Influence of nutrient availability on the reproduction and offspring growth in *Vicia grandiflora*

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Experiments were conducted to study the impact of nutrient availability on reproductive output and maternal effects. In plants grown under higher nutrient conditions, the reproductive biomass, the number of pods per plant, the number of seeds per plant and the total seed biomass were greater. Germination percentage was greater in large seeds, but small seeds germinated earlier than large ones. Mean total content of nitrogen increased with increasing seed size and was higher in high nutrient treatment. A correlation analysis of plants of the two nutrient treatments showed that seed number per pod was negatively correlated with pod number per plant with no correlation between mean seed mass per plant and the total number of seeds produced per plant. Nutrient levels significantly affected root/shoot ratio which decreased as nitrogen supply increased. This study showed that *Vicia grandiflora* reproduction and offspring competitiveness can strongly influence fertilization regimes used in different agronomic crops and rotations.

Keywords: Maternal effects, nitrogen, seed nitrogen content, seed size.

SEXUAL reproductive stage is one of the most critical stages in the lifecycle of a plant[1]. Reproductive success of flowering plants is determined by environmental conditions experienced during growth and maturation. Varying environmental conditions such as light quality, light intensity, temperature, macronutrients and water availability determine many aspects of potential success of the sexual reproductive phase, including seed size and production, percentage and rate of germination, and seedling establishment[2,3].

Nutrient availability to the maternal plant can affect seed production and seed traits[4]. For example, seed numbers, seed size and percentage of germination of *Senecio vulgaris* were decreased substantially with nutrient limitation[5].

Experiments were conducted on an annual legume *V. grandiflora* Scop., relatively independent of mycorrhizal associations. The species is native to central and western Asia, the Caucasus and eastern and southeastern Europe[6]. *V. grandiflora* was introduced into Poland in the 20th century, where it infests arable land but may also occur on roadsides and pastures. Since the plant grows in habitats that may vary abruptly both temporally and spatially in nutrient content, it is likely that the progeny of a single plant may germinate and develop in an environment that differs in nutrient concentration from that of the parent. It seems important, therefore, to study the intergenerational effects of nutrient addition.

Despite its widespread occurrence, critical data is lacking on its biology and ecology. The present study examines the influence of soil nutrition level on growth and reproduction, and maternal nutrition conditions (i.e. nutrient status of mother plants) on germination of seeds and offspring growth of *V. grandiflora*. The following hypotheses were tested: (i) Maternal nutrient limitation may decrease seed mass and germination success of *V. grandiflora*; (ii) Large seeds have greater germination percentage than small ones, and (iii) Nitrogen concentration in the seeds from low maternal nutrient treatment will be lower than that in seeds from high maternal nutrient treatment. This study addresses two research questions: (1) do maternal nutrient environments affect seed mass, seed number per pod and germination in *V. grandiflora*? (2) how are relative growth rate (RGR) and root/shoot ratio (RSR) of *V. grandiflora* influenced by N availability.

Pods of *V. grandiflora* were collected from plants in a natural population (51°43′00″N 44°14′N; 19°28′00″E; 89 m amsl) growing along roadside near Lodz, Central Poland, on 5 August 2015. The pods were taken from at least 100 different plants and opened by hand. Ripe seeds were stored under laboratory conditions (21–25°C, 40–45% relative humidity) for two weeks. Prior to sowing, the seeds were moist-chilled at 4°C for four weeks. Twenty seeds were sown into 0.7 l experimental plastic pots filled with two-thirds sandy soil and one-third clean sand. This potting soil had a total C content of 0.7% and a C/N ratio of 16. The pots were placed in plant growth chambers with a 16/8 h photoperiod, a temperature regime of 25/15°C and 65% humidity. Seedling emergence reached its peak two weeks after sowing. Three days after the initial emergence of seedlings, the plants were transplanted (one per pot) into square 3 l pots containing soil as above. The pots were randomly arranged on benches in an unheated glasshouse during May and June 2016 with natural light of a 14–16 h day length and a temperature range of 20–25°C, and relative humidity varying from 50% to 80%. To reduce variation in initial seedling size, only seedlings of the same height were used in the experiment. The pots were randomly assigned to two nutrient treatments: (i) high nutrient (40 ml 1/2 Hoagland's solution per plant or (ii) low nutrient (40 ml 1/8 Hoagland's solution with the same volume as above). The seedlings were watered daily (200 ml per pot) and received weekly applications of the complete nutrient solution. A randomized complete block design with five replicates consisting of two 5-pot sets each representing two fertilization treatments (low, high) was used.

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The experiment was stopped when the plants reached maturity 10 weeks after sowing. A set of 25 plants grown in each nutrient treatment were harvested as all pods ripened. Shoots were separated from roots at the soil level and the roots were washed free of soil. The pods were hand-threshed, seeds counted and weighed. Both roots and shoots were dried at 60°C for 3 days; then they were weighed. Vegetative mass was the sum of root and shoot mass.

A two-way factorial experiment was conducted to determine the effects of seed size and maternal nutrient regime on seed germination. Seeds from a previous experiment were randomly selected from each of 25 plants from each nutrient treatment. Fresh seeds from each treatment were conditioned by placing in a cold room at 4°C for four weeks. Following stratification, the seeds were classified visually into small (<2.8 mm in width) and large (>2.8 mm in width) size classes using an ocular micrometer ×10 magnification. Across nutrient treatments, the seeds were designated as small (= 30.04 mg ± 0.56) or large (12.64 ± 0.23 mg). Seed mass was determined by weighing 5 samples of 20 seeds each from each of the two size classes. The seeds were placed in sterile 9-cm diameter petri dishes (four replicates of 25 seeds per dish) between two sheets of sterilized filter paper and moistened with 7 ml of sterile distilled water. In all, 16 petri dishes were used: two seed size classes (small, large) × two nutrient treatments (low, high) × five replicates. Petri dishes were placed in temperature- and light-controlled environmental growth chambers with a 14/10-h photoperiod, a temperature regime of 22/10°C (mean daily maximum and minimum temperatures in Central Poland in late April and May) and 70% humidity. Irradiance in the growth chamber was 300 μmol $m^{-2} s^{-1}$ of photosynthetic photon flux density (PPFD). Distilled water (5 ml) was added to each dish every two days to prevent desiccation. Germination (radicle emergence) was examined daily for 21 days and seedlings removed at each count. Non-germinated seeds were tested for viability with tetrazolium chloride[7] and only viable seeds were used to calculate the rate and percentage of seed germination.

The seeds were dried (85°C) and weighed. Ten samples of 100 seeds (4 seeds from each of 25 randomly chosen plants) were randomly selected from low and high maternal nutrient treatments each. The harvested seeds were pooled from different seed-size classes of each individual plant and mixed. Total nitrogen content was determined on dried seed material with a modified Kjeldahl nitrogen procedure[8].

The effects of maternal nutrition and concurrent nutrient regime on offspring growth were evaluated. Fifty seeds for each maternal nutrient treatment (low, high) were initially chosen and sorted into two (large and small) size classes as described above. Next, the seeds were moist-chilled as described above and then germinated in a growth chamber for two weeks under the following conditions: 22 ± 0.5°C day and night, average humidity was 60 ± 5%. After two weeks the seedlings were transplanted, one per pot, into 3 l pots filled with a 3 : 1 (v/v) peat and perlite and were grown in an unheated glasshouse during September 2016 with natural photoperiod of 12 h. The positions of the pots were randomized. Ten seedlings were harvested for dry weight determination at the start of experimental treatments (first harvest). A completely randomized design was used with two-factorial combination of high and low maternal nutrient treatment and two seed-size classes (large versus small) × four replicates. Ten seedlings originating from each seed-size class (small versus large) were grown under two nitrogen treatments as NH_4NO_3 at two concentrations: 8 mM (the high offspring nutrient) and 0.5 mM (the low offspring nutrient). The seedlings were watered (200 ml) every other day. The respective high or low offspring nutrient was applied with every irrigation. Ten seedlings from each seed-size class–N combination were harvested five weeks after the plants were transferred to the pots (final harvest). The seedlings were dried to constant mass at 60°C for four days, and total biomass was determined. Subsequently, roots and shoots (leaves + stem) were separated and their dry weights recorded.

Significant differences in the number of pods per plant, seeds per plant, seeds per pod, and differences in mean seed mass, total seed mass per plant and differences in coefficient of variation of seed mass between nutrient treatments (low and high) were estimated using all-pair Student's *t* test comparisons. The germination rate was estimated using Timson's germination velocity as per the formula G/t, where G is seed germination percentage each day and t is the total germination period[9]. Prior to statistical analysis, germination values and values of the Timson's index were log-transformed to keep homogeneity of variance. For the germination percentage and germination rate, two-way analysis of variances (ANOVAs) were conducted with maternal nutrient level and seed size class as fixed effects. Analysis of covariance (ANCOVA) models were conducted to test the effects of seed mass (covariate) and maternal nutrient conditions (low versus high) on the nitrogen content of seeds. The relation between RGR and RSR was tested with a linear regression analysis. Based on the harvest data, two growth indices were calculated, i.e. relative growth rate (RGR, mg × mg^{-1} × day^{-1}) and RSR. RGR was calculated using the formula[10]: $RGR = (\ln W_2 - \ln W_1)/(T_2 - T_1)$, where W_2 is the average dry weight (in mg) of seedlings at the end of the experiment, W_1 the average initial dry weight (in mg) determined from newly emerged seedlings after transplantation, T_2 the final harvest time and T_1 is the time of transplanting in all treatments. RSR was determined according to the formula: $RSR = R/S$, where R was root dry weight and S was dry weight of shoot.

The three-way ANOVA was performed to determine if there was an interaction effect among three independent

Table 1. Seed and pod traits for plants of *Vicia grandiflora* grown under two soil nutrient conditions

Maternal nutrient conditions	No. of pods (plant^{-1})	No. of seeds (plant^{-1})	No. of seeds (pod^{-1})	Mean seed mass (mg)	Total seed mass (g plant^{-1})
Low	$5.8^b \pm 0.8$	$35.6^b \pm 1.4$	$4.5^b \pm 0.4$	27.38 ± 1.21	$1.02^b \pm 0.45$
High	$11.2^a \pm 2.5$	$50.8^a \pm 8.5$	$7.2^a \pm 0.8$	30.01 ± 3.78	$1.53^a \pm 0.78$
F value	11.2	11.8**	9.2*	3.8^{ns}	7.4*
P value	$P < 0.001$	$P < 0.01$	$P < 0.05$	$P > 0.05$	$P < 0.05$

Data are mean ± SD ($n = 25$ plants). Different letters within a column indicate significant difference (Student's *t* test, $P < 0.05$)

Table 2. Nodule number, whole plant and individual nodule dry weight for plants of *V. grandiflora* grown under two soil nutrient conditions

	Nutrient conditions	
	Low	High
No. of nodule (plant^{-1})	16.0 ± 3.0^b	8.0 ± 2.0^a
Total nodule dry wt (mg plant^{-1})	22.0 ± 1.45^a	10.3 ± 1.8^b
Mean nodule dry wt (mg plant^{-1})	1.25 ± 0.52^a	1.29 ± 0.45^a

Data are the means ± SD of four replicates. Means within a row followed by the same letter are not significantly different ($P < 0.05$).

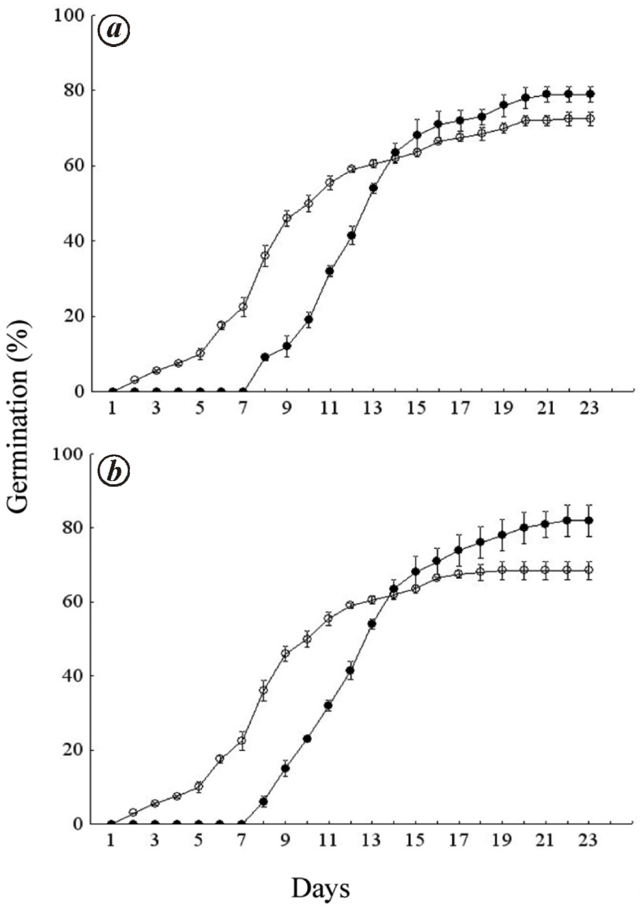

Figure 1. Percentage of cumulative germination of seeds of *V. grandiflora* during 23 days after sowing in relation to parental nutrient regime and seed size class (open bars, small seeds; closed bars, large seeds). ***a***, low; ***b***, high nutrient regime.

variables (i.e. seed size class, maternal nutrient conditions and offspring nutrient conditions) on the dependent variable (RGR).

The number of pods per plant, the number of seeds per plant and seeds per pod, and total seed mass per plant varied significantly between the nutrient treatments. However, mean individual seed masses from the low and high nutrients were not significantly different ($F = 3.8$, $P > 0.05$) (Table 1). Exposure to the lower level of nutrient treatment led to plants with fewer pods ($F = 11.2$, $P < 0.001$) and lower total seed mass ($F = 7.4$, $P < 0.05$). The seed number per pod increased with increasing nutrient from 4.5 ± 0.4 in the low nutrient treatment to 7.2 ± 0.8 in the high nutrient treatment ($F = 9.2$, $P < 0.05$).

The number of root nodules declined as the rate of nutrient application increased. The mean nodules dry weight per plant did not significantly ($P > 0.05$) vary between the treatments (Table 2).

Correlation analyses in the two nutrient treatments revealed that the mean seed mass was not significantly correlated, either positively or negatively, with the number of seeds per pod, whereas the number of pods per plant was significantly negatively correlated with the mean number of seeds per pod. In addition, correlations between the number of seeds per plant and the total seed mass per plant were positive and significant (Table 2).

There was no effect of the nutrient level on final germination percentage. Instead, under both nutrient conditions, seed size had a significant effect ($P < 0.05$) on seed germination. Germination percentage was higher for large seeds (>2.8 mm in width), while small seeds (<2.8 mm in width) emerged earlier and more rapidly (Table 3, Figure 1 *a*, *b*). Additionally, there was no significant interactive effect between seed size class and nutrient level on coefficient of velocity ($P = 0.07$) or on final percentage germination ($P = 0.06$) (Table 3).

Seed nitrogen content was significantly greater ($P = 0.0234$) for the high maternal nutrient conditions as compared to the low maternal nutrient conditions. Relationships between seed mass and seed nutrient contents differed between maternal nutrient conditions, as indicated by maternal nutrient conditions × seed mass interactions (Table 4).

Mean RGR was significantly greater in offspring growing under high nutrient conditions as compared to the low

Table 3. Pearson product–moment correlation coefficients amongst seed and pod traits

	No. of pods per plant	No. of seeds per plant	No. of seeds per pod	Mean seed mass	Total seed mass per plant
Low nutrient					
No. of pods per plant	1	−0.707	−0.985*	0.762	−0.578
No. of seeds per plant	−0.621	1	0.863	−0.345	0.980*
No. of seeds per pod	−0.954*	0.879	1	−0.711	0.235
Mean seed mass	0.686	−0.362	−0.784	1	−0.256
Total seed mass per plant	−0.604	0.987*	0.739	−0.205	1
High nutrient					
No. of pods per plant	1	−0.689	−0.967*	0.765	−0.559
No. of seeds per plant	−0.631	1	0.876	−0.434	0.952*
No. of seeds per pod	−0.941*	0.856	1	−0.711	0.287
Mean seed mass	0.666	−0.3452	−0.742	1	−0.261
Total seed mass per plant	−0.623	0.977*	0.679	−0.3455	1

*Significant correlation (0.05 level).

Table 4. Analysis of variance to determine the effects of maternal nutrient level (low versus high) and seed size class (small versus large) on germination characteristics of *V. grandiflora*

Source	Factor	d*f*	*F* value	*P* value
Coefficient of velocity	Nutrient level (N)	1	4.3	$P > 0.05$
	Seed size class (S)	1	26.8	$P < 0.05$
	N × S	1	12.5	$P < 0.05$
Final percentage germination	Nutrient level (N)	1	29.2	$P > 0.05$
	Seed size class (S)	1	38.4	$P < 0.05$
	N × S	1	36.7	$P > 0.05$

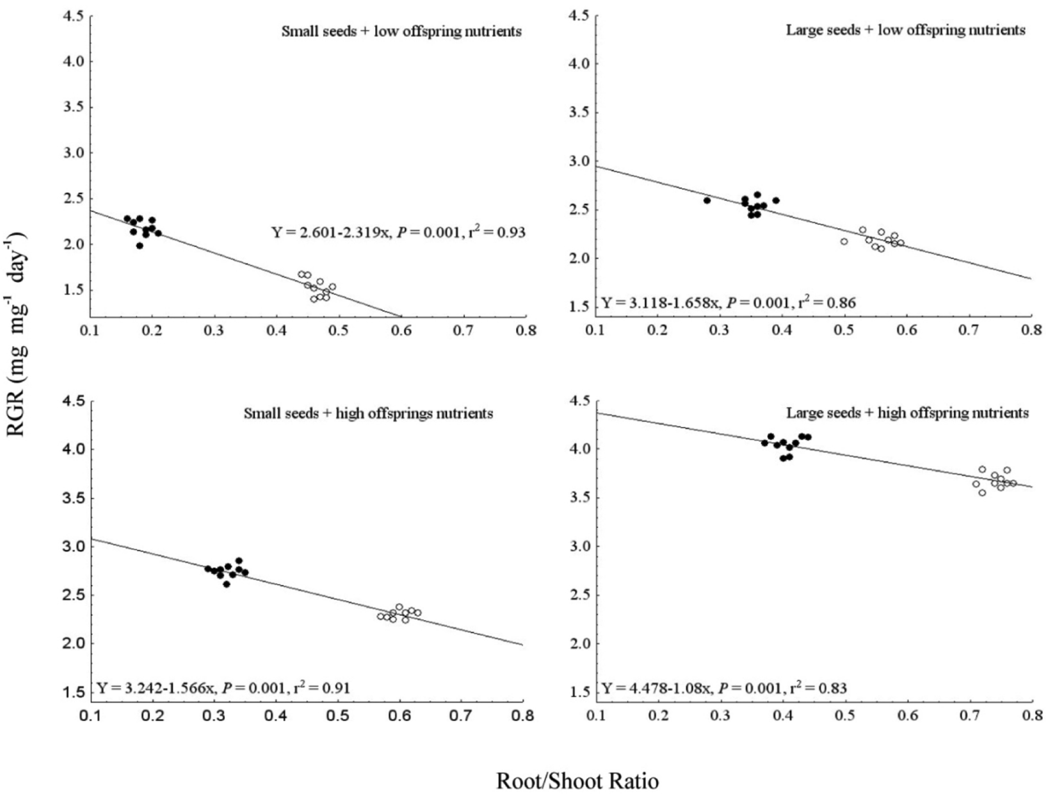

Figure 2. Effects of seed size class (open bars, small seeds; closed bars, large seeds) and offspring nutrient conditions on correlations between relative growth rate (RGR) and root/shoot ratio (RSR) of *V. grandiflora*.

Table 5. ANCOVAs of the effects of seed mass (covariate) and maternal nutrient conditions (low versus high) on the nitrogen content in seeds based on the data pooled across seed size classes

Seed mass	Maternal nutrient conditions	Maternal nutrient conditions × seed mass	Low maternal nutrient conditions (mg seed^{-1})	High maternal nutrient conditions (mg seed^{-1})
22.21	12.66	11.87	0.74 ± 0.02	0.86 ± 0.08

F values for seed mass, maternal nutrient conditions and seed mass × maternal nutrient conditions are given.

Table 6. Results of three-way ANOVA showing effects of seed size class (SSC), maternal nutrient conditions (MNC) and offspring nutrient conditions (ONC) on relative growth rate (RGR, mg × mg^{-1} × day^{-1}) and root/shoot ratio (RSR) of seedlings of *V. grandiflora*

Source of variation	Statistic	d*f*	RGR	RSR
SSC	*F* value	1	89.5	45.4
	P value		**<0.001**	**<0.001**
MNC	*F* value	2	76.9	41.5
	P value		**<0.001**	<0.001
ONC	*F* value	1	125.8	56.3
	P value		**<0.001**	**<0.001**
SZC × MNC	*F* value	2	16.0	12.5
	P value		**<0.001**	**<0.001**
SZC × ONC	*F* value	1	21.4	15.4
	P value		**<0.002**	**<0.003**
MNC × ONR	*F* value	2	35.8	12.4
	P value		**<0.001**	**<0.001**
SZC × MNC × ONC	*F* value	2	19.7	8.9
	P value		**<0.026**	**<0.027**

Significant values are highlighted in bold.

nutrient conditions ($P < 0.001$). RGR was significantly ($P = 0.001$) and negatively correlated with RSR in all experimental variants (Figure 2).

Results of three-way ANOVA showed significant effects for seed size class × maternal nutrient conditions × offspring nutrient conditions. Larger seeds produced larger seedlings than small seeds (Table 5).

The first hypothesis – that maternal nutrient limitation should decrease seed mass of *V. grandiflora* – was incorrect. The mean seed mass differed significantly among plants but did not differ significantly between the two nutrient treatments. These results indicated that the total seed mass per plant was mainly determined by the number of seeds produced. This is consistent with the pattern found in *Senna obtusifolia*[3] and *Lupinus perennis*[11]. In contrast, many other studies found individual differences in seed mass[12,13], which may arise from environmental maternal effects or genetic maternal effects, or both.

In this study, the number of seeds per pod increased with increasing nutrient, suggesting that limitation of nitrogen availability was an important factor influencing the number of seeds produced per pod in *V. grandiflora*. The same is true for another legume, *Cercidium floridum*[14]. In this study, the number of seeds per pod was not correlated with individual seed mass, while the total seed mass per plant was mainly determined by the number of seeds produced. In addition, mean seed masses were similar for both nutrient treatments. Therefore the results showed no general effect of the maternal nutrient conditions on individual seed mass.

The second hypothesis – that large seeds have greater germination percentage than small ones – was correct. Germination percentage of the bigger seeds (>2.8 mm in width) was higher, whereas the smaller seeds (<2.8 mm in width) emerged earlier and more rapidly. These results disagree with those reported by Zhang and Maun[15] that seed germination percentage was not affected by seed mass. Better and rapid germination of heavier seeds may be attributed to their bigger storage reserves[16]. Bewley and Black[17] suggested that proportion of protein, lipid, nitrogen and carbohydrate provided readily available energy which stimulated germination. Better growth of *V. grandiflora* seedlings which emerged from the heavy seeds is linked with larger nitrogen and energy contents of these seeds as compared to the light ones, which concurs with findings for other species[3].

RSR is a parameter that reflects plant response to growing conditions. The resulting data from this study indicate that offspring growing under low nutrient conditions accumulated more biomass in roots than that growing under high nutrient conditions. This means that when nutrient becomes limited, in accordance with optimal partitioning theory, *V. grandiflora* partitions more biomass to roots than to shoots, inhibiting its ability to compete. This finding is similar to those reported by Bonifas[18] for *Abutilon theophrasti* and by McConnaughay and Coleman[19] for *Chenopodium album*.

The resulting data from this study also indicate that RGR was significantly negatively correlated with RSR in both high and low maternal nutrient regimes, which concurs with findings for other species, for example for *Betula papyrifera*[20].

Previous studies reported that increase in the nutrient concentration in the growing environment often led to the production of heavier seeds and greater concentrations of nutrients[21]. The third hypothesis – that nutrient concentration in the seeds from low maternal nutrient treatment will be lower than that in the seeds from the high maternal nutrient treatment – was also correct. In this study, the nitrogen content of seeds was higher under the high nutrient conditions as compared to the low nutrient conditions. A hypothesis may be put forward that soil nutrient content during plant growth influenced its content in the resulting seeds. Another study on other species also showed that nitrogen concentration of seeds did increase in response to maternal nutrient status[3].

In this study, correlation analysis of two nutrient treatments showed that the number of pods per plant was significantly negatively correlated with the mean number of seeds per pod. This result indicates that there was a trade-off between pod size and the number of seeds rather than between seed size/mass and the number of seeds. This result disagrees with that reported by Wulff[22], who found no correlation between mean seed mass per plant and total number of seeds produced per plant. The numbers of pods and seeds are affected by environmental conditions during flowering, seed set[23] and genetic factors[24].

The present results imply that reproductive characters were significantly affected by nutrient treatments. Seed mass is much less influenced by soil nutrient conditions than the number of pods and number of seeds per pod. Both germination percentage and germination rates are affected by seed mass. The nitrogen concentration showed a significant increase in the seed in response to an increase in the parental nutrient status. In addition, poor growth of *V. grandiflora* seedlings under low nutrient conditions indicates that this species is not tolerant of infertile soils.

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Variation studies in surface micromorphology on seed coat and endosperm of *Ensete superbum* (Roxb.) Cheesman: a conservation concern species of India

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***Ensete superbum* (Roxb.) Cheesman is an endemic wild banana species of Western Ghats, northeastern hills of India and northern Thailand. The white powdery endosperms of the seeds are widely used to treat**

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