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Form of the Nitrogen Source Affects the Response of the Two Congeneric *Rumex* Species to Phosphorus Nutrition in The Nile Delta Coast

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Abstract

Phosphorus and nitrogen are limiting plant nutrients in young and old weathered soils, respectively. We investigated the N × P interaction on performance of *Rumex dentatus* and *R. pictus*. Plants were grown on washed sand and supplied with 11 mM N either as nitrate or ammonium and 0.01, 0.15, 0.40, 1.0 and 1.5 mM P. *Rumex*, particularly *R. dentatus*, preferred nitrate over ammonium as N source. Nitrate nutrition favored succulence of *R. dentatus* but the reverse was true in *R. pictus.* The optimum P supply of *Rumex* spp. under nitrate (0.4 mM) was lower than that under ammonium nutrition (1.5 mM P). *R. dentatus* exhibited less number of leaves but longer and wider blades compared with *R. pictus*. Allocation of plant biomass to root was favored under ammonium nutrition and P deficiency, particularly in *R. pictus*. Increasing P supply increased Chl a and carotenoid concentrations but reduced Chl b concentration. Soluble sugars were higher under nitrate nutrition compared with ammonium only in *R. pictus*, with limited effect of P supply. The higher proline concentration under ammonium compared with nitrate nutrition and under P deficiency is not a consequence of impaired protein synthesis and suggests that ammonium might be stressful, particularly to *R. dentatus*. Only in *R. dentatus*, nitrate nutrition led to higher phenolic concentration and DPPH scavenging activity but to lower malondialdehyde content relative to ammonium. Increasing P supply increased phenolic concentration and DPPH scavenging activity but reduced malondialdehyde content. The concentrations of K+ and Na+ in the shoot were non-significantly affected by the form of N but exhibited marked genotypic variability in favor of *R. dentatus.* Increasing P supply non-significantly affected shoot K+ concentration but reduced Na+ concentration. Shoot nitrogen concentration was higher in *R. dentatus* than *R. pictus* and under nitrate over ammonium nutrition only in *R. pictus.* The increase in P supply increased P concentration particularly in the ammonium-fed plants. Phosphorus concentration of the shoot was significantly higher in *R. pictus* than *R. dentatus* under ammonium nutrition but the reverse was true under nitrate nutrition.

Keywords: Rumex dentatus, Rumex pictus, nitrogen, phosphorus, minerals.

Introduction

Phosphorus is required for a wide variety of functions in the plant, e.g. as a structural component in nucleic acids, phospholipids and phytin of seeds, in the phosphorylated

intermediates of plant metabolism and in activation of enzymes (**Hawkesford et al., 2012**). Along with N and K, adequate P supply is necessary for optimum plant growth and performance and must be added periodically to the cultivated lands for sustained productivity. However, similar to K and unlike N, P is a non-renewable resource. While N reservoirs occur in the atmosphere, as the inert dinitrogen gas (N_2), which can be fixed either symbiotically or by free living prokaryotes, P is derived mainly from rock phosphate. Globally P reserves are subjected to severe depletion. Whereas P deprivation is common in old severely weathered soils that have high concentrations of the P-sorbant oxides and hydroxides of aluminium and iron, N is limiting to plant productivity in young soils of low content of organic matter (**Lambers et al., 2015**).

Based on the current rates of consumption, the global P reserves are estimated to be halved (relative to their levels at the onset of the 20th century) very soon in 2040 or if we are fortune in 2060 (Lambers et al., 2006). Surprisingly, while the global P reserves are being depleted, P levels in many agricultural soils are building up, because 80–90 % of the P applied as fertilizer is sorbed by soil particles, and thus is rendered unavailable for most crop plants. There is an urgent need to manipulate crops that are more efficient at acquiring inorganic P (Pi) from impoverished soil and/or with high internal P use efficiency. Equally, it is increasingly important to cultivate crops that reduce the off-site effects of P fertilization, thus reducing the risks of polluting streams and rivers (Simpson et al., 2011).

Meanwhile, agricultural soils that have been fertilized or over-fertilized for decades, contain substantial amounts of P not readily accessible by crop plants since these domesticated plant species mostly lack the necessary specific root adaptation mechanisms to acquire P from the otherwise recalcitrant resources (Lambers et al, 2006). By comparison, the efficient P-acquisition traits are rather well-expressed in the wild relatives. To acquire soil orthophosphate (Pi) more efficiently, crop plants must develop some traits primarily concerning the root. These qualifying traits include increasing investment of the plant biomass in the production of root, production of roots with special architecture and performance and production of cluster or proteoid roots. Alteration of root architecture involves inhibition of primary root growth in favor of promotion of lateral root growth and enhancement of root hair development. Proteoid roots represent a combination of both morphological and functional adaptations (McKay Fletcher et al., 2020). These morphological adaptations are triggered by changes in P partitioning within the plant which is mediated by growth regulators such as auxins, ethylene, cytokinins, nitric oxide (NO), reactive oxygen species (ROS) and abscisic acid (ABA) (Niu et al., 2013). The functional adaptation traits include release of acid phosphatases, protons and/or carboxylates into the rhizosphere which increases the concentration of soluble Pi in the soil and hastens P availability to the efficient species as well as to their neighbors (He et al., 2020). Nevertheless, there is a trade-off in which the species most proficient at acquiring P have ephemeral roots with high susceptibility to soil-borne pathogens (Laliberté et al., 2015).

Unlike nitrate, which is readily mobile in the soil, Pi is highly soil-immobile in contrast to its high mobility in the plant. Diffusion of mineral nutrients, particularly Pi, is further slowed down in dry soil (Lambers and Plaxton, 2018), which aggravates the P problem in arid lands. Increasing Pi delivery to roots via mass flow can be achieved by enhanced transpiration rates; but this would be at the expense of a plant's water-use efficiency. Diffusion of Pi toward the root can be increased by increasing the moisture content of dry soil, or by increasing the Pi concentration in soil solution through release of Pi from complexed, sorbed or organic forms of P.

Rumex is one of the most important genera of Polygonaceae, with cosmopolitan distribution in temperate regions. Rumex species have a long history of domestic herbal use because of their high content of vitamins, particularly vitamins A and C as well as minerals, especially iron (Vasas et al., 2015). It is a gentle laxative, compared with rhubarb, with valuable cleansing properties and is useful for treating a wide range of skin problems. Rumex dentatus L. (dentate dock) is an annual glabrous herb with slender, erect stems of 70-80 cm in height. In grows wildly in abandoned lands, canal banks and cultivated fields. It has been used as a leafy vegetable in the Mediterranean diet (Mashaly et al., 2015). A related species, Rumex pictus Forssk., is annual glabrous herb, with 10-30 cm high stems, decumbent and highly branched at

the base. It dominates the sand formations (dunes and flats) in the Nile Delta coast (Mashaly et al., 2008). This study investigates the effect of form of nitrogen on phosphorus nutrition of two congeneric species of Rumex, R. dentatus and R. pictus which exhibit different ecological niches and growth habits.

Materials and Methods

The study area

The study area is located within the northern part of the Nile Delta, and involved the cultivated lands about 10 km south of the sea coast (for Rumex dentatus L.) and the sand plains and sand dunes immediately at the sea shore (for R. pictus Forssk.) (Figure 2.1). The soil characteristics of the two sites are presented in Table 2.1.

Soil analysis

Three soil samples (0-10 cm in depth) were collected from each site. The three samples were air-dried, mixed into a homogenous sample, passed through a 2 mm sieve, and packed in paper bags for analysis. Soil texture was determined according the procedures of (Jackson, 1962). The pH, electrical conductivity (EC), carbonate, bicarbonate, and minerals were determined in the soil extract (1:5). The pH and EC were measured using a pH meter (Hanna HI 2210) and conductivity meter (HI 9835), respectively. Potassium, sodium and calcium of the soil extract were measured by using a PFP7 Flame Photometer. Chloride content of the soil extract was determined by titration against standard AgNO3 using 5% potassium chromate as an Indicator (Jackson, 1962). The organic carbon content of the soil was determined by titration against FeSO4 as described of Piper (1947). Determination of carbonates and bicarbonates in the soil extract were according to the method described by Jackson (1962). This method is volumetric, using 0.1N HCl and phenolphthalein and methyl orange as indicators. The concentrations of N and P were determined using an auto-analyzer (QuikChem, Series 8000, Lachat Instruments Inc., USA).

Plant material

Seeds of Rumex dentatus L. were collected from the cultivated lands 50 km south of the New Damietta city and seeds of Rumex pictus Forssk. from the coastal sand plains at Gamasa city, north of Egypt. Soil analysis of the natural habitats of the two Rumex species is presented in Table 1. Fruits were collected from mature plants during the period May-June, 2017 and left to dry in the sun until constant weight. Dry fruits were hand-rubbed to extract seeds, and the seeds were screened for homogeneity in shape and color and stored in paper bags before cultivation.

Interaction of N form and phosphorus on growth and physiology of Rumex spp.

Uniform seeds of R. dentatus L. and R. pictus Forssk. were washed thoroughly with tap water and planted in plastic pots of 30 cm diameter, full of 5 kg of water-washed sand, three seeds per pot. Seeds were watered with 0.2 mM CaSO4, and the emerging seedlings, while at the rosette stage, were successively thinned to one per pot. After 30 days from sowing, seedlings received a full nutrient solution containing 11 mM N supplied either as NO3- or NH4+. The macronutrient composition of nutrient solutions is presented in Table 2.

In addition to macronutrients, nutrient solutions contained the following micronutrients (µM): Mn (as MnSO4.4 H2O) 5, Cu (as CuSO4.5 H2O) 0.5, Zn (as ZnSO4.7 H2O) 0.5, B (as boric acid) 25, Mo (as Na2MoO4.2 H2O) 0.25 and Co (as CoSO4) 0.1. Phosphorus was added to the nitrate and ammonium solutions as NaH2PO4 at levels of 0.01, 0.15, 0.40, 1.0 and 1.5 mM. The pH was adjusted to 6-6.5 by using drops of 1 N HCl and 1 N NaOH, if required.

Plants were grown in a greenhouse at the Faculty of Agriculture, Alexandria University during the period from November 2017 to May 2018. Irradiance ranged from 1500 to 2000 µmol m-2 s-1 from natural sunlight, with a temperature of 27/20 °C in a 14/10 h light/dark period and relative humidity of about 70% in average.

Table 1 Analysis of the soil from the natural habitats of *R. dentatus* and *R. pictus*.

Characteristic	*R. dentatus*	*R. pictus*
Texture	Sandy clay loam	Sandy
EC (dS/m)	1.18	1.14
pH	7.25	7.18
Organic carbon (% DW)	1.77	0.8
CO_3^{2-} (mg/g DW)	0	0
HCO_3^- (mg/g DW)	204	183
N (mg/g DW)	168.8	122
P (mg/g DW)	11	4.1
K (mg/g DW)	28	24.1
Ca (mg/g DW)	54.1	46.8
Mg (mg/g DW)	24	16.8
Na (mg/g DW)	130.1	126.2
Cl (mg/g DW)	101	133.6
Fe (µg/g DW)	9.0	11
Zn (µg/g DW)	11	2
Mn (µg/g DW)	11	2
Cu (µg/g DW)	116	61

Table 2 The composition of macronutrients in the ammonium and nitrate solutions

Nitrate solution			**Ammonium solution**		
Chemical	**mM**		Chemical	mM	
KNO_3	5	N = 11 K = 5 Ca = 3 Mg = 1 S = 8 Cl = 2 Na =16	$(NH_4)_2SO_4$	5.5	N = 11 K = 5 Ca = 3 Mg = 1 S = 9 Cl = 6 Na = 0
$Ca(NO_3)_2$. 4 H_2O	3		$CaCl_2$	3	
$MgCl_2$	1		$MgSO_4$. 7 H_2O	1	
Na_2SO_4	8		K_2SO_4	2.5	
FeEDTA	0.1		FeEDTA	0.1	

Plants were harvested 170 days from the application of nutrient solutions. At harvest, plants were thoroughly washed from sand, separated into shoot and root, blotted gently and fresh weights, plant height and leaf dimensions were recorded. An aliquot of fresh leaves was kept frozen at -10 °C for estimation of photosynthetic pigments and metabolites. Dry weights were recorded after drying of the fresh plant material at 80 °C for 48 h and were corrected for the leaf portion kept frozen.

Plant analysis

Estimation of photosynthetic pigments

Photosynthetic pigments were determined according to the method described of **Wellburn and Lichtenthaler (1984).** Frozen leaf discs were macerated in 80% acetone using a cold mortar and pestle in dim light with a pinch of magnesium carbonate to neutralize the plant acids. The slurry was centrifuged at 18000 × *g* for 5 min and the clear extract was brought up to volume with 80% acetone and the absorbance was read at 470, 646 and 663 nm using a UNICO 7200 series spectrophotometer. The concentrations of chlorophyll a, chlorophyll b and carotenoids were calculated (µg ml^{-1}) using the following equations:

Chlorophyll a = 12.21 E663 – 2.81 E646

Chlorophyll b = 20.13 E646 – 5.03 E663

$$\text{Carotenoids} = \frac{(1000\ E_{470} - 3.27\ \text{Chla} - 104\ \text{Chl b})}{229}$$

Estimation of proline content

According to **Bates et al. (1973)**, a known weight of frozen leaves was homogenized in 4 ml of 3% sulfosalicylic acid and the slurry was centrifuged at 18000 × *g* for 10 min. To 1 ml of the clear extract, 1 ml glacial acetic acid and 1 ml of the acid ninhydrin reagent were added. The reaction mixture was boiled in a water bath for 60 min. After cooling in an ice bath, 4 ml toluene were added and the contents were mixed thoroughly. The upper toluene phase was separated into a glass cuvette and absorbance was read at 520 nm. Proline concentration was calculated from a standard curve in the range of 0-100 µg proline. Acid-ninhydrin reagent was freshly prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml of 6 M phosphoric acid, with agitation, until dissolved.

Determination of soluble sugars

A known weight of the frozen leaf material was extracted in 1 ml of boiling 80% ethanol for 30 minutes and the mixture was centrifuged at 8000 × *g* for 10 min. Extraction was repeated with fresh 80% ethanol, followed by centrifugation and extracts were bulked. The supernatant was evaporated to dryness at 70 °C, re-dissolved in distilled water and an aliquot was completed to 1 ml by distilled water, mixed carefully with 3 ml of the anthrone reagent (8.6 mM anthrone in 80% v/v H_2SO_4) and heated in a water bath at 80 °C for 10 min. After cooling in an ice bath, absorbance was read at 623 nm against the reagent blank. Total soluble sugars were estimated from a glucose calibration curve in the range of 0 to 100 µg glucose (**Schlüter and Crawford, 2003**).

Assay of protein

A known weight of the frozen leaf material was grinded in liquid nitrogen using a pestle and mortar, and the slurry was extracted in 600 µl of 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.4) containing 1 mM EDTA and 6 µl of 500 mM of phenylmethane sulfonyl fluoride (PMSF) prepared in methanol. The debris was removed by centrifugation at 6000 × *g* for 10 min at 4 °C. Protein content of leaves was assayed according to the method of **Bradford (1976).** An aliquot of the supernatant was raised to 1 ml with

distilled water and mixed with 5 ml Coomassie brilliant blue reagent. After standing for 5 min at room temperature, absorbance was read at 595 nm using a Spectronic 20 D spectrophotometer. Protein concentration was calculated using a standard curve of bovine serum albumin (BSA) in the range 0 - 100 µg. Coomassie brilliant blue reagent was prepared by dissolving 100 mg of Coomassie brilliant blue G-٢٥0 in 50 ml of 95% ethanol mixed with 100 ml of 85% phosphoric acid. The stock dye reagent was kept at 4 °C and diluted immediately before use; where 15 ml of the stock reagent was raised to 100 ml with distilled water and filtered whenever necessary.

Digestion of plant material and determination of mineral content

Dried plant material was ground into a fine powder and digested in the sulfuric acid/hydrogen peroxide mixture as described by **Allen et al. (1986)**. Temperature was increased gradually until complete digestion of plant material. After cooling, the extract was made up to volume with distilled water. A blank test was prepared by heating the digestion mixture without plant material. Digestion mixture was prepared by mixing 0.42 g selenium and 14 g lithium sulfate hydrated in a one-liter flask. To the mixture 350 ml H_2O_2 (100 volume) and 420 ml conc. H_2SO_4 were added slowly in order with cooling. Potassium and sodium were determined in the clear extract by using a Jenway PFP7 flame photometer. The concentrations of N and P were determined using an auto-analyzer (QuikChem, Series 8000, Lachat Instruments Inc., USA).

Assay of free phenolics

Free phenolics were extracted by incubating a known weight of the frozen leaves in 95% ethanol for 2 hours at room temperature in a rotary shaker at 250 rpm. The mixture was centrifuged at 8000 × *g* for 10 minutes at room temperature. Free phenolic acids were estimated according to the method of **Ainsworth and Gillespie (2007).** An aliquot of the ethanolic extract was raised to 1 ml with distilled water and 3.5 ml of 0.2 N Folin-Ciocalteu reagent were added. After 3-5 min., 2.5 ml of 7.5% Na_2CO_3 were added and the mixture was incubated at 45 °C for 15 min., then cooled to room temperature and absorbance of the resulting blue color was measured at 765 nm. Phenolics were estimated with reference to a standard curve using gallic acid in the range of 0-100 µg; and the concentration was expressed as µg gallic acid equivalent (µg GAE) g^{-1} FW.

DPPH scavenging activity

The capacity of the leaf extract to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of **Hatano et al. (1988).** An aliquot of 0.3 ml of the ethanolic extract was mixed with 2.7 ml of DPPH solution (6×10^{-5} mol l^{-1}). The mixture was shaken vigorously and left to stand for 60 min in the dark to attain stable absorbance. The reduction of the DPPH radical was measured by monitoring continuously the decrease in absorbance at 517 nm. The DPPH scavenging activity was calculated as a percentage of DPPH discoloration using the equation:

$$\text{DPPH scavenging activity} = \frac{(A_d - A_c)}{A_d} \times 100$$

where A_c is absorbance of the sample and A_d absorbance of the DPPH solution.

Assay of lipid peroxidation

Lipid peroxidation of the leaf tissue was assayed by measuring the malondialdehyde (MDA) content following the method of **Heath and Paker (1968).** An aliquot of the frozen leaf material was grinded in liquid nitrogen using pestle and mortar and extracted in 1.3 ml of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged 8000 × *g* and the supernatant was used for the assay of MDA. The reaction mixture – containing 0.5 ml of the supernatant and 2 ml of 0.5% thiobarbituric acid prepared in 20% (w/v) trichloroacetic acid - was incubated in a water bath at 95 °C for 30 min. After cooling in an ice bath, the reaction mixture was centrifuged at 12000 × *g* for 10 min. The absorbance was read at 532 nm against the reagent blank. Correction for unspecific absorbance was performed by subtracting the absorbance at 600 nm. MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Experimental design and statistical analysis

The experiment was factorial with three factors and 4 replications in a completely randomized design. The main factors were 1) plant species with two levels: *R. dentatus* and *R. pictus*, 2) nitrogen form with two levels: nitrate and ammonium both at 11 mM N and 3) P nutrition with five levels: 0.01, 0.15, 0.40, 1.0 and 1.5 mM. Data were subjected to three-way ANOVA using SPSS version 22, followed by mean separation according to the Duncan's multiple range test at $p<0.05$.

Results

Plant growth was highly significantly ($P<0.01$) affected by the main factors: plant species, nitrogen source and level of phosphorus, except for the non-significant effect of species and nitrogen form on shoot water and plant height and of phosphorus level on the root weight ratio (RWR) and the width/ length ratio of blade. The effect of the factor interactions was less evident than the effect of the main factors, ranging from just significant ($P<0.05$) to mostly non-significant (**Table 3**).

The effect of genotype on shoot dry weight was very weak under ammonium nutrition with comparable values in the two *Rumex* species. But, the genotypic variability emerged convincingly under nitrate nutrition with superiority of *R. dentatus* above *R. pictus*. In turn, the differential effect of nitrogen form was evident only in *R. dentatus*, in which shoot dry weight was higher in nitrate-fed than ammonium-fed plants versus a mild preference of nitrate by *R. pictus*. Increasing P level of the medium increased shoot dry weight with a tendency towards saturation beyond 0.4 mM P. The magnitude of increase was generally higher under ammonium than under nitrate nutrition and in *R. dentatus* than *R. pictus*. The overall increase in shoot dry weight across the whole range of P amounted to 38% and 115% for nitrate- and ammonium-fed *R. dentatus*, respectively and 29% and 44% for nitrate- and ammonium-fed *R. pictus*, respectively (**Fig. 1A**).

Root dry weight was significantly higher in *R. dentatus* above *R. pictus*. However, the differential effect of N from was evident only in *R. dentatus*, with preference of nitrate over ammonium versus a comparable root dry weight in the two nitrogen forms of *R. pictus*. Increasing P level of the medium led to progressive increase in root dry, with the effect being most pronounced in nitrate-fed *R. dentatus*. The overall increase in root dry weight across the whole range of P amounted to 64% and 112% for nitrate- and ammonium-fed *R. dentatus*, respectively versus non-significant increases of 23% in the average for nitrate- and ammonium-fed *R. pictus* (**Fig. 1B**).

The RWR was significantly higher in *R. dentatus* above *R. pictus*. However, the differential effect of N from was evident only in *R. pictus*, with higher RWR under ammonium compared to nitrate nutrition, particularly under low P levels versus comparable values in the two nitrogen forms of *R. dentatus*. The effect of P level on the RWR varied according to plant species and from of N. In ammonium-fed plants, increasing P supply from 0.01 to 0.4 mM reduced RWR of *R. dentatus* by 21%, followed by 20% increase as P level further increased up to 1.5 mM, versus a 34% decrease across the whole P range in *R. pictus*. In nitrate-fed plants, RWR of the two species was non-significantly affected by P level of the medium (**Fig. 1C**).

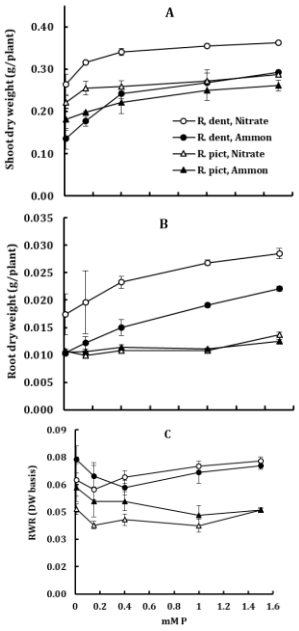

Figure 1 Shoot dry weight (A), root dry weight (B) and the root weight ratio (RWR, C) of *R. dentatus* and *R. pictus* in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO_3^- or NH_4^+. Each value is the mean of 4 replicates ± SE.

Table 3 Three-way ANOVA showing the effect of the main factors (*Rumex* species, N form and level of P) and their interaction on plant growth.

Variable and source of variation	df	F	P	Variable and source of variation	df	F	P
Shoot dry weight				Leaf width			
Species (Sp.)	1	9.029	0.004	Species (Sp.)	1	357.3	0.000
N form (N)	1	96.39	0.000	N form (N)	1	13.53	0.001
P level (P)	4	33.00	0.000	P level (P)	4	23.19	0.000
Sp. × N	1	15.67	0.000	Sp. × N	1	0.265	0.609
Sp. × P	4	3.109	0.022	Sp. × P	4	1.804	0.140
N × P	4	0.825	0.515	N × P	4	1.052	0.388
Sp. × N × P	4	0.428	0.788	Sp. × N × P	4	1.614	0.182
Root dry weight				Leaf width/length ratio			
Species (Sp.)	1	134.4	0.000	Species (Sp.)	1	51.87	0.000
N form (N)	1	29.87	0.000	N form (N)	1	18.26	0.000
P level (P)	4	9.541	0.000	P level (P)	4	2.182	0.082
Sp. × N	1	29.06	0.000	Sp. × N	1	0.219	0.641
Sp. × P	4	4.076	0.005	Sp. × P	4	3.167	0.020
N × P	4	0.401	0.808	N × P	4	4.838	0.002
Sp. × N × P	4	0.408	0.802	Sp. × N × P	4	2.699	0.039
RWR				Shoot water			
Species (Sp.)	1	80.07	0.000	Species (Sp.)	1	2.228	0.141
N form (N)	1	2.716	0.105	N form (N)	1	0.277	0.601
P level (P)	4	1.219	0.312	P level (P)	4	4.728	0.002
Sp. × N	1	3.226	0.078	Sp. × N	1	156.0	0.000
Sp. × P	4	2.721	0.038	Sp. × P	4	3.379	0.015
N × P	4	1.366	0.257	N × P	4	5.710	0.001
Sp. × N × P	4	0.801	0.529	Sp. × N × P	4	5.618	0.001
Number of leaves				Root water			
Species (Sp.)	1	263.4	0.000	Species (Sp.)	1	297.1	0.000
N form (N)	1	1.182	0.281	N form (N)	1	9.482	0.003
P level (P)	4	14.23	0.000	P level (P)	4	15.20	0.000
Sp. × N	1	0.170	0.682	Sp. × N	1	40.68	0.000
Sp. × P	4	11.78	0.000	Sp. × P	4	13.05	0.000
N × P	4	1.993	0.107	N × P	4	4.495	0.003
Sp. × N × P	4	2.007	0.105	Sp. × N × P	4	3.749	0.009
Leaf length				Plant height			
Species (Sp.)	1	96.61	0.000	Species (Sp.)	1	0.450	0.505
N form (N)	1	2.455	0.122	N form (N)	1	0.037	0.848
P level (P)	4	9.684	0.000	P level (P)	4	7.273	0.000
Sp. × N	1	0.090	0.765	Sp. × N	1	0.620	0.434
Sp. × P	4	0.591	0.671	Sp. × P	4	0.454	0.769
N × P	4	2.871	0.030	N × P	4	0.425	0.790
Sp. × N × P	4	0.600	0.664	Sp. × N × P	4	0.448	0.773

Number of leaves was significantly higher in *R. pictus* than *R. dentatus* with non-significant effect of the form of N. Irrespective of the form of N, increasing P level of the medium from 0.01 to 1.5 mM almost doubled the number of leaves in *R. pictus* with non-significant effect in *R. dentatus* (**Fig. 2A**). Blade length was significantly higher in *R. dentatus* than *R. pictus* with non-significant effect of the form of N. Increasing P supply increased blade length, and the effect was more evident in *R. dentatus* than *R. pictus* and under ammonium than under nitrate nutrition. Increasing P supply from 0.01 to 1.5 mM increased blade length by 24% and 39% in nitrate- and ammonium-fed *R. dentatus*, respectively versus respective increases of 7% and 33% (both are non-significant) for *R. pictus* (**Fig. 2B**).

Blade width was significantly higher in *R. dentatus* than *R. pictus* with non-significant effect of the form of N. Increasing P supply increased blade width; but the effect was more evident in *R. pictus* than *R. dentatus* and under nitrate than under ammonium nutrition. Increasing P level of the medium from 0.01 to 1.5 mM increased blade width by 37%and 21% in nitrate- and ammonium-fed *R. dentatus*, respectively versus respective increases of 76% and 48% for *R. pictus* (**Fig. 2C**). The blade width/length ratio was significantly higher in *R. dentatus* than *R. pictus* and under ammonium than under nitrate nutrition. The differential genotypic and N form effects were most evident at the low P supply. Increasing P level of the medium from 0.01 to 1.5 mM increased blade width/length ratio of nitrate-fed *R. dentatus* and

nitrate-fed *R. pictus* by an average of 11% and that of nitrate-fed *R. pictus* by 64% but with a 15% reduction in the ammonium-fed *R. dentatus* (**Fig. 2D**).

Plant height was non-significantly affected by genotype and form of N. Increasing P supply from 0.01 to 1.5 mM increased plant height by an average of 23% for all genotype × N from combinations (**Table 4**). Shoot water was subjected to significant genotype × N form interaction; being higher in *R. dentatus* than *R. pictus* under nitrate nutrition while the reverse was true under ammonium nutrition. Likewise, shoot water was higher under nitrate than ammonium nutrition in *R. dentatus* but the reverse was true for *R. pictus*. Increasing P supply had non-significant effect on shoot water content except for the significant increase in ammonium-fed *R. pictus* across 0.01 to 0.15 mM P with steady values at higher P levels (**Table 4**). Root water content was significantly higher in R. *dentatus* than *R. pictus* and under ammonium than nitrate nutrition for *R. pictus* but with non-significant effect of N form in *R. dentatus*. Increasing P supply had non-significant effect on root water content of *R. dentatus*, irrespective of N form. By contrast, root water content of *R. pictus* was significantly increased by 11% and 6% under nitrate and ammonium nutrition, respectively across the whole range of P supply (**Table 4**).

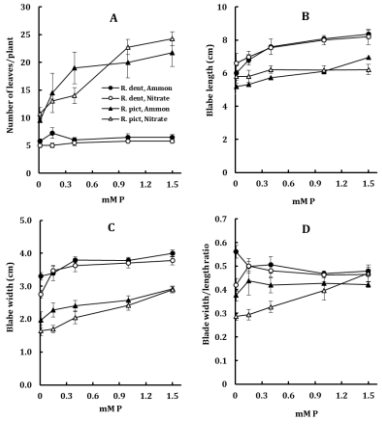

Figure 2 Number of leaves (A), blade length (B), blade width (C) and the blade width/length ratio (D) of *R. dentatus* and *R. pictus* in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO_3- or NH_4+. Each value is the mean of 4 replicates ± SE.

Plant biochemistry was highly significantly ($P<0.01$) affected by the main factors: plant species, nitrogen form and level of phosphorus, except for the non-significant effect of species and level of P on shoot protein and the non-significant genotype effect on shoot phenolics. Similar to the growth measurements, the effect of the factor interactions on plant biochemistry was less evident than the effect of the main factors (**Table 5**).

Chlorophyll a concentration was significantly higher in the leaves of *R. pictus* than *R. dentatus*, particularly under high P supply. The differential effect of N form was evident only in *R. dentatus*, in favor ammonium nutrition but with no effect in *R. pictus*. Increasing P supply increased Chl a concentration, particularly in *R. pictus* and under nitrate nutrition. The increase in Chl a concentration across the whole range of P supply amounted to 3.5 and 2.2 folds in nitrate- and ammonium-fed *R. dentatus*, respectively and to 4.3 and 3.5 folds in nitrate- and ammonium-fed *R. pictus*, respectively (**Fig. 3A**).

Chlorophyll b concentration of the leaves was higher in nitrate-fed *R. dentatus* than the other three species × N form combinations. In contrast to the response of Chl a, increasing P supply decreased Chl b concentration with different patterns according to plant species and N form; but the magnitude of decrease was generally more evident in *R. pictus* than *R. dentatus* and it was more evident under ammonium nutrition in *R. dentatus* but under nitrate nutrition in *R. pictus*. The decrease in Chl b concentration across the whole range of P levels was less severe (42%) in nitrate-fed *R. dentatus* than the other species × N form combinations with an average reduction of 71% (**Fig. 3B**).

Carotenoid concentration was significantly higher in *R. pictus* than *R. dentatus*, and under nitrate than ammonium nutrition. The genotypic difference as well as the N form effect was most evident at high P levels, where carotenoid content of nitrate-fed *R. pictus* exhibited the highest level relative to the other species × N form combinations. Phosphorus supply differentially affected carotenoid content according to plant species and form of N. Increasing P level of the medium increased carotenoid concentration by an average of 42% in nitrate- and ammonium-fed *R. dentatus* and by 91% in nitrate-fed *R. pictus* versus 20% reduction in ammonium-fed *R. pictus* (**Fig. 3C**).

Total soluble sugars (TSS) were markedly lower in the ammonium-fed *R. pictus* than the other three species × N form combinations. Increasing P supply from 0.01 to 1.5 mM increased TSS of ammonium-fed *R. dentatus*

and nitrate-fed *R. pictus* by 28% and 54%, respectively with non-significant effect in nitrate-fed *R. dentatus* and ammonium-fed *R. pictus* (**Fig. 4A**). Proline concentration was significantly higher in *R. dentatus* than *R. pictus* under ammonium nutrition with weak genotypic difference under nitrate nutrition. It was generally higher in ammonium-fed than nitrate-fed plants. Increasing P supply from 0.01 to 1.5 mM decreased proline concentration by an average of 40% for all species × N form combinations (**Fig. 4B**).

Table 4 Effect of nitrogen form and level of phosphorus on plant height and water content of shoot and root of *R. dentatus* and *R. pictus*. Nitrogen was supplied as NO_3^- or NH_4^+ at a level of 11 mM. Each value is the mean of 4 replicates ± SE. ≠

N form and level of P (mM)	Plant height (cm)	Shoot water content (% FW)	Root water content (% FW)
		Rumex dentatus	
Nitrate			
0.01	21.1 ± 2.68a	95.10 ± 0.60de	92.89 ± 0.83efg
0.15	21.3 ± 2.02a	95.05 ± 0.36de	93.35 ± 0.80fg
0.40	23.2 ± 0.68abcd	95.12 ± 0.44de	93.03 ± 0.28efg
1.00	26.8 ± 0.99cd	94.99 ± 0.28de	94.33 ± 0.29fg
1.50	27.3 ± 0.57d	95.26 ± 0.12de	94.93 ± 0.21g
Ammonium			
0.01	21.8 ± 1.78ab	91.53 ± 0.60ab	93.37 ± 1.01fg
0.15	21.2 ± 0.96a	91.58 ± 0.36ab	91.88 ± 0.30defg
0.40	22.8 ± 1.87abc	91.47 ± 0.44ab	91.37 ± 0.59def
1.00	26.0 ± 0.49bcd	92.33 ± 0.28bc	91.27 ± 0.43cdef
1.50	25.8 ± 1.25bcd	91.77 ± 0.12ab	91.68 ± 0.42defg
		Rumex pictus	
Nitrate			
0.01	21.2 ± 0.67a	91.36 ± 0.77ab	81.21 ± 2.14a
0.15	21.3 ± 0.71a	90.12 ± 1.65a	80.85 ± 2.53a
0.40	22.3 ± 1.88ab	91.39 ± 0.62ab	81.42 ± 1.27a
1.00	25.2 ± 2.29abcd	91.98 ± 0.33bc	81.01 ± 0.35a
1.50	26.1 ± 2.77bcd	93.62 ± 0.34cd	89.85 ± 0.62bcde
Ammonium			
0.01	21.1 ± 1.18a	92.04 ± 1.40bc	83.93 ± 2.14a
0.15	22.3 ± 1.03ab	95.77 ± 0.38e	82.32 ± 2.53a
0.40	23.7 ± 1.28abcd	95.65 ± 0.82e	87.89 ± 1.27bc
1.00	24.3 ± 1.55abcd	95.60 ± 0.49e	87.38 ± 0.35b
1.50	25.7 ± 1.08bcd	96.11 ± 0.28de	88.61 ± 0.62bcd

Protein concentration of leaves was comparable in the two species and under the two nitrogen forms. It exhibited an average increase of 30% upon increasing P supply from 0.01 to 0.15 mM, followed by 25% reduction with further increase up to 1.5 mM in *R. dentatus* and *R. pictus* under nitrate nutrition with non-significant effect under ammonium nutrition (**Fig. 5A**). Leaf malondialdehyde (MDA) concentration was significantly higher in *R. pictus* than *R. dentatus*, and under nitrate than ammonium nutrition in *R. dentatus* but with no effect of N form in *R. pictus*. Increasing P supply from 0.01 to 1.5 mM halved the MDA content in a progressive manner in *R. dentatus* and across the range 0.01-0.15 mM in *R. pictus* independent of the N form (**Fig. 5B**).

Phenolics concentration of leaves was significantly higher in the nitrate-fed *R. dentatus* than the other three species × N form combinations. Increasing P supply from 0.01 to 1.5 mM doubled phenolics concentration in nitrate- and ammonium-fed *R. dentatus* and ammonium-fed *R. pictus* but increased it by 64% in nitrate-fed *R. pictus* (**Fig. 6A**). DPPH scavenging activity was significantly higher in the ammonium-fed *R. pictus* than the other three species × N form combinations. Increasing P supply from 0.01 to 1.5 mM increased DPPH scavenging activity by 59% and 49% in *R. dentatus* and *R. pictus*, respectively independent of the N form (**Fig. 6B**).

Table 5 Three-way ANOVA showing the effect of the main factors (*Rumex* species, N form and level of P) and their interaction on plant biochemistry.

Variable and source of variation	df	F	P	Variable and source of variation	df	F	P
Chl a				Protein			
Species (Sp.)	1	33.09	0.000	Species (Sp.)	1	3.157	0.081
N form (N)	1	7.931	0.007	N form (N)	1	10.53	0.002
P level (P)	4	593.3	0.000	P level (P)	4	2.255	0.074
Sp. × N	1	18.59	0.000	Sp. × N	1	3.068	0.085
Sp. × P	4	24.06	0.000	Sp. × P	4	0.306	0.873
N × P	4	1.486	0.218	N × P	4	1.427	0.236
Sp. × N × P	4	0.455	0.769	Sp. × N × P	4	2.583	0.046
Chl b				Malondialedhyde			
Species (Sp.)	1	88.93	0.000	Species (Sp.)	1	73.97	0.000
N form (N)	1	23.62	0.000	N form (N)	1	10.06	0.003
P level (P)	4	36.56	0.000	P level (P)	4	26.58	0.000
Sp. × N	1	78.62	0.000	Sp. × N	1	15.66	0.000
Sp. × P	4	3.156	0.020	Sp. × P	4	3.585	0.014
N × P	4	3.752	0.009	N × P	4	0.177	0.949
Sp. × N × P	4	7.187	0.000	Sp. × N × P	4	0.565	0.689
Carotenoids				DPPH scavenging activity			
Species (Sp.)	1	65.75	0.000	Species (Sp.)	1	77.87	0.000
N form (N)	1	23.17	0.000	N form (N)	1	63.85	0.000
P level (P)	4	9.222	0.000	P level (P)	4	389.1	0.000
Sp. × N	1	0.269	0.606	Sp. × N	1	87.57	0.000
Sp. × P	4	2.539	0.049	Sp. × P	4	8.428	0.000
N × P	4	4.135	0.005	N × P	4	2.511	0.051
Sp. × N × P	4	2.236	0.076	Sp. × N × P	4	4.692	0.002
TSS				Phenolics			
Species (Sp.)	1	74.76	0.000	Species (Sp.)	1	3.746	0.058
N form (N)	1	98.23	0.000	N form (N)	1	8.436	0.005
P level (P)	4	7.482	0.000	P level (P)	4	76.83	0.000
Sp. × N	1	32.94	0.000	Sp. × N	1	6.048	0.017
Sp. × P	4	1.040	0.394	Sp. × P	4	3.372	0.015
N × P	4	2.125	0.089	N × P	4	1.377	0.253
Sp. × N × P	4	7.830	0.000	Sp. × N × P	4	2.852	0.031
Proline							
Species (Sp.)	1	22.30	0.000				
N form (N)	1	220.1	0.000				
P level (P)	4	163.6	0.000				
Sp. × N	1	48.31	0.000				
Sp. × P	4	4.938	0.002				
N × P	4	3.769	0.008				
Sp. × N × P	4	1.105	0.363				

Three-way ANOVA revealed significant effects of most of the main factors and their interactions on mineral composition of shoot. However, the most affected variable was nitrogen with significant ($P<0.05$) to highly significant ($P<0.01$) effects of the three main factors and their interactions, followed by the N/P ratio. By contrast, the least affected variable was the K/Na ratio, with significant effects only of level of P as well as the species × N and N × P interaction (**Table 6)**.

Nitrogen concentration of the shoot was significantly higher in *R. dentatus* than *R. pictus* and under nitrate over ammonium nutrition in *R. pictus* with weak effect of N form in *R. dentatus.* In nitrate-fed *R. dentatus*, N concentration of the shoot exhibited 37% increase with the increase in P level of the medium from 0.01 to 0.15 followed by 14% reduction with further increase in P level up to 1.5 mM but with non-significant changes in the ammonium-fed plants. In *R. pictus*, N concentration of the shoot increased by 30% across the whole range of P in nitrate-fed plants and by 90% post 0.4 up to 1.5 mM P in ammonium-fed plants (**Fig. 7A**).

Phosphorus concentration of the shoot was significantly higher in *R. pictus* than *R. dentatus* under nitrate nutrition but the reverse was true under ammonium nutrition. Regarding the effect of N form, ammonium nutrition led to higher P

concentration of the shoot above nitrate nutrition in *R. dentatus* but such an effect was absent in *R. pictus*. Increasing P supply from 0.01 to 1.5 mM increased shoot P concentration by 30% (non-significant) and 150% in nitrate- and ammonium-fed *R. dentatus*, respectively and by 100% and 80% in nitrate- and ammonium-fed *R. pictus*, respectively (**Fig. 7B**).

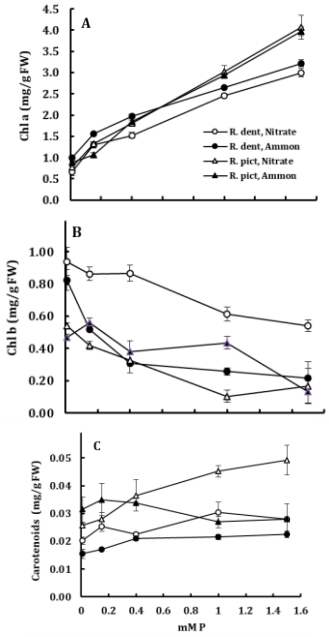

Figure 3 Concentrations of chlorophyll a (A), chlorophyll b (B) and carotenoids (C) in the leaves of R. dentatus and R. pictus in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO3- or NH4+. Each value is the mean of 4 replicates ± SE.

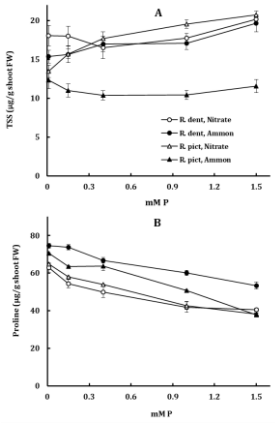

Figure 4 Concentrations of total soluble sugars (A) and proline (B) in the shoots of R. dentatus and R. pectus in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO3- or NH4+. Each value is the mean of 4 replicates ± SE.

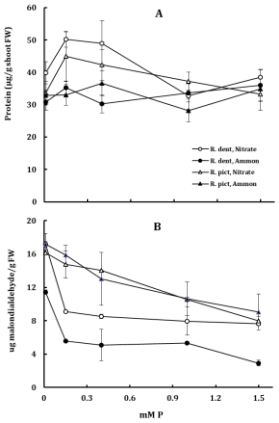

Figure 5 Concentrations of protein (A) and malondialdehyde (B) in the shoots of R. dentatus and R. pictus in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO3- or NH4+. Each value is the mean of 4 replicates ± SE.

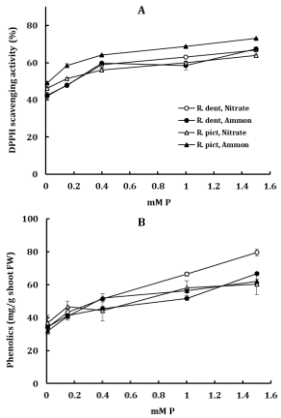

Figure 6 DPPH scavenging activity (A) and concentration of phenolics (B) of the shoots of *R. dentatus* and *R. pictus* in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO_3- or NH_4+. Each value is the mean of 4 replicates ± SE.

The N/P ratio was significantly higher in *R. dentatus* above *R. pictus* under nitrate nutrition with no genotypic difference under ammonium nutrition. Generally, the N/P ratio was higher under nitrate over ammonium nutrition, particularly in *R. dentatus*. In *R. dentatus*, the N/P ratio was reduced by 20% with the increase in P level of the medium post 1 up to 1.5 mM under nitrate nutrition; but in ammonium-fed plants, the reduction amounted to 50% across the moderate P levels (0.01-0.4 mM) with steady low levels at higher levels. In *R. pictus*, the N/P ratio was increased by 35% with the increase in P level of the medium from 0.01 to 0.4 mM, followed by 50% reduction with further increase in P level up to 1.5 mM under nitrate nutrition. In ammonium-fed *R. pictus*, the reduction amounted to 40% with the

increase in P level of the medium from 0.01 to 0.15mM, followed by 75% increase with further increase in P level up to 1.5 mM (**Fig. 7C**).

The concentrations of K and Na in the shoot were markedly higher (about twice) in *R. dentatus* than *R. pictus* with non-significant effect of form of N (**Fig. 8A**). Increasing P supply non-significantly affected shoot K concentration; however, Na concentration was reduced by 22% and 32% in nitrate- and ammonium-fed *R. dentatus*, respectively upon increasing P supply from 0.01 to 1.5 mM with non-significant effect in *R. pictus* (**Fig. 8B**). The K/Na ratio of the shoot was comparable in the two *Rumex* species and under the two N forms. Increasing P supply from 0.01 to 1.5 mM had non-significant effect on K/Na ratio of nitrate-fed but increased that of ammonium-fed *R. dentatus* by 66%. In *R. pictus*, increasing P supply from 0.1 to 1 mM reduced K/Na ratio of nitrate-fed plants by 29%, followed by 29% increase with further increase in P supply up to 1.5 mM, but the increase amounted to 45% across the whole range of P supply in ammonium-fed plants (**Fig. 8C**).

Table 6 Three-way ANOVA showing the effect of the main factors (*Rumex* species, N form and level of P) and their interaction on shoot mineral content.

Variable and source of variation	df	F	P	Variable and source of variation	df	F	P
Shoot N				Shoot K+			
Species (Sp.)	1	199.5	0.000	Species (Sp.)	1	1703	0.000
N form (N)	1	9.909	0.003	N form (N)	1	10.93	0.002
P level (P)	4	15.21	0.000	P level (P)	4	1.035	0.397
Sp. × N	1	4.661	0.035	Sp. × N	1	1.814	0.183
Sp. × P	4	12.32	0.000	Sp. × P	4	1.410	0.242
N × P	4	11.55	0.000	N × P	4	8.232	0.000
Sp. × N × P	4	3.774	0.008	Sp. × N × P	4	7.437	0.000
Shoot P				Shoot Na+			
Species (Sp.)	1	3.017	0.088	Species (Sp.)	1	403.1	0.000
N form (N)	1	50.10	0.000	N form (N)	1	5.509	0.022
P level (P)	4	10.21	0.000	P level (P)	4	6.380	0.000
Sp. × N	1	29.54	0.000	Sp. × N	1	3.629	0.062
Sp. × P	4	0.653	0.627	Sp. × P	4	2.718	0.038
N × P	4	2.268	0.072	N × P	4	0.295	0.880
Sp. × N × P	4	2.716	0.038	Sp. × N × P	4	0.456	0.768
Shoot N/P ratio				Shoot K/Na ratio			
Species (Sp.)	1	51.83	0.000	Species (Sp.)	1	3.621	0.062
N form (N)	1	126.4	0.000	N form (N)	1	0.016	0.899
P level (P)	4	4.590	0.003	P level (P)	4	3.943	0.007
Sp. × N	1	37.59	0.000	Sp. × N	1	6.847	0.011
Sp. × P	4	1.824	0.136	Sp. × P	4	1.817	0.137
N × P	4	9.285	0.000	N × P	4	4.461	0.003
Sp. × N × P	4	3.419	0.014	Sp. × N × P	4	1.403	0.244

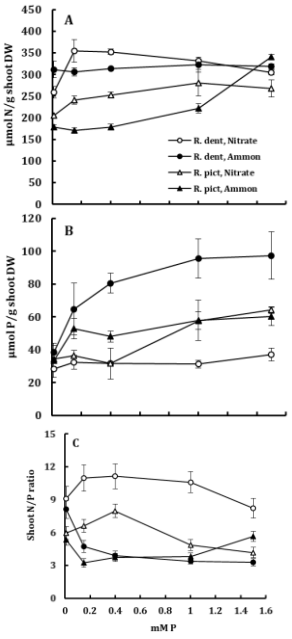

Figure 7 Concentrations of nitrogen (A), phosphorus (B) and the nitrogen/phosphorus ratio (C) in the shoots of *R. dentatus* and *R. pictus* in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO_3- or NH_4+. Each value is the mean of 4 replicates ± SE.

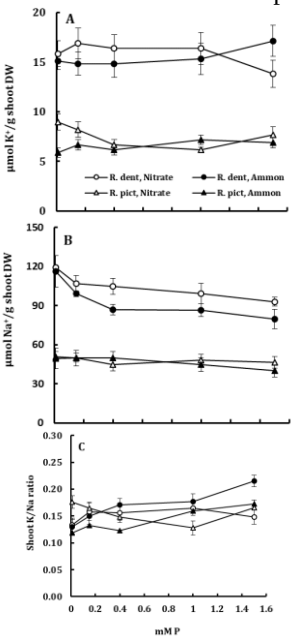

Figure 8 Concentrations of potassium (A), sodium (B) and the potassium/sodium ratio (C) in the shoots of R. dentatus and R. pictus in response to increasing level of P in a hydroponic culture with 11 mM N supplied either as NO3- or NH4+. Each value is the mean of 4 replicates ± SE.

Discussion

Nitrate and ammonium are the major N sources for higher plants. Compared with ammonium, nitrate is an oxidized anionic form of N; therefore it is more mobile in the soil, more available but less utilizable by plants and safer (Miller and Cramer, 2004). In agricultural soils, nitrate occurs at higher concentrations than ammonium but the reverse is true in unfertilized soils. Soil analysis revealed higher levels of all nutrients, including nitrogen, in the fertilized agricultural soil of R. dentatus than the coastal sand plains of R. pictus.

Plant species differ in their preference to either ammonium or nitrate. Generally, plants adapted to acid soils (calcifuge and wetland species) prefer ammonium whereas those adapted to calcareous high pH soils (cal¬cicole species) prefer nitrate (Britto and Kronzucker, 2002). The present work reveals preference of nitrate by the two Rumex species, particularly R. dentatus. In addition, the superiority of R. dentatus growth over R. pictus was particularly evident under nitrate nutrition. This justifies application of nitrate rather than ammonium for R. dentatus, which is widely consumed as a leafy vegetable. This should be done with application of cautious doses to avoid buildup of free non-assimilated nitrate in the plant foliage which can impact human health. In accordance with the present findings. nitrogen form differentially affected plant height in two amaranth varieties, and the genotypic variability was quite evident under nitrate treatment (Munene et al., 2017). However, preference of nitrate by cotton genotypes was not associated with genotype × N form interaction, and plant performance was not affected by the form of N (Iqbal et al., 2020). The present work suggests that ammonium nutrition favors plant succulence (more water content of the foliage) in R. pictus while the reverse was true for R. dentatus which exhibited higher water content under nitrate nutrition. The effect on R. pictus can be explained in view of the similarity between NH3 and water in molecular sizes and polarity, allowing NH3 to permeate water channels (Hawkesford et al., 2012).

Phosphorus is the second key plant nutrient after nitrogen. It plays several important roles regarding energy metabolism (in the form of ATP and its analogues) in addition to involvement in phospholipids and nucleic acids (Byrne et al., 2011). Phosphorus concentration of leaves can vary widely without affecting plant performance, because Pi concentration in the cytosol is adjusted within a narrow range utilizing the vacuolar Pi as a buffer (Mimura et al., 1990). The optimal P content for plant

growth is in the range of 0.3 - 0.5% DW, but it might be much lower in plants adapted to P-impoverished soils (Lambers et al., 2011). The P supply optimum for shoot growth of Rumex spp. under nitrate nutrition (0.4 mM) was lower than that required under ammonium nutrition (1.5 mM P), and the growth promoting effect of increasing P supply was more evident under ammonium than nitrate nutrition and in R. dentatus than R. pictus.

In P-deficient plants, reductions in the number of leaves and leaf expansion are the most obvious symptoms (Lynch et al., 1991). Leaf expansion may be impaired as a consequence of reduced root hydraulic conductivity (Clarkson et al., 2000). The vigorous species (R. dentatus) was, however, characterized with less number of leaves but longer and wider blades compared with R. pictus; and this pattern was particularly evident under ammonium and low P nutrition. However, leaf number was negatively correlated to leaf dimensions (Table 7). Leaf width was more sensitive to P supply in R. pictus than in R. dentatus, but the reverse was true for leaf length.

Compared with shoot growth, root growth was subjected to lesser inhib¬ition under P deficiency, leading to a typical increase in the plant root/shoot ratio; probably as a consequence of the favored partitioning of carbohydrates and P towards the roots, along with net translocation of P from the shoot to the roots (Hawkesford et al., 2012). The more P-responsive R. dentatus was characterized with high RWR relative to R. pictus. It seems that allocation of plant biomass to root (increasing RWR) is favored under ammonium nutrition and P deficiency, and this pattern was particularly evident in R. pictus.

In con¬trast to the severe inhibition in leaf expansion under P defi¬ciency, the contents of protein and chlorophyll are less affected, leading to higher chlorophyll concentration per unit leaf area (Rao and Terry, 1989). A common symptom of P deficiency is, thus, the dark green color of foliage, because leaf expan¬sion is more strongly inhibited than chlorophyll formation (Hawkesford et al., 2012). However, in the two Rumex species increasing P supply increased leaf Chl a and carotenoid concentrations but reduced Chl b concentration. Leaves of R dentatus were characterized with lower Chla and carotenoid contents but higher Chl b content relative to R. pictus. It can be concluded that ammonium nutrition favors Chl a formation whereas nitrate favors Chl b and carotenoid formation. In cotton, ammonium nutrition reduced leaf chlorophyll compared with nitrate nutrition (Iqbal et al., 2020).

One of the symptoms of ammonium toxicity is depletion of the plant carbohydrate reserves to the point of starvation as a consequence of excessive con¬sumption of sugars for ammonium assimilation (Iqbal et al., 2020). Nevertheless, ammonium nutrition led to the highest sugar concentrations in carrot relative to the other N sources (Smoleń and Sady, 2009). By contrast, accumulation of soluble sugars and starch in the leaves is a typical symptom of P deficiency, as a result of either lower phloem loading, lower demand at the sink (Rao et al., 1990) or more suppression of shoot growth compared with photosynthesis (De Groot et al., 2003). In the present work, the effect of N form on sugar concentration of shoot was evident only in R. pictus, where nitrate nutrition led to higher levels of soluble sugars compared with ammonium.

In addition, the effect of P supply was limited with mild increase in total sugar content under high P supply only in nitrate-fed *R. pictus* and ammonium-fed *R. dentatus*.

Under stress conditions, protein synthesis may be inhibited in favor of the accumulation of a number of amino acids (e.g., glycine betaine and proline). These soluble nitrogen compounds are involved in osmotic adjustment, protection of enzymes or detoxification of reactive oxygen species (**Radyukina *et al*., 2008**). Proline accumulation is a well-known response to water and salt stresses in plants (**Hawkesford et al., 2012**). The present findings of higher proline concentration under ammonium nutrition compared with nitrate nutrition, in *R. dentatus* compared with *R. pictus* and under low P supply relative to high P supply suggests that ammonium, as a sole N source might represent stressful conditions, compared with nitrate particularly to *R. dentatus* and under P shortage. Meanwhile, protein concentration was non-significantly different in the two species and under the two nitrogen forms. Only P supply exerted a mild effect, where both P starvation and excess led to marginal reduction in protein content of *Rumex* leaves. This might suggest that the increased proline content of *Rumex* leaves under P deficiency and ammonium nutrition is not a

consequence of impaired protein synthesis.

Phenolics are secondary plant metabolites that might play pivotal role in plant protection against a variety of biotic and abiotic stresses (**Munene et al., 2017**). Accumulation of phenolics has been reported under deficiency of N, K, Ca, B and Cu (**Broadley et al., 2012**). The present work revealed marked effect of N form on shoot phenolics only in *R. dentatus*, where the stressing nitrate nutrition led to higher phenolics concentration relative to ammonium. Increasing P supply increased phenolics concentration. The effect of N form on phenolics accumulation can vary according to plant species and susceptibility to ammonium toxicity. Ammonium nutrition stimulated accumulation of phenolics in the ammonium-sensitive *Matricaria chamomilla* (**Kováčik and Klejdus, 2014**) and amaranth (**Munen et al., 2017**), whereas urea fertilization reduced phenolic concentration in cabbage leaves (**Leja et al., 2005**). In the present work, shoot biomass was positively correlated with leaf phenolics in contrast to the negative correlation between phenolics concentration and plant height of amaranth (**Munen et al., 20217**). DPPH scavenging activity was intimately correlated to leaf phenolics content of *Rumex* is in agreement with the findings of **Munen et al. (2017)** with amaranth but in contrast to **Leja et al. (2005)** with cabbage where the changes in phenolics did not correspond to radical scavenging activity.

Table 7 Correlation analysis of the performance of the two *Rumex* species in response to N form and level of P of the medium in a hydroponic culture.

	NOL	LL	LW	W/L ratio	Sh DW	Chl a	Chl b	Carot	DPPH	Phenol	Proline	SS	Protein
LL	- - -												
LW ratio	- - -	+ + +											
W/L	-	ns	+ + +										
Sh DW	ns	+ + +	+ +	ns									
Chl a	+ + +	+ + +	+ +	ns	+ + +								
Chl b	- - -	ns	ns	+	- - -	- - -							
Carot	- - -	ns	- -	-	ns	+ + +	- - -						
DPPH	+ + +	+ + +	ns	ns	+ + +	+ + +	- - -	+ +					
Phenol.	ns	+ + +	+ + +	ns	+ + +	+ + +	- - -	+	+ + +				
Proline	- - -	- -	ns	ns	- - -	- - -	+ + +	-	- - -	- - -			
SS	-	+ + +	+ + +	+	+ + +	+	ns	- - -	+ + +	+ +	- -		
Protein	ns	ns	ns	ns	-	ns	+	ns	ns	ns	+ +	-	
MDA	ns	- - -	- - -	- -	- - -	- - -	+	ns	- - -	- - -	+ + +	- - -	ns

+ + + and - - - denote very highly significant (P<0.001) positive and negative correlation, respectively, + + and - - highly significant (P<0.01) correlation, + and –significant (P<0.05) correlation and ns non-significant (P>0.05) correlation.

NOL = number of leaves, LL = leaf length, LW = leaf width, W/L = leaf width/length ratio, DPPH = DPPH scavenging activity, SS = soluble sugars

The present findings suggest that malondialdehyde (MDA) concentration of *Rumex* shoot was high under the stress conditions of P shortage with variable effect of N form in the two species, with strong negative correlation with plant growth and pigment content (**Table 7).** Accumulation of malondialdehyde (MDA) in wheat leaves, as a sign of oxidative stress, was more expressed in nitrate-fed plants than in ammonium-fed plants (**Polesskaya et al., 2006**). The advantage of NH_4^+ nutrition to *Spartina alterniflora* was associated with high antioxidant enzyme activities, together with low MDA content (**Hessini et al., 2013**).

Ammonium and nitrate constitute about 80% of the total cations and anions, respectively taken up by plants; therefore, the form of N can determine the uptake of other cations and anions. Generally, compared with nitrate, ammonium nutrition favors accumulation of inorganic anions such as Cl^-, SO_4^{2-} and $H_2PO_4^-$ but reduces the uptake of cations such as K^+, Ca^{2+} and Mg^{2+} (**Britto and Kronzucker, 2002**). Ammonium ion (NH_4^+) resembles K^+ in ionic radius and size of hydration shells (**Howitt and Udvardi, 2000**); therefore, it can compete with K^+ for plant uptake (**ten Hoopen et al., 2010**). Nevertheless, the low K^+ concentrations of NH_4^+-fed plants may lead to up-regulation of K^+ channels in order to improve the plant K^+ uptake (**ten Hoopen et al., 2010**). However, the concentrations of K^+ and Na^+ in the shoot of *Rumex* spp. were marginally affected by the treatments; only the genotypic variability was

marked in favor of *R. dentatus.* Increasing P supply non-significantly affected shoot K^+ concentration but reduced Na^+ concentration, with a consequent increase in the K/Na ratio. The reports about the effect of P supply on mineral composition of plants are few. Increasing P supply had been found to increase concentrations of Na^+ in several legume species; with variable effect on K^+ concentration according to the species **(Andrew and Robins, 1969)**. A negative correlation has been reported between concentrations of P and K^+ in rice straw versus a positive correlation in the grain (**Saleque *et al*., 2001).**

The present work suggests higher uptake of nitrogen by *Rumex* spp. as nitrate than as ammonium. Also, there was a marked genotypic variability in N uptake in favor of *R. dentatus* above *R. pictus.* Thus, for *Rumex* spp., the preference of either N form (nitrate or ammonium) for plant growth and for uptake by the plant are correlated. In agreement with the present findings, nitrate was more available N source to cotton than ammonium (**Iqbal et al., 2020**). By contrast, **Serna et al. (1992)** reported faster absorption of ammonium than nitrate by citrus seedlings; whereas Abbes **et al. (1995**) reported no influence of N form on uptake of N by onion seedlings. The increased P concentration of *Rumex* shoot in response to increasing P supply was more evident in ammonium-fed than nitrate-fed plants. Ammonium nutrition led to higher P concentration of the shoot compared with nitrate nutrition only in *R. dentatus*. Higher concentrations of N and P in watermelon seedlings were found under mixed nitrate and ammonium sources compared with either nitrate or ammonium alone (**Na et al., 2014**). Leaf concentration of N and P of citrus seedlings were higher under ammonium than nitrate nutrition (**Serna et al., 1992**). The differential effect of treatments on the uptake of N and P led to marked effects on the N/P ratio of shoot. Generally, the N/P ratio was higher under nitrate over ammonium nutrition, particularly in *R. dentatus*.

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الملخص العربي

عنوان البحث: تأثير شكل المصدر النيتروجيني في الاستجابة للفوسفور في نوعين من جنس الحميض في المنطقة الساحلية لدلتا النيل

يشكل النيتروجين والفوسفور مغذيات محددة للنبات في الأراضي الحديثة والناضجة على التوالي. تم دراسة التفاعل بين النيتروجين والفوسفور على نمو وأداء نوعين من جنس الحميض هما *Rumex dentatus* و *Rumex pictus*. نميت النباتات في رمل مغسول مع الإمداد بمحلول مغذى يحتوى على ١١ ملى مولار من النيتروجين أما في صورة نترات أو أمونيوم مع تركيزات متدرجة من الفوسفور ٠,٠١ و ٠,١٥ و ٠,٤ و ١ و ١,٥ مللى مولار. فضلت النباتات وخاصة *R. dentatus* النترات على الأمونيوم كمصدر وحيد للنيتروجين. شجعت النترات عصيرية النبات فى *R. dentatus* بينما لوحظ العكس في *R. pictus*. كان المستوى الأمثل للفوسفور في حالة النترات (٠,٤ ملى مولار) أقل منه فى حالة الأمونيوم (١,٥ ملى مولار). امتلك *R. dentatus* عددا أقل من الاوراق ولكن مع أنصال أطول وأعرض من *R. pictus*. شجعت التغذية بالأمونيوم ونقص الفوسفور زيادة المجموع الجذري خاصة في *R. pictus*. أدت زيادة مستوى الفوسفور الى زيادة كلوروفيل أ والكاروتينات ولكن نقص كلوروفيل ب. كان محتوى السكريات أعلى في حالة النترات مقارنة بالأمونيوم فقط في *R. pictus* مع تأثير محدود لفوسفور. تراكم البرولين في أنسجة الساق تحت التغذية بالأمونيوم ونقص الفوسفور والذى يدل على التأثير المجهد للأمونيوم لم يكن راجعا الى نقص البروتين خاصة في *R. dentatus*. فقط فى *R. dentatus* أدت النترات إلى زيادة تركيز الفينولات والنشاط الكاسح للشق النشط DPPH ولكن إلى مستوى منخفض من ألدهيد المالونيك. لم تتأثر تركيزات البوتاسيوم والصوديوم فى المجموع الخضرى بصورة النتروجين ولكنها كانت أعلى في *R. dentatus* عن *R. pictus*. كان تركيز النيتروجين أعلى في *R. dentatus* عن *R. pictus* وفقط فى *R. pictus* كان تركيز النيتروجين أعلى في حالة النترات عن الأمونيوم. زاد الإمداد بالفوسفور من تركيز الفوسفور فى المجموع الخضري خاصة في حالة الأمونيوم. كان تركيز الفوسفور أعلى في *R. pictus* عن *R. dentatus* تحت تأثير الأمونيوم بينما كان العكس صحيحا في حالة النترات.