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Studies on some seed traits of *Iris pumila* L., *Adonis vernalis* L., *Primula veris* Huds. and *Alkanna tinctoria* (L.) Tausch.

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Summary: In this study we summarize the results of a five-years period concerning seed traits examinations on *Iris pumila, Adonis vernalis, Primula veris* and *Alkanna tinctoria,* with special attention to seed dimensions, seed mass and other traits concerning plant fitness, to their variability and the relationship among them. We found tight correlation between seed weight and seed dimensions in *Adonis* and *Primula,* at the same time no correlation exists among the same characters in *Alkanna tinctoria.* Consequently, the seed weight and seed dimensions can be used as synonyms in the form of „seed size" only after preliminary detection of correlations among them.
The variability of seed traits is higher in natural categories (individuals, morphs) than in seed mass categories as speculative groups. When we need homogeneous plant stand (e.g. for an introduction experiment) it is suggested to use seeds pre-selected in this way. For ex situ conservation, where the central goal is to maintain the genetic variability, seeds originated from different individuals are preferred.

Introduction

Seed characters -among others seed weight- contribute plant adaptation and play central role in plant life history through their complex fitness effects (*Venable* & *Brown* 1988; *Stamp* 1990; *Simons* & *Johnston* 2000). Seed size pattern may affect dispersal, dormancy, seedling emergence and survival, and the extent of phenotypic variability of a population. Seed weight varies at a great extent among species and is influenced by environment and plant age (*Salisbury* 1942; *Baker* 1972).

Seed traits of a species or an individual have long been characterized by low infraspecific morphological variability (*Harper* et al. 1970) as a result of intensive stabilizing selection on plants to maintain seed size. In the last three decades however substantial variation has been observed in seed size among populations of the same species (*McWilliams et al.* 1968; *McKee* & *Richards* 1996), between individuals within a population (*Cavers* & *Steel* 1984; *Agren* 1989; *Mehlman* 1993), and even within plant individuals among fruits (*Matthies* 1990; *Obeso* 1993; *Stöcklin* & *Favre* 1994). *Susko* & *Lovett-Doust* (2000) detected also high variability of seed mass among *Alliaria petiolata* populations, and found that it decrease within population (within individuals) and within fruits of an individual of the same species. Sexual morphs of *Silene acaulis* however does not differ significantly in the seed mass and embryo/endosperm ratio (*Delph* et al 1999).

It is a special character of the terminology of seed research that the terms „seed size" and „seed mass" are in most cases applied as synonyms (e.g.: *Seiwa* 2000). It rarely happens, that the „seed size" means really the dimension of seeds. *Simons and Johnston* (2000) for example regard the seed width to be important component of fitness.

In the recent work we summarize the results of examinations of seed traits of *Iris pumila, Adonis vernalis, Primula veris* and *Alkanna tinctoria* in a five-year period with special attention on the relationship among seed dimensions seed mass, and other traits concerning plant fitness. These species are involved in the ex situ conservation program of the Botanic Garden of the University of Szeged.

Our work has the following objectives in detail:

detection
- -the seed length and width of test plant species,
- -the variability of these characrers at the level of population, individuals and morphs,
- -the relationship among seed dimensions and other seed traits,

revealing
- -the differences among seed mass and seed number categories in trait's variability and relationships,

measuring - water uptake, germination and seedling survival of individuals in different seed weight classes.

Material and methods

The work was carried out on planted or semi-natural populations of the Botanic Garden of University of Szeged in the years 1995-2000.

Iris pumila and *Primula veris* have capsules with numerous seeds, the other two species have indehiscent one-seeded fruit (achene in *Adonis vernalis* and nutlet in *Alkanna tinctoria*). In the following we term the propagules of these latter two species as „seeds".

Description of populations and characters measured:

Iris pumila

The test populations were established in 1993 using rhizome particles with two buds originated from five natural populations of the same area. *Iris* is clonal plant, on morphological-anatomical basis it is not possible to separate ramets and genets therefore in this species we detected the seed dimensions only at the level of populations. Seed length and width has been measured in 10 individuals per populations in the vegetation period of 1995.

Adonis vernalis

The population were settled also with rhizome particles bearing two buds in 1996. Measurements were done in 1999: the length, width, mass and number of the seeds were detected in every flower of 11 individuals.

Primula veris

The sample originated from the semi natural population existing in the Botanic Garden of the University of Szeged for a long period. *Primula* is a distylous species composed of two morphs: pin plants with stigma at the throat of the corolla tube (long-styled morph), and thrum plants with stigma in the middle of the corolla tube (short-styled morph).

Matured capsules were harvested before dehiscence from 50 pin and 48 thrum individuals in 1998. Seed number per capsule was recorded, and all seeds were weighed as a group to the nearest 0.01 mg. Division of seed weight per capsule by seed number per capsule was used to estimate mean seed weight in each fruit. According to the mean seed weight three classes were distinguished: low (mean seed weight is under 0.4 mg), medium (mean seed weight is between 0.4 and 0.7 mg), and high (mean seed weight is over 0.7 mg). Capsules developed on pin (P) and thrum (T) plants were examined separately, thus, there was a total of six categories of fruits (P1, P2, P3, and T1, T2, T3).

Twenty capsules (all originated from different individuals) were chosen randomly in each category. Length and width of 15 seeds (or all seeds if the total was < 15) in each capsule was measured. Twenty seeds (or all seeds if the total was < 20) from 10 capsules of each category were separated, water-air-pumped and soaked in distilled water overnight to soften seed coat. Swollen seeds were cut with razor blade, the cutting surface was parallel to the dorsal flat side of the seed. The area of the whole cutting surface and of the embryo was measured on the dorsal part of the seeds. The amount of endosperm was estimated by subtracting the area of the embryo from the area of the whole surface. Length/width ratio of seeds and endosperm/embryo ratio was also examined.

Alkanna tinctoria

The population of natural origin was settled in 1997, using individuals with rhizome length of 30 cm. In 1998 individuals producing few (<1000) and many (1001<) seeds were separated. 100-100 seeds of three individuals from each category were placed in petri dishes on moistened filter paper. Dishes were kept in continuous darkness at 4°C. The water uptake of the seeds were measured periodically in the first 14 days and the total water uptake was also calculated. Seeds were checked for germination every day under dissecting microscope. The germinating seeds (protrusion of the radicle) were put in pots separately and the seedling survival were followed.

Statistical analysis

Means and coefficient of variations [CV%: (standard deviation/mean) x 100] of examined traits were calculated. For comparing the characters one-way and two-way ANOVA was used. The relationships among traits were detected by using Pearson correlation coefficients, analysis of covariance or multiple regression analysis, depending on the nature of traits.

Software

The seed dimensions and the endosperm/ embryo ratio were measured by using Image-Pro Plus 3.0 image analysis program. Data analysises were performed using Statistica and Excel 7.0.

Results and discussion

According to seed dimensions the four test species can be divided into two categories. *Iris* and *Adonis* comprise the large seed category, the other two the small one (*Table 1*).

There were no significant differences in seed length and width among the five *Iris* populations. The seed dimensions differ signifinantly among individuals in *Adonis vernalis*.. It was turned out from the two-way ANOVA of seed length and

Table 1 Seed dimensions: means and coefficients of variation

Species			dimensions of seeds (mm)		coefficient of variation %	
			seed length	seed width	seed length	seed width
Iris pumila			4.55	3.60	4.50-10.20[a]	2.10-8.20 [a]
Adonis vernalis			4.35***	3.05**	3.20-13.10[b]	3.90-14.50 [b]
Primula veris.	morphs	pin	1.41*	1.12	13.36	12.54
		thrum	1.37*	1.11	14.35	13.20
	seed weight categories	low	1.17***	0.96***	9.21	7.18
		medium	1.41***	1.12***	6.58	6.95
		high	1.57***	1.26***	6.23	5.55
Alkanna tinctoria	seed weight categories	low	2.24	1.79	5.93	7.49
		high	2.28	1.77	6.06	6.61

a: minimum and maximum of populations
b: minimum and maximum of individuals
p < 0.05:*, p<0.01:** ,p<0.001:***

Table 2 Means and coefficient of variations of other seed characters examined

Species		characters	mean	CV%
Adonis vernalis		total seed mass(mg)	433.08***	80.00
		weight of one seed(mg)	9.92 ***	32.82
		seed number/flower	38.15***	72.94
Primula veris	morphs: pin	seed lenght/seed width	1.27**	5.97
		endosperm (unit)	0.82	24.75
		embryo(unit)	0.23	29.52
		endosperm/embryo	4.05	29.66
	thrum	seed length/seed width	1.24**	5.58
		endosperm (unit)	0.88	24.74
		embryo (unit)	0.24	26.78
		endosperm/embryo	3.73	17.85
seed weight classes:	low	seed length/seed width	1.23*	6.33
		endosperm (unit)	0.63	12.82
		embryo (unit)	0.16	14.72
		endosperm/embryo	4.35	32.28
	medium	seed length/seed width	1.27*	5.81
		endosperm (unit)	0.86	16.26
		embryo (unit)	0.23	15.88
		endosperm/embryo	3.69	19.28
	high	seed length/seed width	1.26	5.24
		endosperm (unit)	1.06	12.38
		embryo (unit)	0.03	11.59
		endosperm/embryo	3.64	10.69
Alkanna tinctoria	seed weight classes.: low	water uptake(mg)	0.85	–
		no. of germinated seeds	24.0	–
		no. of seedlings	10.0	–
	high	water uptake(mg)	1.15	–
		no. of germinated seeds	13.0	–
		no. of seedlings	7.0	

p < 0.001:***, p < 0.01: **, p < 0.1: *

seed width of *Primula veris*, that the seed weight classes differed significantly concerning both of these traits, while there was slight difference in seed length and no significant difference in seed width between the two morphs (*Table 1, Figure 1*). Parallel with the seed weight both seed length and width increased. The level of significance was higher in the speculatively selected weight classes than that of the morphs. In the seed weight classes of *Alkanna tinctoria* there is slight difference between the seed dimensions.

The variability of seed dimensions among populations of *Iris* and among individuals of *Adonis* is high. In *Primula veris* with reference to a natural biological unit (morphs), the variability of seed dimensions was one order of magnitude higher, than that of the speculative (and pre-selected) seed weight classes. The variability within seed weight classes showed falling tendency with the increase of seed weight.

The variability of seed dimensions was almost the same in *Alkanna tinctoria* (*Table 1*), than in *P. veris*.

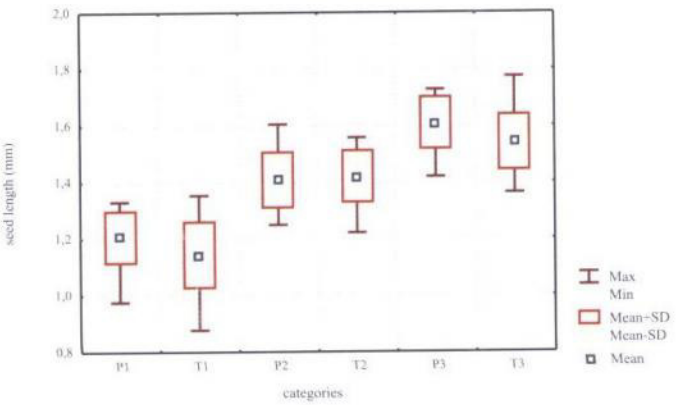

Fig. 1. *Primula veris:* mean, standard deviation, minimum, and maximum value of seed length in each category

The high significance level of differences and the large coefficients of variation prove, that *Adonis vernalis* population can be characterized by highly different individuals concerning seed mass, weight of one seed and average seed number per flower (*Table 2*).

The degree of differences among the six categories of *Primula veris* in seed shape characterized by seed length/seed width ratio depended on the compared categories. There was a strong difference between the two morphs: pin seeds were „slimmer" than thrum ones. Slight difference could also be found among seed weight classes: seeds from the first class were „dumpier" than seeds from the other two ones.

Two-way ANOVA of endosperm amount and embryo size showed almost the same pattern than seed length and seed width: the three seed weight classes could be distinguished unambiguously ($p < 0.001$***), but differences between the two morphs were negligible. Endosperm amount/embryo size ratio did not show significant differences among the six categories thus, all the categories could be characterized by the same value.

The amount of water uptake in *Alkanna* seeds until the beginning of the germination differed among the seed weight classes. In the high weight category the amount of absorbed water exceeded the 53 % of the seed weight, while in the lower mass class it was only 44 %. At the lower seed mass category the number of germinated seeds is higher and the survival of the seedlings is better than in higher one (*Figure 2*) in contrast with the results of *Seiwa* (2000). On the other hand *Platenkamp and Shaw* (1993) found, that small seeds has been associated with failure to germinate in the year of production. We suppose, that the reason of this phenomenon is that large seeds remain dormant for longer period (probably for years).

The coefficients of variation of traits summarized in *Table 2* are high among individuals (*Adonis vernalis*) but much lower between morphs and among seed weight classes of *Primula veris*. The seed length/seed width ratio seems to be very stable character, the differences of CV%-s are negligible.

Relationships among characters: No correlation exists between seed length and width by *Iris* populations

At the level of individuals of *Adonis vernalis* there were significant correlations among all seed characters examined (*Table 3*). In this case the use of seed weight and seed dimension as synonyms does not result uncertainity in the examination of contribution of seed character to plant adaptation or fitness.

Table 3 Correlation coefficients of seed characters in *Adonis vernalis*

	length	width	total seed weight	weight of one seed
length	1			
width	0.783**	1		
total seed weight	0.722**	0.635**	1	
weight of one seed	0.804**	0.804**	0.714**	1
seed amount	0.638**	0.564**	0.964**	0.601**

$p < 0.01$:**

Analysis of covariance between seed length and seed width in *Primula veris* revealed, that in all the six categories there was tight relationship between the two traits ($r = +0.693$***) in agreement with our former work (*Mihalik* et al. 1999), and a common regression coefficient could be used ($b = +0.512$). Among the means of each category a tight correlation could be detected ($r = +0.991$***, *Figure 3*).

The relationship between endosperm amount and embryo size was also tight ($r = +0.670$***), and there was a common regression coefficient in all the six categories ($b = +0.179$).

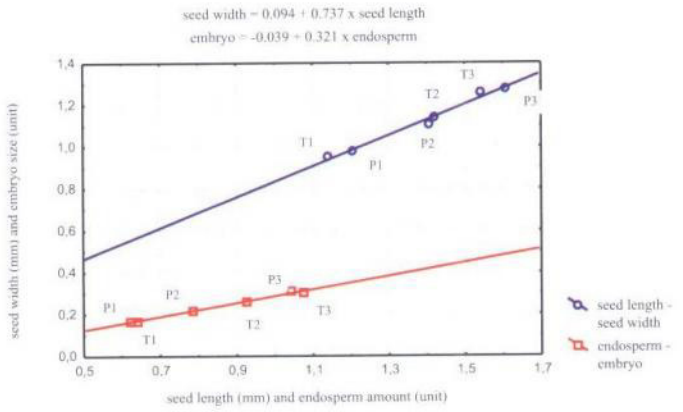

Fig. 2. *Primula veris:* means of seed length-seed width, and endosperm amount-embryo size in each category

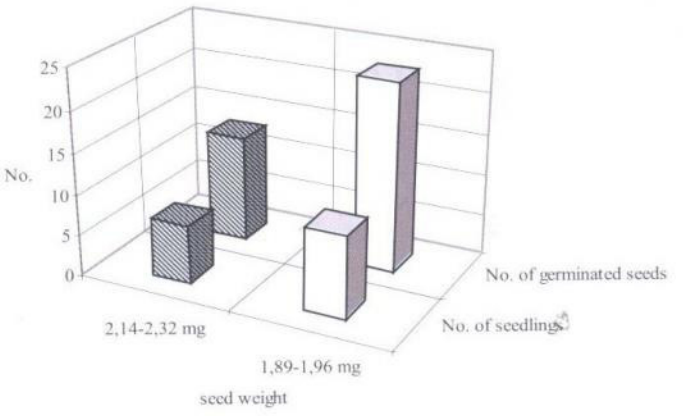

Fig. 3. *Alkanna tinctoria:* number of germinated seeds and seedlings in seed weight categories

The means of each category were fitted closely to a rising line (r= 0.994***), similarly to the length-width relationship (*Figure 3*).

Relationships among seed size parameters, endosperm amount and embryo size were examined by partial correlation coefficients in the three seed weight classes. Seed length had no effects on either endosperm amount or embryo size, while significant positive correlations were found in the case of seed width in all the classes.

It is proved from the data of *Table 4*, that there were only weak correlations among seed dimensons, the number of germinating seeds and seedlings. We emphasize the low positive correlation between seed length and number of germinating seeds as well as the number of seedlings, simultaneously with the negative correlation between seed

Table 4. Correlation coefficients of the seed characters of *Alkanna tinctoria*

	length	width	absorbed water	No. of germinated seeds
length	1			
width	0.133	1		
absorbed water	0.085	0.153	1	
No. of germinated seeds	0.308	–0.485	0.127	1
No. of seedlings	0.119	–0.414	–0.156	0.640

width and the former two traits. It means, that seed shape should probably influence the strength of germination and seedling survival in this species. We suppose, that third dimension of the seed (thickness) contribute at higher extent in the correlation among germination and seed dimensions. To reveal this qestion is one task of the further work.

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