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Genetic Differentiation and Phenotypic Plasticity I. Responses in Three *Plantago lanceolata* L. Populations upon Changes in Mineral Supply

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Abstract: Three populations of *Plantago lanceolata* L. were analyzed for genetic differentiation and phenotypic plasticity. Eight randomly taken samples of each population were grown at two nutrient levels and subjected to alterations in mineral supply. Growth and root respiration was followed during the experiment. With respect to all measured characteristic genetic differentiation on population level was demonstrated. Overall phenotypic plasticity of the measured characteristics and differences in estimated genetic variation were present. High relative growth rate was correlated with high root respiration. High relative growth rate was correlated with high root respiration and high Ca^{2+} - Mg^{2+} - stimulated ATPase activity in roots. Ecological significance of the results and correlation with habitat properties are discussed.

Key wards: Genetic differentiation, *Plantago lanceolata,* phenotype, plasticity, Genotype.

Introduction

The set of environmental conditions, to which an organism is exposed, exerts considerable influence on its development. This particularly is evident in plants, because of their immobility. Plants show morphological and physiological adjustments to their particular environment, a response referred to as phenotypic plasticity. The way in which an organism reacts to different environmental conditions is perhaps one of the most important characteristics for its survival. Plasticity forms a short-term response to a changed environment. Phenotypic plasticity was studied in several *Plantago* species and we are interested in the role of genetic variation and phenotypic plasticity in populations of *Plantago* species in maintaining themselves in their habitats. Genotypes within a population may differ widely in phenotypic plasticity, depending upon the number of available plastic plant characteristics and the extent of the plasticity of the plant factor in question. The size of genetic variation in a population and the gene flow will determine the possibility to develop new genotypes, which are better adapted to the changed environment. Thus, phenotypic plasticity and genetic variation present possibilities for short- term and long-term adaptations of plant population.

The plant chosen for this study is the cosmopolitan *Plantago lanceolata* L., a perennial rosette herb, which is an obligatory cross-pollinating (Sagar and Harper, 1969; Primack, 1978, 1980), with a high degree of heterozygosity. Genetic variation within populations is generally higher than between populations (Fowler and Antonovics, 1981; Van Tienderen, 1992). The genetic variation enables the development of more or less specialized genotypes in *P. lanceolata*, for instance genotypes with tolerance to heavy metals (Pollard, 1980). In the field genetic differentiation in *P. lanceolata* was demonstrated with respect to light by Teramura (1983) and Van Hinsberg (1997 and 1998) and Van Hinsberg and Tienderen (1997), to the height of the vegetation (Warwick and Briggs, 1979) and to time of flowering by Van Tienderen and van-der Toom (1991a, b). Boecher (1943) observed many morphological traits, like leaf length and size of rosette. Several characteristics of photosynthesis are highly plastic, resulting in a broad photosynthetic optimum response to light intensity (Teramura and Strain, 1979) and Van Hinsberg (1997). A degree of plasticity was also concluded from results on root respiration and shoot to root ratio (Lambers *et al*., 1981), and on parameters of nitrogen metabolism (Stulen *et al*., 1981). The diversity of species is rather high (Van der Aart, 1999).

These data suggested that adaptation to local conditions of populations of *P. lanceolata* is mainly based on plastic responses of individual plants. This hypothesis was tested in the present work by studying root respiration and growth of plants of three populations of *P. lanceolata* to change in a level of mineral nutrition.

Materials and Methods

Populations of *P. lanceolata:* From each of three populations, Egyptian (Ismailia)(Egypt), Merrevliet (Netherlands) (P) and Heteren (Netherland) (H), eight seed samples were taken at random. Each sample consisted of seeds collected from a single plant. Egyptian location is a desert with mainly dry and poor vegetation. The mean seed weight of this population was 0.5 mg and germination occurred in spring. Merrevliet is a silted up branch of a small river in Netherlands, formerly used as farm land. The mean seed weight of this population was 2mg and the germination period is in autumn. Heteren (H) is former farm land, now used as hay meadow (Table 1). The vegetation is dominated by tall grass species and the diversity of species is rather low.

Growth conditions: Seeds were placed on wet filter paper in petri dishes. After about 5 days seed coats of ungerminated seeds were cut open to provide a high germination percentage (c. 98%). After 14 days seedlings were transferred into a nutrient solution at half the strength of that given by Smakman and Hofstra (1982), at pH 6.0. Twenty sets of three plants each were grown on 30 L tanks. After 2 days half of the plants were transferred to a full nutrient solution (100%). The other plants were transferred to a diluted nutrient solution (2% of the full nutrient solution). On day 32 after sowing half of the plants grown in a full nutrient solution were transferred to a diluted nutrient solution (100%-2%). Half of the samples were kept as control (100%) plants. At the sam time half of the plants grown in a 2% solution were transferred to full nutrient solutions (2%-100%) and the other half were kept as control (2%) plants. Nutrient solutions were, replaced twice a week. The experiment was done in a growth chamber at 20°C. Relative humidity of the air was about 60% and light intensity was about 50 W $m^{-2,}$ supplied by Philips MF140W/338 and Philinea 120W in a ratio of 10:1 during 12 days-1. The total duration of the experiment was about 55 day.

Growth parameters: Plants were harvested twice a week. The

plant material was dried overnight at 90± 2°C. The dry weights of shoot and root were determined separately and from these data the shoot to root ratio was calculated.

Root respiration: Respiration of the roots was measured with a YSI (Yellow Springs Instruments) oxygen meter (model 53). Intact roots, excised from the shoot, were placed in a cuvette containing an aerated culture solution similar to that used for growth. The cuvette was carefully sealed, excluding any air bubbles in the solution and an electrode mounted in the cuvette measured the decrease in oxygen concentration of the solution. Thereafter, the culture solution was replaced by a solution containing all the nutrients of the culture solution (except Fe) and 25 mM SHAM. Fe was omitted since it chelates with SHAM, pH was 6.0 and the temperature of the solution was kept at 20± 2°C. The decrease in oxygen concentration of this solution was measured, and the difference between the two measurements ascribed to the activity of the alternative respiratory pathway, which is sensitive to substituted hydroxamic acids, like SHAM (Schonbaum *et al.*, 1971). In some determinations 5 mM cyanide was used for measuring the capacity of the alternative pathway, when the cytochrome pathway was inhibited.

ATPase and protein determination: Differential centrifugation of the microsomal membrane fraction from roots has been described earlier (Kuiper and Kuiper, 1979a, b). The ATPase activity was determined at 30± 2°C in 1mL volume containing 3mM ATP, 40 mM histidine- HCl buffer (pH 6.5), $MgCl_2$ or $CaCl_2$ at various concentrations (0mM- 10mM), and enzyme (0.1mL) as a last addition. The reaction was stopped after 30 min by adding 0.1 mL of 33% TCA and the reaction vessels were placed in ice. Four ml water was added to each tube in order to dilute the protein precipitated by TCA. Inorganic phosphate was determined by the ammonium molybdate-$SnCl_2$ method (Lindeman, 1958). ATPase activity was calculated on a protein basis . For protein determination the protein precipitated with TCA was spun down by centrifugation, the pellet dissolved again in 0.1M NaOH, and then protein content was determined according to Lowry *et al.* (1951), using serum albumin as ± 2 a standard. This experiment was carried out in control growth chamber in Hetren, Netherlands.

Results

Effect of mineral nutrition on growth: The shoot and the root growth of the eight samples of each population were averaged (Figs. 1 and 2). In all treatments the growth rate of the shoot of the H (Heteren) plants was higher than that of the other two populations. Also in root growth, the H plants had the highest and the E (Egypt) plants the lowest biomass development. Large differences in root growth were observed between the populations, comparing root growth of 100% and 2% plants of the three populations. Root growth of E plants was unaffected by nutrient strength. Root growth of 100% the M (Merrevliet) plants was higher than root growth of 2% plants but the reverse was observed in H plants.

The RGR (relative growth rate) of shoots and roots of the H plants was higher than that of the other two populations, although the differences were not significant (Table 2). The variation within the RGR values of the E plants was constantly high.

The responses of the shoot to root ratio of plants of the three populations to mineral supply were similar, except, that shoot to root ratio in 2% plants of the M populations was higher than that of 2% E- and H plants (Table 3). Generally the variation within the shoot to root ratios of the E plants was the highest.

Differences in growth among the populations of *P. lanceolata* were arranged from low to high (Table 4). The dry weights of 100% and 2% plants increased in the order E> M> H. The differences between dry weight of the E and M populations was small and non significant due to a high variation in E population. The weight of M plants always was larger than

Table 1: Some characteristics of habitats of the three populations of *P. lanceolata*. E, Egypt; M, Merrevliet (Netherland); H, Heteren (Netherland); P, phosphorus; N, nitrogen.

Characteristics	E	M	H
Total P (ppm)	120	1230	800
Total N (ppm)	1300	22800	2800
Total organic matter (%)	2-20	86	33
Moisture content of soil (%)	4-30	69-86	18-44
Soil	Sand	Peat	Clay
Height of vegetation (cm)	15	35	70

Table 2: Relative growth rate of shoot and root, average of plants of three populations of *P. lanceolata*, grown at two mineral levels. E, Egypt; H, Heteren. M, Merrevliet

Mineral levels	Localities	Shoot	Root
100%	E	0.095± 0.041	0.091± 0.047
	M	0.128± 0.027	0.106± 0.020
	H	o.147± 0.021	0.129± 0.012
2%	E	0.091± 0.047	0.095± 0.042
	M	0.086± 0.015	0.084± 0.018
	H	0.122± 0.019	0.107± 0.021

(n= 8)

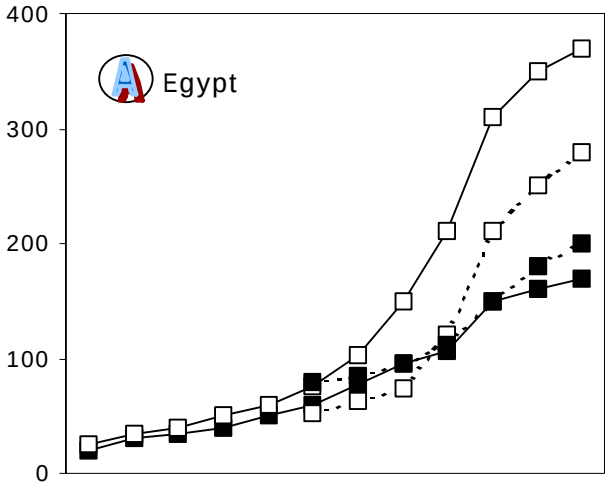
Table 3: Shoot to root ratio of plants of three populations of *P. lanceolata*. E, Egypt; M, Merrevliet ; H, Hetern.

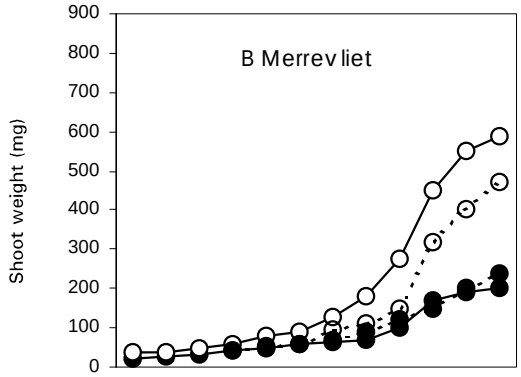
Localities	Days after sowing	Treatments			
		100%	2%-100%	100%-2%	2%
E	25	2.8± 0.6			1.7± 0.7
	33	3.5± 0.9	1.3± 0.7	3.5± 0.9	1.1± 0.4
	44	3.8± 0.8	2.7± 1.1	2.0± 0.8	1.6± 0.6
	49	3.8± 1.1	3.7± 1.0	1.8± 0.3	1.4± 0.5
	53	3.4± 1.0	4.0± 1.0	1.7± 0.7	1.5± 0.5
M	25	3.8± 0.4			2.3± 0.2
	33	3.3± 0.4	2.0± 0.2	3.3± 0.6	2.4± 0.2
	44	3.9± 0.6	3.1± 0.3	3.0± 0.4	2.1± 0.3
	49	3.5± 0.4	3.4± 0.4	2.1± 0.3	2.2± 0.3
	53	3.6± 0.5	3.50± 0.5	2.1± 0.7	1.7± 0.2
H	25	2.5± 0.3			0.9± 0.1
	35	3.5± 0.3	1.5± 0.1	3.5± 0.2	1.5± 0.1
	44	3.9± 0.3	2.5± 0.1	2.6± 0.2	1.1± 0.1
	49	3.7± 0.3	2.9± 0.2	1.5± 0.1	1.4± 0.1
	53	3.7± 0.3	3.0± 0.2	1.6± 0.1	1.4± 0.1

(n= 40)

Table 4: Arrangement of dry weights of shoot (Fig. 1) and root (Fig. 2) and shoot to root ratio (Table 2) in three populations of *P. lanceolata*, with an indication of significance level: xxx, p< 0.005 ; xx ,< p 0.01; x, < p< 0.025. (Time, 53 days after sowing).

Nutritional Regime	Growth parameter	Range low----------high
100%	dry weight of shoot	E-----x--------M---------xxx--------H
	dry weight of root	E-----x--------M---------xx----------H
2%	dry weight of shoot	E and M----------xxx----------H
	dry weight of root	E and M----------xx-----------H





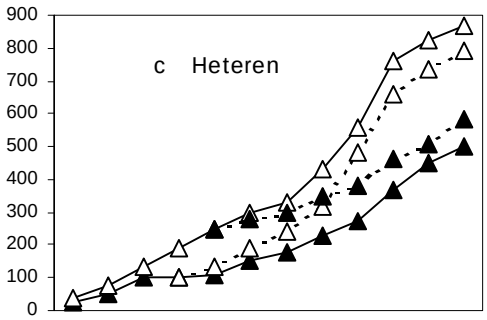
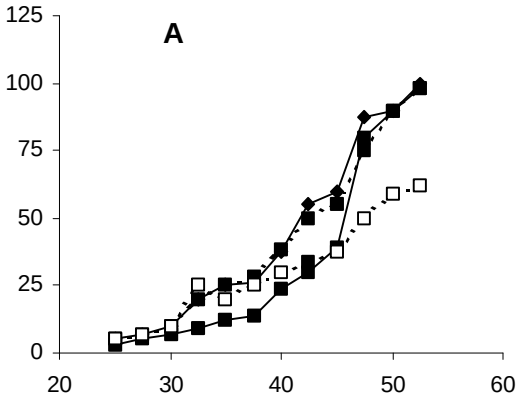
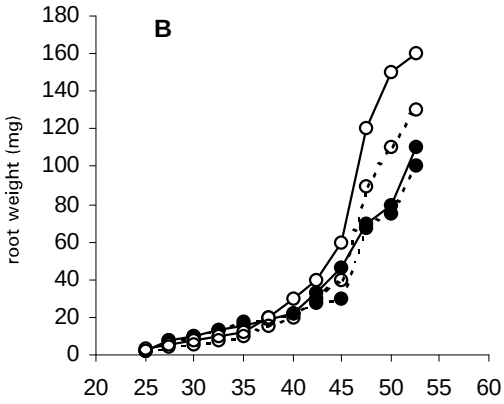


Fig. 1:
lanceolata of the three population, Egypt





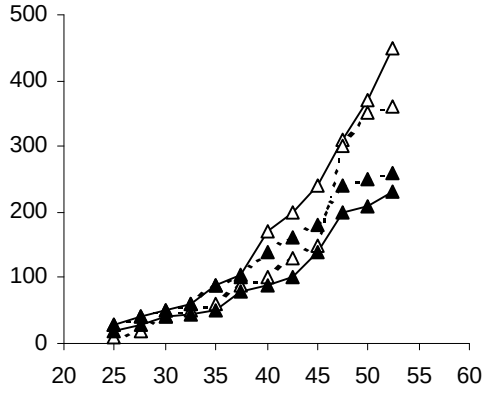
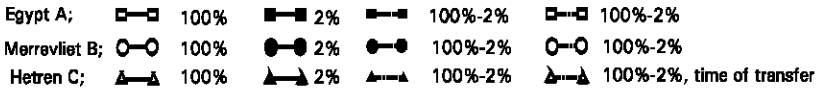


Fig. 2: Dry accumulation in root.



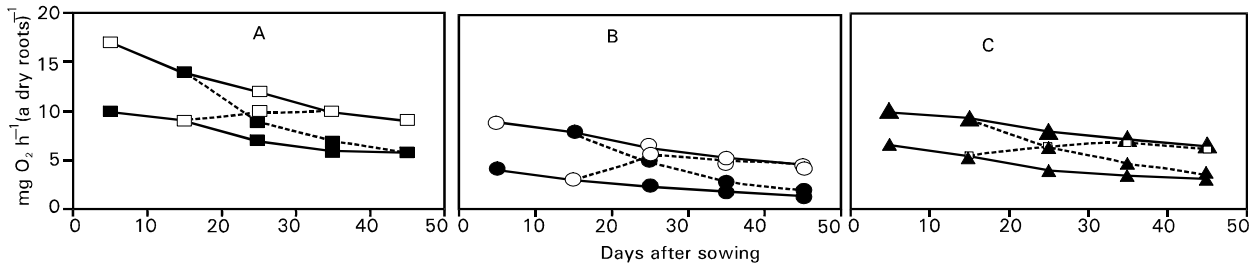


Fig. 3: Activity of the Cytochrome pathway. Otherwise like Fig. 1.

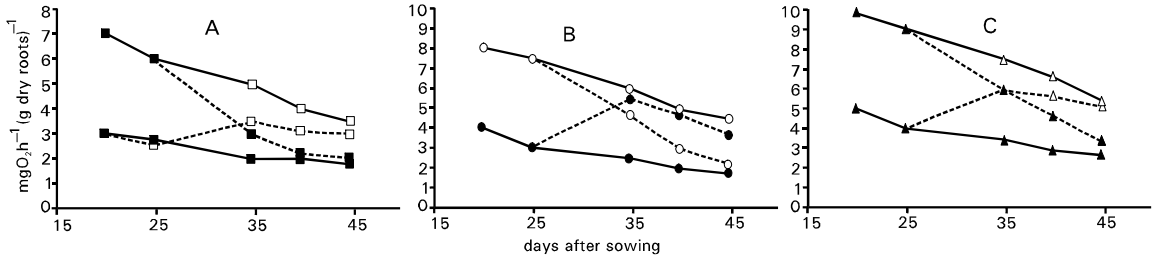


Fig. 4: Total root respiration. Otherwise like Fig. 1.

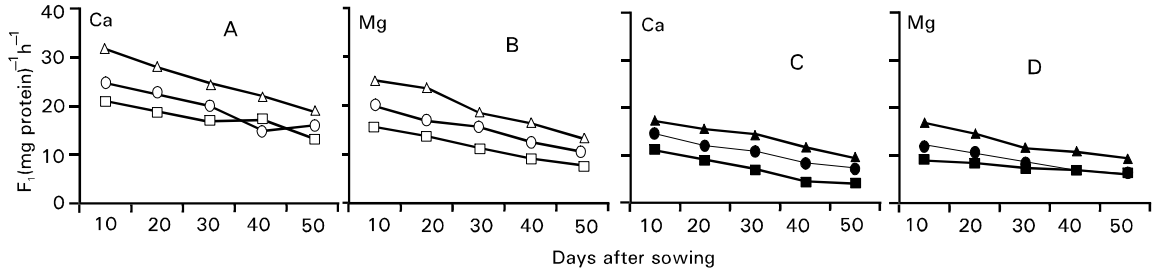


Fig. 5: Root ATPase activity of 100% (A & B)& 2% plants (C & D) of three populations of *P. lanceolata*. Otherwise like Fig. 1.

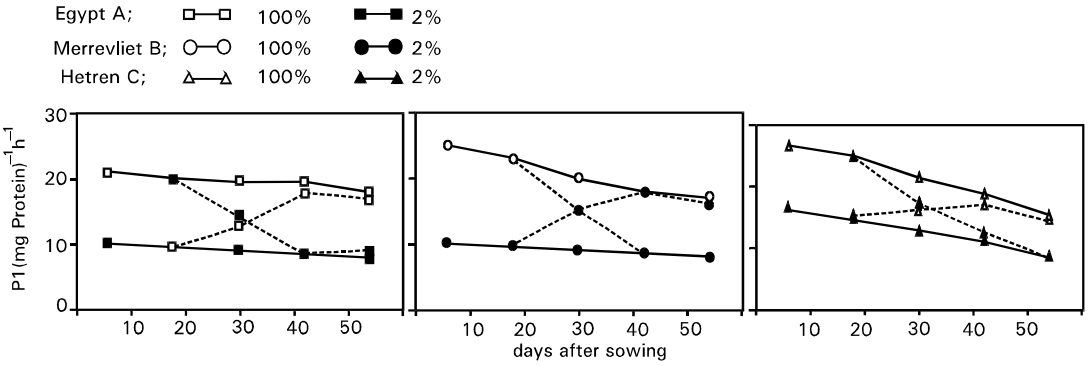


Fig. 6: Root -Ca^{2+} ATPase activity. Otherwise like Fig. 1.

that of E plants.

Effect of alternation of the level of mineral nutrition on growth: Shoot and root growth of transferred plants (100%-2% and 2%-100%) showed a quick response to alternation of the nutrient solution (Figs. 1 and 2). The shoot growth of 100-2% and 2% plants of both populations E and M were similar after about 14 days. The root growth of 2-100% plants of the E population was lower than in the other three treatments, and shoot to root ratio of these plants was even higher than that of 100% plants on day 53 after sowing (Table 3). During the experiment shoot to root ratios of the transferred plants generally showed a complete adaptive response to the new level of mineral nutrition.

Effect of mineral nutrition on root respiration: Total root respiration (Fig. 4) and the activity of the cytochrome pathway (Fig. 3) of plants of the three populations were affected by

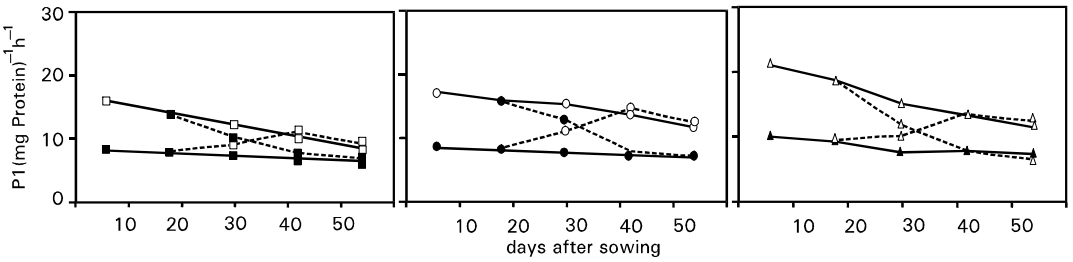


Fig. 7: Root -Mg^{2+} ATPase activity. Otherwise like Fig. 1.

nutritional strength. The respiration of 2% plants was always lower than that of the 100% plants. Total root respiration of 2% plants was always lower than that of the 100% plants. Total root respiration and the cytochrome respiration decreased in all plants during the experiment. The decrease in 100% plants of the H population was larger than in E and M plants, although the proportional decrease was almost similar, viz 48, 45 and 40% for H, M, and E 100% plants, respectively.

Activity of the alternative pathway did not differ between 100% and 2% plants of the H population (Table 5). In plants of the E and M populations this activity was higher in 100% plants than in 2% plants. The variation in the measured values of root respiration of the E population was remarkable. Total root respiration and the activities of both respiratory pathways were arranged from low to high (Table 6). The H plants possessed the highest respiratory activity, except the activity of the alternative pathway of 100% plants on days 42 and 49. The respiratory activity of the E plants was always lower than that of the M plants. Because of the large variation in respiratory values of E plants in most cases no significant level was achieved.

Effect of alternation of mineral nutrition on root respiration: Respiration of transferred plants (100%-2% and 2%-100%) responded very quickly, especially the cytochrome pathway (Fig. 3) upon a change in mineral strength to that level, which corresponded to the new situation. The response of the alternative pathway of the 2-100% plants was remarkably stable.

Table 5: The activity of the alternative pathway (mg O_2 g^{-1} h^{-1}) of plants in the three populations of *Plantago lanceolata*. E, Egypt; M, Merrevliet; H, Heteren.

	Day after sowing	Treatments			
		100%	2%-100%	100%-2%	2%
E	21	5.1± 2.7			3.5± 1.9
	28	3.5± 2.0	3.6± 2.1	3.5± 1.9	3.6± 1.9
	35	2.9± 1.6	2.8± 1.4	2.7± 1.6	2.4± 1.9
	42	3.6± 1.6	3.4± 1.6	1.9± 1.3	1.6± 0.9
	49	3.5± 1.9	3.5± 1.9	1.8± 1.1	1.5± 0.9
M	21	6.1± 2.2			4.5± 1.3
	28	5.2± 2.1	3.8± 1.3	5.2± 2.2	3.8± 1.3
	35	3.7± 1.6	3.8± 1.0	3.6± 1.3	2.9± 0.9
	42	3.6± 1.1	3.4± 1.1	3.0± 1.1	2.8± 1.1
	49	3.8± 1.2	3.6± 0.9	2.8± 0.8	2.9± 1.1
H	21	6.9± 3.0			5.2± 1.3
	28	5.0± 2.1	4.2± 1.1	5.0± 2.0	4.8± 1.3
	35	4.0± 1.4	4.3± 1.1	3.1± 1.1	2.9± 0.7
	42	3.9± 1.2	3.2± 0.9	2.8± 1.0	2.8± 0.7
	49	2.8± 0.9	3.0± 0.7	2.5± 0.9	2.7± 0.7

(n= 40)

Effect of pH on root ATPase activity: The pH optimum of the microsomal ATPase activity with and without addition of cations was about 6.5 for preparations of all populations. Also, no difference in affinity for ATP was observed.

Table 6: Arrangement of total root respiration and the activity of both pathways (Fig. 3 and Table 5) in roots of plants of three populations of *P. lanceolata* with indication of significance level. E, Egypt; M, Merrevliet; H, Heteren; xxx,,p< 0.005; xx, 0.005< p< 0.01; x,0.01< p< 0.025. (time, from day 21 to 42).

Nutritional regime	Growth parameter	Range low----------high
100%	Total respiration	E and M--------xx----------H
	Alternative activity	E ----------x---------M-------and H
2%	Total respiration	E and M--------x----------H
	Alternative activity	E----------x--------M and H

Table 7: SE values, expressed as percentage of the average of a population, of physiological characteristics measured in 100% plants of three populations of *P. lanceolata*. E, Egypt; M, Merrevliet; H, Heteren;(time, last measurement).

Characteristics	Population			
	E	M	H	
V_{max} Mg^{2+} _ATPase	10.5		7.2	6.0
V_{max} Ca^{2+} _ATPase	11.8		6.4	5.8
Total root respiration	19.2		11.1	114
RGR of shoot	15.3		7.5	6.7
RGR of root 12.3	5.1	3.3		
Shoot to Root ratio	10.4		4.9	2.9

(n= 8)

Effect of mineral nutrition on root ATPase activity: V_{max} values for the Ca^{2+} – and Mg^{2+} – stimulated ATPase activity of roots of plants from three populations of *P. lanceolata* were followed during the experiment. Stimulation by Ca^{2+} generally was higher than Mg^{2+} (Fig. 5). The stimulation by both cations of ATPase activity in 100% plants was higher than that in 2% plants. The plants of the H population showed the highest V_{max} values and in the plants of the M population root ATPase activity generally was higher than that in E population. The significance of these differences could not be proven, since the variation in plants of the E population was large.

Effect of alternation of mineral nutrition on root ATPase activity: Transferred plants (100%- 2% and 2%- 100%) of the three populations showed a rapid response upon a change in mineral supply (Fig. 6 and 7). After 5 days, the transferred plants showed intermidiate V_{max} values between those of 100% and 2% plants. In most cases the adaptive response was completed after 10 days.

Discussion

The present results confirmed the hypothesis that *Plantago lanceolata* L. possesses a quick and adaptive response to mineral nutrition. In addition, transferred plants of each population responded similarly to an alteration of mineral supply, indicating that no large genetic differences for such an adaptive response were present. In conclusion, phenotypic plasticity for mineral

strength is characteristic for *P. lanceolata. P. lanceolata* also shows plastic and adaptive responses to changes in light energy flux (Van Hinsberg, 1997, 1998). These included stable photosynthesis over a wide range of light energy fluxes (Bjoerkman and Holmgren, 1966; Teramura and Strain, 1979 and Van Hinsberg, 1997). The rate of formation of new leaves also appeared to be stable for *P. lanceolate.* Plastic and adaptive responses are evident for energy metabolism of this species. A decreased growth implies a lower need for energy, a lower respiratory activity via the cytochrome pathway and redistribution among the respiratory pathways (Lambers *et al.*, 1981). Transfer from 100% to 2% solution resulted in retarded growth, a lower shoot to root ratio, a lower root respiration , all factors contributing to a stable energy metabolism. Stulen *et al.* (1981) observed an unchanged NO_3 content in the shoot of 100%-2% plants of plants of *P. lanceolata*, while shoot growth was retarded (Lambers *et al.,* 1981 and Jarzomski *et al.,* 2000). Evidently, shortage of nitrogen (or phosphorus; Bieleski, 1973 and Poot *et al.*, 1997) induced a lower growth rate. A receptor, sensitive to a dilution of mineral supply, might explain a lower growth rate, which in its turn caused a lower root respiration and root ATPase activity. Plants, transferred to a full nutrient solution (2-100%), showed a rise in growth of root respiration and root ATPase activity.

In several aspects the plants of the H population showed the highest metabolic activity, i.e in RGR and in respiration. Thus, genetic differentiation was present in the studied populations. Our results on genetic differentiation in physiological parameters are supported by information on morphological properties. In the area of Egypt the prostrate form of *P. lanceolata* dominates during the whole growth period, in the M population only in spring time; later it is replaced by a more erect habit due to growth of vegetation. Leaf angle and leaf length is genetically differentiated among these populations (Mook *et al.*, 1981). This adaptive and morphological specialization (Warwick and Briggs, 1979 and Van Tienderen and van-der Toom, 1991a, b) can be related to the management of the vegetation. Egypt has been grazed by sheep and goats for centuries, while Heteren and Merrevliet are both hayfields and managed for that purpose for decennia. Vegetative reproduction (Teramura, 1983) and time of flowering (Van der Aart, 1984 and Van Tienderen and van-der Toom, 1991a, b) of *P. lanceolata* seem to depend on light regime. Individuals of *P. lanceolata* from the E population (range) flower late, while plants from the H and M populations (hayfield) flower earlier, in correlation with time of mowing. The present results on growth (Figs. 1 and 2) and RGR (Table 1) give a consistent picture of a fast increment of the biomass in the H population. The slow growth in plants of the E population could not be distinguished significantly from that of the M population, even though differences in growth between M and E plants often were larger than those between M and H plants. Merrevliet and Heteren are both hayfields with a higher total phosphorous and nitrogen content than Egypt area (Table 1), although these values do not represent the available amounts of phosphorus and nitrogen. The persistence of an overall lower growth rate in E plants, at growth chamber conditions, indicates a genetic background for slow growth, and may be interpreted as an adaptation to the lower nutrient availability and water supply. Selection for a higher growth potential may be correlated with a larger vegetation height (longer leaves) and with a higher content of organic matter and water. In conclusion, genetic differentiation between populations is present in *P. lanceolata* (Fowler and Antonovics, 1981; Hooglander and Lumaret, 1993).

Noe and Blom (1980) reported the occurrence of two varieties within *P. lanceolat* var. *sphaerostachya* Mert. and Koch. (rosette diameter, 6.4 cm, low RGR and from this paper, low root respiration) and var. *lanceolata* (rosette diameter, 11.9 cm, high RGR and from this paper, high root respiration and low root ATPase activity). Both varieties were present in the E population and this may explain the relatively higher population than those of the other two populations.

It is suggested, that differences in variation among the populations may be ascribed to genetic variation, although instability can not be ruled out completely. Results on alloenzyme variation of the same populations support this conclusion.

Generally, *Plantago lanceolata* was characterized by a high degree of phenotypic plasticity and heterozygosity. Specialization may be obtained at the expense of phenotypic plasticity. Specialization to flooding at the expense of plasticity to light intensity (Menges and Waller, 1983; Van Hinsberg, 1997; and Sims and Kelley, 1998). Specialization may be related to an increasing degree of homozygosity (Parker and Kereitman, 1982), implicating a loss of genetic information, which may be reflected in a lower regulatory gene variation (Scandelios and Baum, 1982). Specialization is often accompanied by a substantial loss of DNA (Groenendijk *et al.*, 1997), which seems to be related to a smaller capacity for establishment in less suitable environments, as for example to soil pH (Foy *et al.*, 1980). The results of this work show, that growth of populations of *Plantago lanceolata* was genetically differentiated for low growth potential and may result in a less competitive ability to other plants. Phenotypic adaptive plasticity will function here in a way, which provides a sufficient supply of water and nutrients. Highly plastic responses will enable individual plants to follow the increase in vegetation height and to maintain the photosynthesis.

Conclusively phenotypic plasticity of plant characteristics may have adaptive value, when changes in the environment will take place within the generation time of the plant (Ernst, 1983). In annuals the need for seed production dominates and phenotypic plasticity may be a helpful mechanism in this respect (Bradshaw, 1965). Phenotypic plasticity in perennial plants may be important for several reasons. Plasticity in morphological traits enables the plant to respond to changing vegetation (Brown, 1983; see present study on M and H populations) or to a changing vegetation to reproductive period (*Rumex acetosella* L., Farris and Schaal, 1983 and Matumura *et al.*, 1990; Waite and Hutchings, 1982; the Egyptian population, present paper). *Plantago lanceolata* L. may be considered a generalist species, and besides some population differentiation, phenotypic plasticity plays an important role. The lack of alloenzyme genetic variation, its ecological distribution and a high degree of hetrozygosity support this conclusion(Nevo, 1978; Price *et al.*, 1981, Hooglander & Lumaret, 1993 and Wolff, 1990).

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