D. O. Extremadura). In Portugal, *N. cavanillesii* is restricted to two localities, Ajuda and Montes Juntos, in the Alto Alentejo region (Malato-Beliz 1977; Rosselló-Graell & al. 2004) corresponding to the species' western range limit in the Iberian Peninsula. This taxon presents in this country a low area of occupancy and population size, and fragmentation. Moreover, both *N. cavanillesii* Portuguese populations are affected by the establishment of the Alqueva dam in the Guadiana basin. Thus, *N. cavanillesii* is a species of conservation interest in Portugal.

In this study the role of breeding system on reproductive success of *N. cavanillesii* was evaluated. The specific aims are reflected in the following questions:

what is the breeding system of the species,

is there a pollinator limitation for fruit set,

is there any evidence of inbreeding depression in the studied population,

if not, does target species show any evidence for a decline in fitness after outcrossing between the two Portuguese populations?

Methods

The field work was carried out in Ajuda population during the flowering period of 2003. Controlled pollinations were performed to determine the effect of pollen source on fruit set, seed set, seed weight, final seed germination percentage, germination rate (T_{50}), radicle vigour and the number of days until the appearance of first leaf.

Hand pollination experiments

The flowers were randomly selected before anthesis and five pollination treatments were performed using 60 flowers in each one: 1) natural pollination, unbagged and untouched flowers; 2) spontaneous autogamy, bagged and untouched flowers; 3) induced autogamy, bagged flowers pollinated with pollen from the same flower; 4) within-population xenogamy, bagged flowers cross-pollinated with a mixture of pollen from at least ten arbitrarily selected flowers from the same population, and 5) between-population xenogamy, bagged flowers cross-pollinated with a mixture of pollen from at least ten randomly flowers from Montes Juntos population.

For treatments 3, 4 and 5, flowers were emasculated before anther dehiscence using a fine forceps to avoid pollen contamination and then hand-pollinated. Flowers producing fruits were recorded for each treatment. Mature fruits were collected and the seeds per fruit (seed set) were counted and weighted with a high precision balance (accuracy ± 0.0001 g). Mean fruit set, mean number of seeds per capsule and mean seed weight as well as standard deviations were calculated for each treatment.

Seed germination tests

The seeds from each treatment were placed in Petri dishes (25 seeds per dish) on wet filter paper with distilled water and maintained at 15ºC in growth chambers with a 16 h:8 h photoperiod. Previous experiments have shown that *N. cavanillesii* seeds reveal optimal germination rates under these conditions (Rosselló-Graell & al. 2002). Depending on the cases, four to eight replicates were used per treatment. Seeds from both spontaneous autogamy and induced autogamy were used together for the autogamous treatment germination assay. Germination parameters recorded were final seed germination percentage, T_{50}, defined by the time needed to reach 50% of the final germination value, radicle vigour, measured by root length on the seventh day after germination, and the days until the appearance of first leaf. All parameters were recorded each two days.

Data analysis

To evaluate the effect of pollen source on studied parameters different statistical analysis were carried out. One-way ANOVA ($P < 0.05$) was used when no significant deviations from normality were found in the variables. Scheffe test ($P < 0.05$) was applied for comparison of means. Germination percentage was analysed after arc-sine transformation. Non-parametric Kruskall-Wallis and Mann-Whitney tests were applied to compare treatments when variables were not normal. All statistical analyses were performed with SPSS package 10.0.

Fitness

The fitness reduction due to lowered genetic variation could be exposed early in the life of plants (Menges 1991; Waser & Price 1994). Thus, several authors had used fruit set, seed set, seed weight germination and even percentage of offspring survival or growth rate to calculate inbreeding depression for target species (Charlesworth 1988; Stevens & Bougourd 1988; Waser & Price 1994; Fischer & Matthies 1997; Quilichini & al. 2001; Navarro & Guitián 2002). In that sense and in order to estimate offspring fitness (W) we used the following expression for each pollination experiment:

$$W = w1 \text{ x } w2 \text{ x } w3 \text{ x } w4 \text{ x } w5 \text{ x } w6 \text{ x } w7$$

where: $w1$ = fruit set; $w2$ = seed set; $w3$ = seed weight; $w4$ = final seed germination percentage; $w5 = 1/T_{50}$; $w6$ = radicle vigour and $w7$ = 1/number of days until the appearance of first leaf. As with the values of T_{50} and number of days until the appearance of first leaf, the higher the value the lower the fitness is, we used the inverse of these variables for the calculation of the fitness coefficients. All variables were previously transformed by dividing the variable by the highest value obtained in each treatment. W ranges between 0-1.

Results

Hand pollination experiments

N. cavanillesii produced fruits and seeds from both self- and cross-pollination treatments (Tab. 1). Significant differences in fruit set were found among treatments ($H = 29.982$, $P < 0.001$). Flowers that were cross-pollinated set more fruits than self- or induced autogamy ($U = 1020$, $P < 0.001$ and $U = 1350$, $P < 0.01$, respectively). No significant differences were found between control and cross-pollinated plants (U = 1560, P = 0.142).

The seed set significantly differed among treatments (H = 31.050, P < 0.001) and xenogamous pollinations set more seeds than induced autogamous crosses (U = 249.500, P < 0.05) (Tab. 1). No statistically significant differences were detected in seed weight among treatments (H = 8.794, P = 0.66).

Seed germination

No significant differences in final germination percentage were detected among treatments (F = 2.094, d.f. = 3; P = 0.132) (Tab. 2). Germination rate differed significantly when treatments were compared (H = 7.929, P < 0.05). T_{50} was higher in between-populations xenogamy than in within-population xenogamy (U = 7.500, P < 0.01). Radicle vigour and the number of days until the appearance of first leaf did not differed significantly among treatments (U = 27.302, P = 0.203 and U = 5.546, P = 0.134, respectively) (Tab. 2).

Fitness

Within-population xenogamous crosses provided the highest reproductive fitness coefficient value (0.72), whereas autogamous crosses had the lowest reproductive fitness coefficient (0.18) (Tab. 3). The results show too a better performance of offspring from within-population than from between-population crosses (0.72 and 0.58, respectively).

Table 1. Mean and standard deviations for fruit set, seed set and seed weight after different pollination treatments (n = 60) of *Narcissus cavanillesii*.

Pollen transfer	Fruit set	Seed set	Seed weight (mg)
Control	0.50±0.50	4.5±2.7	1.54±0.43
Spontaneous autogamy	0.20±0.40	3.0±1.4	1.57±0.38
Induced autogamy	0.38±0.49	2.5±1.3	1.76±0.28
Within-population xenogamy	0.63±0.48	5.6±3.5	1.63±0.45
Between-population xenogamy	0.60±0.49	5.7±1.8	1.44±0.22

Table 2. Mean and standard deviations for final germination percentage, germination rate, radicle vigour and number of days until appearance of the first leaf after different pollination treatments of *Narcissus cavanillesii*.

Pollen transfer	Germination (%)	T_{50} (days)	Radicle vigour (mm)	Days until first leaf
Control	94±8	12±1.6	5.2±1.1	10.2±3.2
Autogamy	87±8	12.5±1.9	5.1±2.0	10.0±2.71
Within-population xenogamy	79±18	10.7±1.5	5.9±1.6	9.9±3.10
Between-population xenogamy	87±7	13.1±1.3	6.1±1.8	9.7±3.4

Table 3. Reproductive fitness coefficients (*W*) for each pollen transfer experiment in *Narcissus cavanillesii*. *w1* = fruit set; *w2* = seed set; *w3* = seed weight; *w4* = final seed germination percentage; *w5* = 1/T_{50}; *w6* = radicle vigour and *w7* = 1/number of days until first leaf. The maximum reproductive fitness coefficient value is highlighted in bold. As with the values of T_{50} and number of days until the appearance of first leaf, the higher the value the lower the fitness is, we used the inverse of these variables for the calculation of the fitness coefficients. All fitness components were transformed to obtain a coefficient that might range from 0 to 1, being 1 the value with the highest fitness.

	w1	***w2***	***w3***	***w4***	***w5***	***w6***	***w7***	***W***
Control	0.79	0.78	0.94	0.94	0.90	0.87	0.95	0.41
Autogamy	0.60	0.52	0.96	0.87	0.86	0.82	0.97	0.18
Within-population xenogamy	1	0.98	1	0.79	1	0.95	0.98	**0.72**
Between-population xenogamy	0.95	1	0.85	0.87	0.82	1	1	0.58

Discussion

Results show that this species has a mixed mating system allowing for seed production from both xenogamous and autogamous crosses. However, the results of the control treatment suggest that there is a significant predominance of fruits set from xenogamous crosses than from autogamous ones. This can be explained by the species' floral structure, protogyny and pollinator assemblage that all together facilitate outcrossing processes in the studied population. Although most *Narcissus* species are protandrous (Barret & al. 1996), *N. cavanillesii* presents protogyny. Protogyny facilitates cross-pollination and thus prevents self-fertilization (Dafni 1992; Snow & al. 1996; Richards 1997). Moreover, *N. cavanillesii* has an open corolla exposing sexual structures that improves cross-pollination chance. A previous study in the same population revealed that *N. cavanillesii* flowers were mainly visited by Hymenoptera, like *Halictus* sp. and *Megachile* sp. Less frequent visits were made by some Diptera and some honeybees (Marques & al. 2007). The absence of significant differences in fruit set between control and xenogamous crosses suggests that pollinator activity is not a limiting factor for fruit production (Albert & al. 2001).

A comprehensive and realistic estimate of the effects of inbreeding in nature should include measurements of the fitnesses of cross and self offspring (Stevens & Bougourd 1988) but has seldom been examined in sensitive plant species (Ellstrand & Elam 1993). In many plant species, inbreeding is associated with reduced performance of selfed progeny relative to those produced by outcrossing (Menges 1991; Quilichini & al. 2001). The level of inbreeding depression may vary with the mating system and may also be a function of population size (Ellstrand & Elam 1993).

The results show a significant reduction in seed set after selfing and reproductive fitness coefficients show a better performance of progeny from within-population crosses when compared with the progeny from the other treatments. The differences in reproductive fitness coefficient between xenogamous and autogamous crosses indicate that inbreeding depression may occur in the studied population after autogamous crossings. Nevertheless, because in our study progeny mainly proceeds from xenogamous crosses we can expect that the population presents low levels of inbreeding depression.

Despite these results, the capability of clonal reproduction of *N. cavanillesii* should be considered due to the implications on population genetic variation (increased crossings between close relatives) in order to accurate determine the levels of inbreeding depression of the studied population.

Results show the importance of xenogamous crosses for offspring performance and to maintain population genetic variation. In that sense, management strategies involving the conservation of *N. cavanillesii* Portuguese populations should focus on the preservation of pollinators community and preventing habitat fragmentation in order to minimize inbreeding depression processes.

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