



Phosphorus fertilization rate and dynamics in early development of three giant reed (*Arundo donax* L.) ecotypes

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ARTICLE INFO

Keywords:

Biomass plants
Phosphorus use efficiency
Peroxidase
Bioconcentration
Translocation
CO₂ assimilation

ABSTRACT

Recently, biomass crops attracted considerable attention as a possible alternative to fossil fuels; nevertheless, the interest in its agronomic practices such as irrigation and fertilization is still unclear. This study aims at determining the appropriate phosphorus (P) fertilization management in the early development stage of giant reed (*Arundo donax* L.). The uptake, transportation, and fertilization use efficiency of P were reported in three ecotypes of giant reed, i.e., STM (Hungary), BL (the USA), and ESP (Spain). Somatic embryo-derived plantlets were developed under glasshouse environment in clean sand (quartz) augmented with 0, 25, 50, 100, and 150 mg P kg⁻¹, added as monosodium phosphate. P-treated plants possessed a fibrous root system characterized by many lateral roots, while control plants (received no P) had a root system distinguished with long axial roots with very few lateral branches. Phosphorus uptake significantly varied according to plant ecotype, plant parts, and applied P concentration. Giant reed ecotypes showed different responses to uptake and accumulation of P, where P contents in the STM, ESP and BL ecotypes were 392, 413, and 445 μg g⁻¹, respectively. The accumulation of P in different plant parts was as follows: leaf blade > culm > root. The treatment of 50 mg P kg⁻¹ displayed the highest P content in all plant parts. The BL and STM ecotypes showed the same translocation factor (TF) of 4.96, while the ESP ecotype exhibited a lower TF of (4.03). The treatment of 50 mg P kg⁻¹ revealed the highest TF (5.94). The BL ecotype showed P use efficiency (PUE) of 7.08 compared with 6.49 and 5.85 for the ESP and STM ecotypes, respectively. The 50 mg P kg⁻¹ exhibited the highest PUE (13.37). The BL ecotype displayed the highest CO₂ assimilation rate (12.46 μmol photons m⁻² s⁻¹). Also, the rate of CO₂ assimilation increased upon increasing the rate of the applied P. Our results reveal that the appropriate P fertilization rate of the ESP, STM, and BL ecotypes is 25, 25, and 50 mg P kg⁻¹, respectively.

1. Introduction

Phosphorus (P) is an indispensable macronutrient for plants. The vital function of P in plants is bioenergy; it is a main constituent of adenosine nucleotides (ADP and ATP), which conserves sunlight energy in chemical compounds during the photosynthesis process and also in respiration reactions [1]. It, also, has a key role in modulating activity of several enzymes involved in phosphorylation, activation of proteins, regulation of metabolic pathways, and participation in cell fission and

signal transduction [2]. The response of plants to P deficiencies is quite different from that of other nutrients, where it is generally manifested in decreased production of chlorophyll. Alternatively, deep-green development purpling of shoot part has been cited for P-deprived plants [3,4]. This dimming or purpling is a result of the amassing of biosynthesized compounds by photosynthesis, which is utilized as a result of the declined energy abstracted from chemical substances in the plant.

Many symptoms also appear on plants lacking adequate P supply. For example, reproductive tissues are obstructed by P deprivation as a result

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<https://doi.org/10.1016/j.biombioe.2023.106805>

Received 14 July 2022; Received in revised form 20 January 2023; Accepted 10 April 2023

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of flower initiation retarding, diminished number of flowers, and deformation of seed [5] suppression in leaf expansion [6] and the number of leaves [7]. Moreover, P deficiency caused a reduction in cell division in corn [8] and the hydraulic conductivity of the root, owing to a reduction in genes encoding aquaporins [9]. Consequently, P deficiency reduces root growth [6]. Furthermore, the root cell elongation might increase [10].

Although it is ranked the 11th most plentiful element in the Earth's crust, P concentration in agricultural soils is usually very low. Even though P might be present in sufficient quantities (theoretically) in soils, its phyto-availability depends on several factors such as soil type, soil pH, redox potential, organic matter, and the structure of the soil microbiome. Phosphorus forms complexes with iron, aluminum (in too acidic soils), and calcium (in too alkaline soils); these complexes, however, are not available for absorption by plant roots [11]. Therefore, continual P fertilization is a necessity for growing cash crops [1]. Adequate fertilization is necessary for effective crop production to achieve maximum yield. Being one of the three macronutrients besides N and K, the size of the world trade of P fertilizer in 2021 reached about USD 61 billion [12]. However, the P fertilizer industry brings potential threats to the environmental components, including air, water, and soil. Phosphate rock, as one of the main sources of P fertilizers, could contain high levels of potentially toxic elements and radioactive ones such as uranium and thorium. Also, the application of P fertilizers at high rates or wrong time might adversely affect aquatic ecosystems and reduce the quality of water bodies due to the leaching of excess P into water streams, which is referred to as eutrophication [13,14]. Thus, determining the appropriate rate and application time of P fertilization is critical.

Fertilizer use efficiency, particularly of NPK, is a major and decisive factor in selecting suitable perennial lignocellulosic crops for cultivation as well as enhancing their productivity [15,16]. Yet, perennial lignocellulosic crops showed different responses to NPK fertilization. For instance, N fertilization positively improved the yield of elephant grass (*Pennisetum purpureum* Schum.) [17–19], while P fertilization revealed an insignificant impact [19]. In case of giant reed (*Arundo donax* L.), biomass yield increased by 4 tons ha⁻¹ year⁻¹ upon NPK fertilization at a rate of 200, 80, and 200 kg ha⁻¹, respectively [17]. However, another study observed that fertilizing giant reed with N at a rate of 140 kg ha⁻¹ reduced biomass productivity or at least showed no effect [20]. Switchgrass (*Panicum virgatum* L.) displayed different responses to NP fertilization, where 1 kg of N added resulted in a biomass yield of 55–100 kg [15,21,22]; however, the optimum N fertilization is 80 kg N ha⁻¹ year⁻¹ [23]. On the other hand, P fertilization increased the biomass yield of switchgrass by 44–49%, especially when P was applied at a rate of 28 kg ha⁻¹ year⁻¹ [17].

Contrary to what may be misunderstood that the term biofuel is a modern term (recently defined), the concept of biofuel is old since man discovered fire, where wood, dung, and charcoal have been used in cooking and heating; and are still used in many countries of the world until now [24]. Cellulosic ethanol has been produced since 1903 using sulfite pulp mills in several countries, including Sweden (in 1909), Switzerland (1915–2008), and North America (1946–2000) [25]. However, the real revolution of biofuels and its return into fashion began in the second half of the twentieth century, after many oil crises faced by the world, such as World War I and II, the oil crisis in 1973 during the Arab–Israeli War, the oil crisis in 1979 during the Iranian Revolution, and in 1990 oil price shock during the Gulf War [24]. All of these crises were the main driver of the increased interest in biofuel production as an alternative to fossil fuels. Consequently, many countries, such as the US and Brazil, have started the so-called large-scale production of biofuels. The production of ethanol, as one of the main components of biofuels, has been started depending on the edible plant-based biomass such as starch (maize), sugar (sugar cane and sugar beet), and oil (rapeseed and soybean) feedstock referred to as first-generation biofuels. Later, interest emerged in what is known as the

second generation of biofuels, which are defined as biofuels that are manufactured from non-food feedstocks, mainly lignocellulosic crops (miscanthus, giant reed, and switchgrass), woody crops (poplar), agricultural wastes or municipal solid wastes, to avoid competition over croplands with strategic crops [26]. Perennial lignocellulosic crops have shown the ability to adapt and grow in poor lands, which provides an opportunity to avoid competition with agricultural food crops ensuring the sustainable production of biofuels [27–29]. However, the success of crop establishment has often been undermined by marginal lands as a result of the harsh growth conditions plants encounter in these harsh lands [30].

Giant reed is one of the most important candidates for second-generation ethanol production due to its large biomass yield [27,31]. Furthermore, the giant reed requires low inputs, particularly fertilizers, in addition to low-maintenance costs since it shows high resistance to pests and insects [31]. It is a typical biomass crop, exhibiting several attractive properties such as activated carbon [32], pulp and paper [33], and biomass production [34]. Also, it can be used as a biofiltration material for wastewater treatment [35].

Understanding plant responses to nutrient availability is critical for developing sustainable plant production systems. Besides N, P is the most common limiting factor in plant production systems. Plants can absorb only about 15% of the added phosphate fertilizer to the soil immediately after fertilization, while the rest is converted quickly to insoluble P form [36].

Giant reed plants possess a root system characterized by stout rhizomes and coarse fibrous roots [27]. Many researchers have documented the uptake dynamics and accumulation of various nutrients, including P, in giant reed plants [1,37,38]. Nevertheless, giant reed seedlings used in most of these studies were rhizome-derived plantlets. Rhizomes represent a stock of nutrients due to the remobilization of nutrients during the senescence period and before harvest [1]. Therefore, accumulated nutrients in rhizomes possibly alter the uptake dynamics and plant response to applied fertilizations. Rhizome-derived plantlets are the most common reproductive method of giant reed seedlings; however, micro-propagated seedlings have many advantages, including a fast supply of cost-effectively priced propagules, pathogen-free, low production cost, precise control of plant density in the farm, ease of mechanized plantation, and ease of mechanized harvesting due to the uniformity of the plants [39], in addition to being ideal for studying nutrient uptake dynamics due to its fibrous roots. However, growing somatic embryo-derived plantlets in the first year is critical and needs a strict fertilization program.

Thus, the current study aimed at determining the threshold of P fertilization corresponding to the highest growth rate, development, and productivity of somatic embryo-derived giant reed seedlings using three different ecotypes belonging to different climatic zones. Also, the response of giant reed ecotypes to the elevated rates of P fertilizer was noticed. To meet the objectives of this study, plant biometrics, photosynthesis efficiency, physiological response, P accumulation in different plant organs of giant reed plants, and PUE were observed as well as the translocation and bioconcentration factors of P.

2. Materials and methods

2.1. Plant material source

Plant materials used in this study were somatic embryo-derived plantlets of three different giant reed ecotypes belonging to different climatic zones, i.e., STM (Hungary), BL (the USA), and ESP (Spain). The STM and ESP plantlets were obtained from the University of Debrecen, Hungary, while the BL plantlets were obtained from the University of South Carolina, USA. The plantlets were *in vitro* propagated using somatic embryogenesis at the University of Debrecen, Hungary, as described by Márton and Czákó [40,41].

2.2. Experiment description

The experimental layout was a full factorial split-plot design organized in the randomized complete block design (RCBD) with five repeats. The experiment included two factors: (1) three giant reed ecotypes (STM, BL, and ESP) as the main level and (2) five P treatments (0, 25, 50, 100, and 150 mg kg⁻¹) as the sublevel. Giant reed seedlings were transplanted in 1 kg polyethylene pots (no holes) filled with clean sand (Manufacturer: Aquabau, Budapest, Hungary) in a greenhouse environment, according to Smith et al. [42]. To fulfill the nutritional requirements of the plantlets, the following nutrient solution [42] was applied (mg kg⁻¹): (NH₄)₂SO₄ 10, KCl 10, CaCl₂·2H₂O 2.6, MgCl₂·6H₂O 2, FeCl₃·6H₂O 0.5, and MnSO₄·H₂O 0.1.

Five concentrations of P (0, 25, 50, 100, and 150 mg kg⁻¹) were prepared from monosodium phosphate (NaH₂PO₄, Sigma-Aldrich, St. Louis, USA). The corresponding P concentration of each treatment was added to the growth medium before seedling transplantation in 200 mL distilled water per pot to stay below the saturation percentage of the growth medium, which was 300 mL kg⁻¹ sand. The transplantation of eight-week-old plantlets was carried out at the rate of 2 seedlings per pot in mid of June, and the moisture of the sand medium was kept at 75% of water saturation throughout the whole experimental period by measuring the pot's weight each time it was watered. Six months later, the plants were harvested for analysis.

2.2.1. Determination of P content in giant reed plants and growth medium

Regarding P measurements, giant reed plants were first brushed to remove any attached solid particles to the plant surface and then washed carefully with double-distilled water. After air-drying at room temperature, the giant reed plants were split into the root, culm, and leaf blades, and then Alpha 1–4 LSC Christ lyophilizer (Martin Christ GmbH, Germany) was applied to lyophilize the plant tissues. The dried samples were pulverized by a stainless steel mill (Mill, M 20 Universal, 120 V, Fullerton, California, USA) to a fine powder (particle size <300 μm) and kept in polythene bags for further measurements. Afterward, a 200 mg lyophilized sample was weighed in a Kjeldahl digestion tube and mixed with three mL HNO₃ to be digested at 100 °C for 45 min. After cooling off, two mL H₂O₂ was added to the digested samples after cooling off followed by further heating to 120 °C for another 45 min. The total volume of the digested sample was brought to 10 mL by Milli-Q water (Millipore, Molsheim, France), then filtered by Whatman filter paper no. 40. The P concentration was calorimetrically determined after Sparks et al. [43].

Concerning the determination of P content in the growth medium (sand), the five repeats of the same treatment were collected in one composite sample, air-dried, grounded, and sieved using a 2-mm sieve. For the total P content, a 0.5 g sample was weighed in a Kjeldahl digestion tube and broken down using a mixture of HNO₃:HClO₄ (5/3, v/v) at 140–150 °C for 75 min. After complete digestion, the solution volume was brought to 50 mL with Milli-Q water, and the P content was spectrophotometrically determined according to Sparks et al. [43].

The extractable AL-P₂O₅ in the growth medium (sand) at the end of the experiment was quantified using 0.1 M ammonium-lactate-acetic acid (AL) solution (pH 3.7). The P content was spectrophotometrically determined at 660 nm wavelength [44].

2.2.2. P-related formulas

Based on the P content in plant tissues and growth medium (sand), the following formulas were calculated:

$$\text{Tarnslocation factor (TF)} = \frac{[P]_{\text{culm} + \text{leaf blade}}}{[P]_{\text{root}}} \quad [45]$$

$$\text{Bioconcentration factor (BCF)} = \frac{[P]_{\text{culm} + \text{leaf blade}}}{[P]_{\text{growth medium}}} \quad [46]$$

The agronomic efficiency of applied P (PUE) was calculated as

follows:

$$PUE = \frac{Pf - Pc}{R} \quad [47]$$

Pf = P removed by the harvest for a P treatment (mg kg⁻¹)

Pc = P removed by the harvest for the 0 P treatment (mg kg⁻¹)

R = rate of P applied to a P treatment (mg kg⁻¹)

Since there were no holes in the experimental pots and irrigation was based on the 75% of soil saturation percentage, the absorbed P percentage was calculated using the following equation:

$$\text{Absorbed P \%} = \left[\frac{P_i - P_t}{P_i} \right] * 100$$

where.

Pi = initial P concentration in the growth medium before the experiment

Pt = total P concentration in the growth medium after the experiment

2.2.3. Plant biometrics

Root and shoot, and 2nd internode lengths, root and shoot volumes, number of leaves, internodes, and new tillers, root, culm, and leaf blade fresh and dry masses, and dry matter content of root, culm, and leaf blade were recorded at the end of the experimental period.

2.2.4. Efficiency of photosynthesis and CO₂ assimilation by giant reed under P treatments

The efficacy of photosystem II (the optimum photochemical activity [45]) was determined by measuring the Fv/Fm ratio (a characteristic of the photochemical activity and structural dynamics of photosystem II). The PAM 2100 (Walz, Germany) modulated light fluorometer was used to *in vivo* measure the chlorophyll fluorescence [46]. Briefly, the newest fully expanded leaves were dark-adapted for 20 min, and then the initial fluorescence (Fo) was excited by weak light (0.1 μmol photons m⁻² s⁻¹). The maximal fluorescence (Fm) was induced by a white saturating flash (8000 μmol photons m⁻² s⁻¹) (fast phase of chlorophyll fluorescence). The difference between the maximum and minimum fluorescence is the variable fluorescence (Fv).

The net photosynthesis rate in giant reed leaf blades at room temperature was measured using the LICOR LI-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA). It contains two infrared gas analyzers to determine the mole fraction of CO₂ and H₂O in the air. The light was controlled and stable in the sample chamber, a multiphase flash fluorometer head was used as a light source, and the aperture was 2 cm². The photosynthetic active radiation (PAR) was 865 μmol photon m⁻² s⁻¹, with 90% red (630 nm) and 10% blue (470 nm) light. The ambient CO₂ level was 401.461 μmol mol⁻¹. The measurements were carried out six repeats per leaf in three repetitions on light-adapted leaves. Readings were logged when the measured parameters stabilized but after a minimum of 120 s.

2.2.5. Soluble protein content and peroxidase activity in giant reed

Buffer-soluble protein content in the plant parts (root, culm, and leaf blade) was quantified according to the Bradford method [47] in four replications. Briefly, a 20 mg lyophilized plant sample was suspended in 1 mL solubilizing buffer containing NaCl 0.6%; urea 3.5 M; thiourea 1 M; and NaOH 0.1 M in a 2 mL Eppendorf tube. Samples were mixed thoroughly using a vortex for 1 min at room temperature. For better extraction of soluble protein fraction, Eppendorf tubes were placed in an ultrasonic bath (Sonics VCX 750, Sonics & Materials Inc., Newtown, CT, USA) for 1 h at room temperature (heater power: 109 W and operating frequency: 40 kHz). Afterward, samples were centrifuged at 10,000×g

for 5 min. The buffer-soluble protein content was measured in the supernatant using a UV-160 A spectrophotometer (Shimadzu, Japan) at 595 nm. The standard curve was generated using bovine serum albumin.

The activity of peroxidase (POD, EC 1.11.1.7) in the root, culm, and leaf blade tissues of giant reed plants was detected, according to Roxas et al. [48]. In brief, a 200 mg lyophilized plant sample was thoroughly homogenized in 2 mL phosphate buffer 250 mM (pH 6.8). The supernatant used to spectrophotometrically determine the activity of POD was obtained by centrifugation of homogenate at 10,000×g for 10 min at room temperature. The reaction mixture consists of 1700 µL Na-acetate buffer 0.1 M (pH 5.0), 30 µL 0.3% H₂O₂, 20 µL *o*-dianisidine (10 mg mL⁻¹, dissolved in methanol), and 10 µL sample extract bringing the total volume up to 1760 µL. The POD activity was detected as simple kinetics using a UV-160 A spectrophotometer (Shimadzu, Japan) at 440 nm for 1 min with 10-s intervals. The extinction coefficient of *o*-dianisidine is 11.3 mM⁻¹ cm⁻¹ at 440 nm. The activity of POD (one unit of absorbance mL⁻¹ min⁻¹ g⁻¹ of dry matter) is calculated as follows:

$$\text{Activity (U mL}^{-1}\text{)} = (Y \times 11.3^{-1}) \times 1760 \times 10^{-1}$$

Y = absorbance calculated from the plot; 11.3 = extinction coefficient of *o*-dianisidine; 1760 = total volume in the cuvette (µL); and 10 = sample volume in the cuvette (µL).

2.3. Data analysis

Normality and homoscedasticity tests were run before statistical analysis. Data analysis was done using the SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA). The analysis of variance (two-way ANOVA) was achieved between P treatments and giant reed ecotypes. One-way ANOVA was run for the significant interactions between P treatments and giant reed ecotypes. Separation of means was done by post-hoc test (Tukey's test), and significant differences were accepted at

the levels $p < 0.05$, 0.01, and 0.001. The data were presented as mean ± SD (standard deviation).

3. Results

3.1. Phosphorus content

3.1.1. Uptake and buildup of P in different tissues of giant reed

The uptake and buildup of P in giant reed tissues significantly varied according to the giant reed ecotype, plant tissues, and the concentration of applied P (Table 1). The BL ecotype displayed the highest P content in culm (170.7 µg g⁻¹) and leaf blade (204.9 µg g⁻¹) tissues, whereas the ESP ecotype exhibited the highest P content in the root system (77.5 µg g⁻¹). The results also showed that the highest P content was measured in the leaf blade and followed by culm tissue, while the root system possessed the lowest P content, regardless of the giant reed ecotype. The P content in the leaf blade was almost three times higher than that of the root system in all three ecotypes. Regardless of the ecotype, the P contents in the root, culm, and leaf blade significantly increased upon the P addition up to 50 mg kg⁻¹ and then started to decline linearly. The P contents in the root, culm, and leaf blade at the 50 mg P kg⁻¹ were 116.3, 269.5, and 389.6 µg g⁻¹, respectively (Table 1).

The three giant reed ecotypes similarly responded to the applied P concentrations, where the highest P content in their different tissues was achieved at the treatment of 50 mg P kg⁻¹, except the root of STM ecotype that showed the highest P content when plants received 100 mg P kg⁻¹. The results of the interaction between giant reed ecotypes and applied P concentrations showed that the highest P content in the root (149.1 µg g⁻¹; ESP ecotype), culm (304.8 µg g⁻¹; BL ecotype), and leaf blade (426.5 µg g⁻¹; ESP ecotype) was measured at the treatment of 50 mg P kg⁻¹ (Table 1).

The buildup of P in the plant tissues varied significantly according to

Table 1
Phosphorus content in different tissues of three different giant reed ecotypes and growth medium after 6 months growing on elevated P levels.

	Plant P content (µg g ⁻¹ plant)			Growth medium P content (mg kg ⁻¹ sand)		Absorbed P (%)
	Root	Culm	Leaf blade	Total-P	Extractable-P	
Ecotype						
STM	61.9 ± 1.16 c	149.0 ± 8.3 b	181.0 ± 12.3 c	37.1 ± 3.6 b	14.8 ± 1.6 c	36.8 ± 2.3 b
BL	69.2 ± 1.89 b	170.7 ± 10.3 a	204.9 ± 15.7 a	39.5 ± 3.8 a	19.8 ± 2.3 b	32.9 ± 3.3 c
ESP	77.5 ± 1.08 a	134.0 ± 8.6 c	201.3 ± 14.5 b	29.2 ± 2.3 c	22.4 ± 2.7 a	41.1 ± 3.7 a
Phosphorus (mg kg⁻¹ sand)						
0	37.4 ± 0.92 e	36.0 ± 2.75 e	31.8 ± 3.82 e	0.0 ± 0.00 e	0.0 ± 0.00 e	0.0 ± 0.0 e
25	47.3 ± 10.05 d	103.9 ± 21.82 d	108.1 ± 15.47 d	11.1 ± 0.38 d	0.6 ± 0.52 d	55.8 ± 3.4 a
50	116.3 ± 1.89 a	269.5 ± 33.01 a	389.6 ± 54.53 a	31.9 ± 5.20 c	12.4 ± 2.23 c	36.2 ± 2.8 d
100	86.0 ± 11.62 b	197.8 ± 50.77 b	262.8 ± 57.37 b	55.0 ± 10.45 b	30.0 ± 6.09 b	45.0 ± 3.5 c
150	60.7 ± 6.54 c	149.0 ± 4.35 c	186.4 ± 29.26 c	78.6 ± 20.77 a	51.9 ± 13.35 a	47.6 ± 3.9 b
Interaction						
STM						
0	33.4 ± 0.00 d H	39.2 ± 0.00 e J	28.5 ± 0.00 e M	0.0 ± 0.00 e K	0.0 ± 0.00 d I	0.0 ± 0.0 e I
25	40.2 ± 0.21 e H	99.6 ± 2.92 d H	104.8 ± 3.57 d J	10.6 ± 0.32 d J	0.0 ± 0.00 d I	57.5 ± 1.3 a B
50	78.3 ± 4.58 b D	239.4 ± 1.97 a C	327.0 ± 0.79 a C	26.2 ± 0.30 c I	11.3 ± 0.29 c G	47.6 ± 2.1 b D
100	99.4 ± 0.66 a C	221.1 ± 2.74 b D	278.4 ± 3.14 b E	66.3 ± 0.02 b C	25.5 ± 0.44 b E	33.7 ± 1.9 d G
150	58.1 ± 0.34 c F	145.7 ± 1.24 c EF	166.3 ± 0.00 c H	82.5 ± 0.07 a B	37.1 ± 0.39 a C	45.0 ± 2.2 c E
BL						
0	40.2 ± 0.00 d H	34.7 ± 0.00 e J	31.0 ± 0.00 e LM	0.0 ± 0.00 e K	0.0 ± 0.00 e I	0.0 ± 0.0 e I
25	50.5 ± 0.07 c G	127.5 ± 5.65 d G	94.6 ± 1.42 d K	11.3 ± 0.01 d J	0.9 ± 0.17 d H	54.8 ± 3.4 a C
50	121.4 ± 0.95 a B	304.8 ± 7.58 a	415.4 ± 1.25 a B	36.4 ± 0.53 c G	15.0 ± 0.19 c F	27.3 ± 1.2 d H
100	78.3 ± 6.57 b D	232.8 ± 2.90 b CD	310.7 ± 0.51 b D	52.9 ± 0.57 b E	27.5 ± 0.22 b D	47.1 ± 3.6 b D
150	55.8 ± 2.11 c FG	153.9 ± 1.63 c E	173.0 ± 2.74 c H	97.2 ± 0.45 a	55.7 ± 0.13 a B	35.2 ± 2.8 c F
ESP						
0	38.6 ± 0.00 e H	34.2 ± 0.00 d J	36.0 ± 0.00 e L	0.0 ± 0.00 e K	0.0 ± 0.00 e I	0.0 ± 0.0 d I
25	51.1 ± 0.60 d FG	84.5 ± 0.51 c I	125.0 ± 1.57 d I	11.2 ± 0.25 d J	0.9 ± 0.11 d H	55.1 ± 4.4 b C
50	149.1 ± 0.13 a	264.3 ± 4.19 a B	426.5 ± 0.50 a	33.1 ± 0.33 c H	11.0 ± 0.22 c G	33.8 ± 3.3 c G
100	80.4 ± 2.48 b D	139.6 ± 3.40 b F	199.2 ± 0.44 c G	45.7 ± 0.22 b F	36.9 ± 0.47 b C	54.3 ± 2.9 b C
150	68.1 ± 2.22 c E	147.3 ± 9.26 b EF	220.0 ± 7.24 b F	64.2 ± 0.12 a D	60.0 ± 0.39 a	62.5 ± 5.2 a
Two-way ANOVA (F-value)						
Ecotype	166.203***	323.473***	419.596***	5425.976***	3445.137***	3325.671***
Phosphorus	1657.089***	4502.355***	28968.774***	115235.417***	67528.451***	105235.212***
Interaction	171.351***	116.697***	823.021***	3946.401***	1697.291***	2646.306***

Means in the same column within the same ecotype followed by the different lowercase letters are significant according to the Tukey's test ($p \leq 0.05$) and different uppercase letters are significant regardless of the ecotype according to the Tukey's test ($p \leq 0.05$). Data are Means ± SD and n = 3. *** denotes significance at $p \leq 0.001$.

the applied P concentration and the ecotype of the giant reed (Table 1). The STM and BL ecotypes displayed the highest absorbed P % (57.5% and 54.8%, respectively) upon fertilizing them with the lowest P concentration (25 mg P kg⁻¹). The ESP ecotype showed higher tolerance to the applied P concentration as it absorbed about 62.5% of the applied 150 mg P kg⁻¹. The efficiency of the STM and BL ecotypes to uptake P from the growth medium decreased when the applied P concentrations increased as they exhibited lower absorbed P % than 50%. The ESP ecotype showed a different response to the applied P concentrations, as it accumulated more than 50% of the applied P concentration, except for the treatment of 50 mg P kg⁻¹, which showed an absorbed P % of 33.8%.

3.1.2. Residual P in growth medium

The content of extractable and total P as the residual P fractions in the growth medium (sand) at the end of the experiment showed a significant dependence on giant reed ecotypes, regardless of the applied P concentration (Table 1). The highest residual extractable P content (22.4 mg kg⁻¹) was measured after the ESP ecotype, while the STM ecotype exhibited the lowest residual extractable P content (14.8 mg kg⁻¹). In contrast, the ESP ecotype showed the lowest residual total P content (29.2 ± 2.3 mg kg⁻¹), while the highest residual total P content (39.5 mg kg⁻¹) was measured in the growth medium of the BL ecotype. Based on the applied P concentration and the total P concentration in the growth medium at the end of the experiment, the fixation rate of P was 60.1, 49.9, and 23.3% in the growth medium of the STM, BL, and ESP ecotypes, respectively. The residual extractable and total P contents at the end of the experiment show an exposure-response relationship with the concentration of added P to giant reed plants, regardless of the giant reed ecotypes (Table 1). The residual extractable P content significantly increased from 0.6 to 51.9 mg kg⁻¹ when the applied P concentration changed from 25 to 150 mg kg⁻¹, regardless of the giant ecotype. Likewise, the residual total P content varied from 11.1 to 78.6 mg kg⁻¹ upon increasing the concentration of the applied P from 25 to 150 mg kg⁻¹ sand, respectively. The percentage of the residual extractable P significantly increased with increasing the applied P concentration, as it elevated from 2.4% to 34.6% upon increasing P concentration from 25 to 150 mg kg⁻¹, regardless of the giant reed ecotype. The giant reed ecotypes showed a considerable effect on the concentration of the residual extractable P in the growth medium at the end of the experiment. The ESP ecotype exhibited the highest residual extractable P % at all the P treatments, except for the treatment of 50 mg P kg⁻¹, where the BL ecotype displayed the highest residual extractable P % (30.0%). At the treatment of 150 mg P kg⁻¹, the residual extractable P % was 24.7%, 37.1%, and 40.0% for the STM, BL, and ESP ecotypes, respectively. The STM ecotype showed different effects, as at the lowest P treatment (25 mg kg⁻¹), the residual extractable P % was 22.6% for the STM (as the highest %), followed by 3.5% and 3.6% for the BL and ESP ecotypes, respectively.

3.1.3. Translocation and bioconcentration factors of P in giant reed ecotypes

Root-to-shoot translocation of P showed slight dependence on giant reed ecotypes, while it largely differed according to the applied concentration of P. The ESP ecotype had a translocation factor (TF) of 4.0, while the STM and BL ecotypes exhibited the same TF of 4.9, regardless of the applied concentration of P (Table S1; supplementary materials). The TF of P increased upon increasing the applied concentration of P up to 50 mg kg⁻¹ and then slightly decreased. The highest TF value (5.9) corresponded to the treatment of 50 mg P kg⁻¹; however, at the treatment of 150 mg P kg⁻¹, the TF value was 5.5, regardless of the giant reed ecotype. The results of the interaction between giant reed ecotypes and applied P concentration presented in Fig. 1 showed a different response of giant reed ecotypes to transportation of P when they grew on elevated P concentrations. The STM and BL ecotypes exhibited lower TF at the high P treatments, whereas the ESP ecotype displayed the highest TF at the highest P treatment. For instance, the highest TF value of STM (7.3)

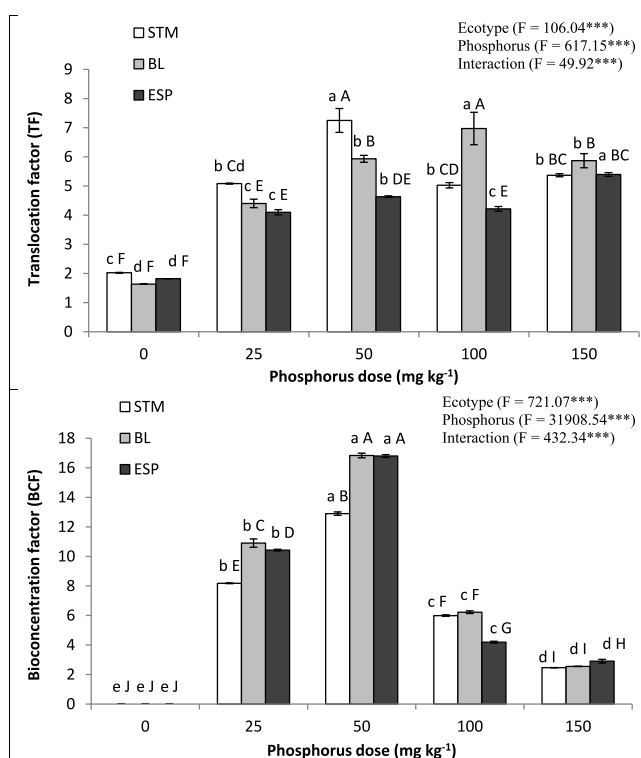


Fig. 1. Translocation (TF) and bioconcentration (BCF) factors of P in three ecotypes of giant reed grown on five elevated P concentrations (0, 25, 50, 100, and 150 mg kg⁻¹ sand) under greenhouse conditions for 6 months. Data are Means ± SD and n = 3. *** denotes significance at $p \leq 0.001$ according to the Tukey's test ($p \leq 0.05$).

corresponded to the treatment of 50 mg P kg⁻¹, while BL and ESP ecotypes possessed the highest TF 7.0 and 5.4 at the treatments of 100 and 150 mg P kg⁻¹, respectively. The BL ecotype exhibited the highest bioconcentration factor (BCF; 7.3), followed by the ESP ecotype (6.9), while the STM showed the lowest BCF (5.9), regardless of the applied P concentration. The BCF of the giant reed plant, regardless of its ecotypes, increased upon increasing the concentration of applied P up to 50 mg kg⁻¹ and then drastically decreased. For example, BCF decreased from 15.5 to 2.6 when the concentration of applied P increased from 50 to 150 mg P kg⁻¹, respectively. The three giant reed ecotypes displayed a similar response to the elevated P concentrations, where BCF values significantly increased with increasing the concentration of added P from 25 to 50 mg kg⁻¹, followed by a sharp reduction resulting in lower values than those measured at the treatments of 25 and 50 mg P kg⁻¹ (Fig. 1). The STM, BL, and ESP ecotypes had their highest BCA values of 12.9, 16.8, and 16.8, respectively, at the treatment of 50 mg P kg⁻¹.

3.2. Plant biometrics

3.2.1. Dry matter content

Dry matter content varied significantly according to giant reed ecotypes and applied P concentrations (Fig. 2 and Fig. S1; supplementary materials). The highest root (14.3%), culm (45.4%), and leaf blade (37.3%) dry matter contents corresponded to the BL, STM, and ESP ecotypes, respectively, regardless of the applied P concentration. On the other hand, applying P at the rate of 150 mg kg⁻¹ soil resulted in the highest root (15.1%) and culm (46.0%) dry matter contents, while the highest leaf blade dry matter content (39.2%) belonged to the treatment of 25 mg P kg⁻¹ soil. The highest root, culm, and leaf blade dry matter contents of the STM ecotype were 14.7, 54.2, and 37.6% at the treatments of 150, 50, and 25 mg P kg⁻¹ soil, respectively. Otherwise, the BL ecotype had its highest root (15.7%), culm (50.7%), and leaf blade

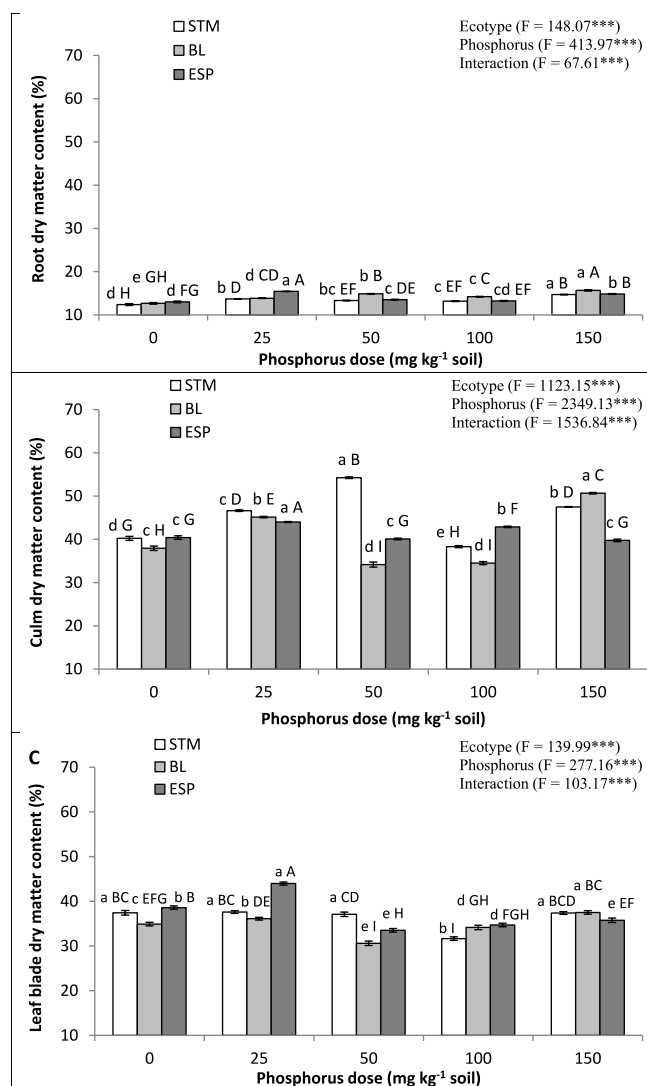


Fig. 2. Dry matter content of three giant reed ecotypes grown on different P concentrations (0, 25, 50, 100, and 150 mg kg⁻¹ sand) under greenhouse conditions for 6 months; (A) root; (B) culm; and (C) leaf blade. Different lowercase letters on the same bars of the same ecotype are significant according to the Tukey's test ($p \leq 0.05$) and different uppercase letters are significant regardless of the ecotype according to the Tukey's test ($p \leq 0.05$). Data are Means \pm SD and $n = 3$. *** denotes significance at $p \leq 0.001$.

(37.5%) dry matter contents at the treatment of 150 mg P kg⁻¹ soil. Similarly, treating the ESP plants with 25 mg P kg⁻¹ soil resulted in the highest dry matter content of root (15.4%), culm (44.0%), and leaf blade (44.0%). Considering the interaction between P treatments and giant reed ecotypes, results revealed that treating the ESP ecotype with 25 mg P kg⁻¹ soil was the best combination.

3.2.2. Vegetative characteristics of giant reed ecotypes under elevated P concentrations

The ESP ecotype had a deeper root system than the STM and BL ecotypes, regardless of the concentration of applied P; however, the differences in root length among giant reed ecotypes are statistically insignificant. The control plants possessed a significantly longer root system (36.9 cm) compared to treated plants with elevated P concentrations; no significant differences were statistically measured among P treatments, regardless of the giant reed ecotype (Table 2).

Shoot length significantly varied among giant reed ecotypes; the ESP ecotype showed the tallest shoot system (50.4 cm), while the BL ecotype

had the shortest shoot system (33.6 cm). All P treatments exhibited almost two times significantly higher shoot length than control plants (received no P); however, the differences between the applied P concentrations were insignificant (Table 2).

Similar to shoot length results, the ESP ecotype displayed the longest 2nd basal internode (4.3 cm), followed by the STM (3.3 cm) and BL (2.9 cm) ecotypes. The control plants exhibited the lowest significant length (2.3 cm) of the 2nd basal internode compared to those treated with P, regardless of the P concentration (Table 2). Plants that grew on 50 mg P kg⁻¹ possessed a 2nd basal internode length of 3.4 cm; however, it increased to 4.1 cm when plants received 100 mg P kg⁻¹. The BL and ESP ecotypes displayed their longest 2nd basal internode length of 3.6 and 5.1 cm at the treatment of 150 mg P kg⁻¹. The longest 2nd basal internode length of the STM ecotype (4.1 cm) corresponded to the treatment of 25 mg P kg⁻¹.

Giant reed ecotypes displayed almost the same volume of the root system, where the differences were insignificant (Table 2). Treating giant reed plants with P significantly increased root volume by 166–228% compared to control plants; nevertheless, P treatments showed insignificant differences. Growing giant reed plants on different P concentrations improved the development of the root system, recording significantly higher volumes than the control, regardless of the giant reed ecotypes. The giant reed ecotypes differed in their P uptake capacity as the STM ecotype showed the highest root volume (11.0 cm³) at the treatment of 25 mg P kg⁻¹, while the BL and ESP ecotypes revealed the highest root volume (11.5 and 9.9 cm³, respectively) by receiving 50 and 100 mg P kg⁻¹, respectively.

Similar to root volume, the shoot volume did not significantly vary among giant reed ecotypes. The application of P significantly improved the shoot volume. For example, plants fertilized with 25 mg P kg⁻¹ had about 10-fold higher shoot volume than the control. The highest shoot volume of the STM and ESP ecotypes were 12.5 and 11.2 cm³ and measured at the treatment of 25 mg P kg⁻¹, whereas the BL ecotype recorded its highest shoot volume (9.5 cm³) under 50 mg kg⁻¹ of P.

The number of new tillers of giant reed plants noticeably varied among the giant reed ecotypes. The BL ecotype exhibited a significantly higher number of new tillers (3.4) than the STM (3.0) and ESP (2.9) ecotypes. Treated plants with P possessed a higher number of new tillers than the control; however, increasing P concentration from 25 to 150 mg kg⁻¹ did not result in significant differences. Each ecotype revealed its highest number of new tillers at different P concentrations. The STM ecotype had the highest number of new tillers (4.2) at the treatment of 25 mg P kg⁻¹, while the treatment of 150 mg P kg⁻¹ resulted in the greatest number of new tillers of the BL (4.0) and ESP (3.3) ecotypes.

The number of leaves changed from 8.4 (for control plants) to 10.4 (for plants that received 50 mg P kg⁻¹); however, the differences were not significant. Similarly, giant reed ecotypes showed a number of leaves ranging from 9.6 to 10.2 with insignificant differences. The STM and BL ecotypes displayed the highest number of leaves (10.7 and 10.1) at the treatment of 25 mg P kg⁻¹, while the ESP ecotype treated with 50 mg P kg⁻¹ exhibited the highest number of leaves compared with the other P treatments.

Likewise, the number of internodes did not significantly vary among giant reed ecotypes. Control plants had a lower number of internodes (7.4) than P-treated plants. The 100 mg P kg⁻¹ treatment showed the highest number of internodes (9.5). The greatest number of internodes of the STM (9.7) and BL (9.1) ecotypes was counted when plants received 25 mg P kg⁻¹. The ESP ecotype showed its greatest number of internodes (10.3) when it grew on 50 mg P kg⁻¹.

3.3. Photosynthesis and CO₂ assimilation of giant reed ecotypes

3.3.1. Efficiency of photosynthesis

The Fv/Fm ratio, as a characteristic of the photochemical activity and structural dynamics of PSII, showed insignificant dependence on giant reed ecotypes or P concentrations (Fig. 4). The BL ecotype had an

Table 2

Vegetative traits of three different giant reed ecotypes (STM, BL, ESP) grown on five elevated P concentrations (mg kg⁻¹).

	Length (cm)			Volume (cm ³)		Number of			
	Shoot	Root	2nd internode	Shoot	Root	Internodes	Leaves	New tillers	
Ecotype									
STM	40.7 ± 9.55 b	22.1 ± 2.98 a	3.3 ± 3.22 b	8.2 ± 0.91 a	8.5 ± 4.14 a	8.7 ± 1.05 a	9.7 ± 1.05 a	3.0 ± 0.99 b	
BL	33.6 ± 7.51 c	24.1 ± 3.11 a	2.9 ± 2.90 b	6.7 ± 0.59 a	8.2 ± 2.75 a	8.6 ± 0.63 a	9.6 ± 0.64 a	3.4 ± 0.38 a	
ESP	50.4 ± 14.07 a	25.4 ± 1.96 a	4.3 ± 2.92 a	7.2 ± 0.87 a	7.9 ± 3.70 a	9.3 ± 1.15 a	10.2 ± 1.06 a	2.9 ± 0.67 b	
Phosphorus (mg kg⁻¹ sand)									
0	23.4 ± 2.63 b	36.9 ± 2.87 a	2.3 ± 0.32 b	1.9 ± 0.51 d	3.2 ± 0.25 b	7.4 ± 0.40 b	8.4 ± 0.40 b	2.1 ± 0.81 b	
25	46.3 ± 9.14 a	19.7 ± 2.93 b	3.9 ± 1.51 a	10.6 ± 0.82 ab	9.6 ± 2.32 a	9.2 ± 0.46 a	10.2 ± 0.46 a	3.3 ± 0.06 a	
50	45.5 ± 9.78 a	17.9 ± 1.99 b	3.4 ± 1.00 a	9.5 ± 0.79 ab	10.5 ± 0.50 a	9.4 ± 0.81 a	10.4 ± 0.81 a	3.5 ± 0.62 a	
100	47.4 ± 10.30 a	22.2 ± 1.64 b	4.1 ± 0.80 a	7.0 ± 0.70 c	9.1 ± 0.29 a	9.5 ± 0.72 a	10.3 ± 0.44 a	3.3 ± 0.31 a	
150	45.3 ± 11.29 a	22.6 ± 3.45 b	3.9 ± 1.31 a	8.0 ± 1.08 bc	8.5 ± 2.63 a	9.0 ± 0.62 a	10.0 ± 0.76 a	3.4 ± 0.56 a	
Interaction									
STM	0	24.5 ± 0.71 d FG	36.2 ± 2.55 a AB	1.9 ± 0.17 c F	1.9 ± 0.72 d CD	3.0 ± 0.79 b C	6.9 ± 0.82 b C	7.9 ± 0.82 b C	1.5 ± 0.37 c D
	25	48.2 ± 2.51 a	17.5 ± 1.78 b C	4.1 ± 0.42 a	12.5 ± 1.77 a	11.0 ± 1.37 a	9.7 ± 1.32 a AB	10.7 ± 1.32 a	3.4 ± 0.42 ab
	50	40.9 ± 2.75 c CD	17.5 ± 1.61 b C	3.7 ± 0.42 ab	9.0 ± 1.37 bc AB	10.5 ± 2.74 a	8.9 ± 1.02 a ABC	9.9 ± 1.02 a	4.2 ± 0.57 a
	100	47.1 ± 3.65 ab	20.3 ± 1.69 b C	4.0 ± 0.73 a	6.8 ± 1.35 c BCD	8.3 ± 1.15 a ABC	9.0 ± 1.06 a ABC	10.0 ± 1.06 a	3.2 ± 0.57 b
	150	42.9 ± 2.27 bc	18.9 ± 1.24 b C	3.0 ± 0.47 b	11.0 ± 1.37 ab	9.5 ± 1.12 a AB	8.8 ± 0.76 ab	9.8 ± 0.76 ab	2.9 ± 0.42 b
BL	0	20.4 ± 2.77 b G	28.4 ± 2.19 a BC	2.2 ± 0.42 b EF	2.2 ± 0.27 b CD	3.6 ± 0.65 b BC	7.6 ± 0.42 a BC	8.6 ± 0.42 a BC	3.0 ± 1.17 a
	25	36.4 ± 3.21 a DEF	23.0 ± 2.85 a BC	3.0 ± 0.88 ab	8.0 ± 2.74 a AB	8.0 ± 2.09 ab	9.1 ± 1.14 a ABC	10.1 ± 1.14 a	3.3 ± 0.27 a
	50	38.8 ± 8.55 a DE	20.1 ± 2.61 a C	2.5 ± 0.59 ab DEF	9.5 ± 4.11 a AB	11.5 ± 5.48 a	9.9 ± 1.02 a ABC	9.9 ± 1.02 a	3.3 ± 0.45 a
	100	37.2 ± 3.91 a DEF	23.4 ± 1.98 a BC	3.4 ± 0.86 a	7.3 ± 2.77 a ABC	9.2 ± 3.72 ab	9.1 ± 1.19 a ABC	10.1 ± 1.19 a	3.6 ± 0.42 a
	150	35.4 ± 3.07 a DEF	25.7 ± 1.48 a BC	3.6 ± 0.41 a	6.4 ± 1.08 ab	8.9 ± 1.08 ab	8.5 ± 0.61 a ABC	9.4 ± 0.55 a	4.0 ± 0.94 a
ESP	0	25.3 ± 2.97 b EFG	46.1 ± 2.91 a	2.9 ± 0.52 b DEF	1.7 ± 0.84 b D	3.1 ± 0.74 b C	7.6 ± 0.37 b BC	8.6 ± 0.37 b BC	1.7 ± 0.32 b
	25	54.4 ± 11.4 a ABC	18.5 ± 1.75 b C	4.6 ± 1.13 ab ABC	11.2 ± 6.03 a AB	9.8 ± 4.96 a	8.8 ± 0.97 ab	9.8 ± 0.97 ab	3.3 ± 0.57 a
	50	56.7 ± 3.56 a AB	16.2 ± 1.57 b C	4.0 ± 0.35 ab	10.0 ± 0.00 a AB	9.5 ± 2.74 a AB	10.3 ± 1.86 a	11.3 ± 1.86 a	3.0 ± 0.00 a
	100	57.8 ± 7.29 a	22.8 ± 3.27 b BC	4.8 ± 1.46 a AB	6.8 ± 1.60 ab	9.9 ± 2.88 a	10.3 ± 1.15 a	10.8 ± 1.04 ab	3.0 ± 0.61 a
	150	57.6 ± 15.7 a	23.3 ± 2.28 b BC	5.1 ± 0.91 a	6.5 ± 2.24 ab	7.0 ± 2.74 ab	9.7 ± 1.96 ab	10.7 ± 1.96 ab	3.3 ± 0.75 a
Two-way ANOVA (F-value)									
Ecotype		43.969***	1.488 (ns)	22.554***	2.702 (ns)	0.312 (ns)	3.036 (ns)	2.335 (ns)	6.657**
Phosphorus		38.910***	18.275***	14.938***	28.893***	16.682***	8.850***	8.459***	15.851***
Interaction		2.115*	2.466*	1.888 (ns)	2.049 (ns)	0.892 (ns)	1.154 (ns)	1.083 (ns)	3.849***

Means in the same column within the same ecotype followed by the different lowercase letters are significant according to the Tukey's test ($p \leq 0.05$) and different uppercase letters are significant regardless of the ecotype according to the Tukey's test ($p \leq 0.05$). Data are Means \pm SD and $n = 3$. ***, **, and \times denote significance at $p \leq 0.001$, 0.01 , and 0.05 , respectively. ns denotes insignificant at $p \leq 0.05$.

Fv/Fm ratio of 0.812, followed by the ESP (0.810) and STM (0.806) ecotypes, regardless of the concentration of applied P. Control plants revealed a higher Fv/Fm ratio (0.825) than giant reed plants treated with different P concentrations; however, this increase was insignificant. The STM ecotype displayed its highest Fv/Fm ratio (0.830) at the treatment of 150 mg P kg⁻¹. Treating the ESP plants with 100 mg P kg⁻¹ resulted in the highest Fv/Fm ratio (0.830). The BL plants showed an Fv/Fm ratio of 0.831 when they received zero P. **3.3.2. Efficiency of CO₂ assimilation.**

Assimilation of CO₂ into giant reed leaves significantly depended on the giant reed ecotypes and the concentration of added P (Fig. 4). The BL ecotype displayed a significantly higher CO₂ assimilation rate (12.46 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) than the STM (9.76 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and ESP (8.46 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) ecotypes. Applying P to giant reed plants, regardless of plant ecotype, significantly increased the rate of leaf CO₂ assimilation. All treated plants with P unveiled higher rates of CO₂ assimilation than control plants. Increasing P concentration resulted in a significant and gradual increase in the assimilation rate of CO₂, where the highest CO₂ assimilation rate (11.78 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$)

corresponded to the treatment of 150 mg P kg⁻¹. The highest CO₂ assimilation rate (14.55 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in leaves of the STM ecotype linked to the treatment of 100 mg P kg⁻¹, while the BL ecotype displayed its highest rate of CO₂ assimilation (16.33 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) when plants grew on the 50 mg P kg⁻¹. Growing the ESP plants on 150 mg P kg⁻¹ displayed the highest CO₂ assimilation rate (13.77 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

3.4. Biochemical changes in giant reed plants under elevated P concentrations

3.4.1. Buffer-soluble protein content

Culm and leaf blade tissues showed more than two times higher buffer-soluble protein content than the root system. Also, control plants (no added P) illustrated significantly higher content of buffer-soluble protein in the root, culm, and leaf blade than the P-treated plants. Giant reed ecotypes and concentration of the applied P significantly affected the content of buffer-soluble protein in different plant tissues (Table 3). The ESP ecotype showed the highest root buffer-soluble

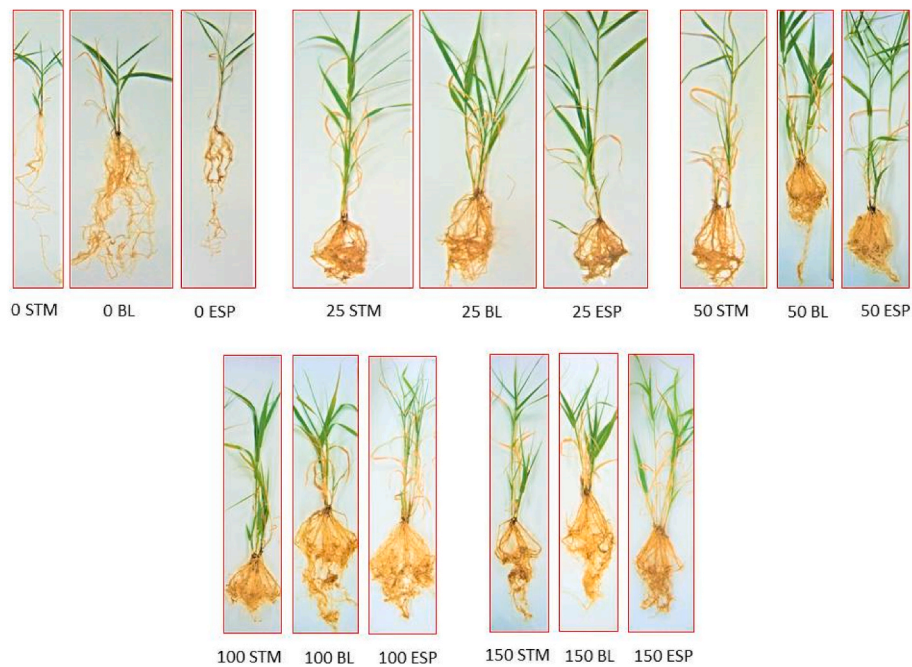


Fig. 3. Morphological features of three giant reed ecotypes (STM, BL, and ESP) grown on elevated P concentrations (0, 25, 50, 100, and 150 mg kg⁻¹) under greenhouse conditions for 6 months.

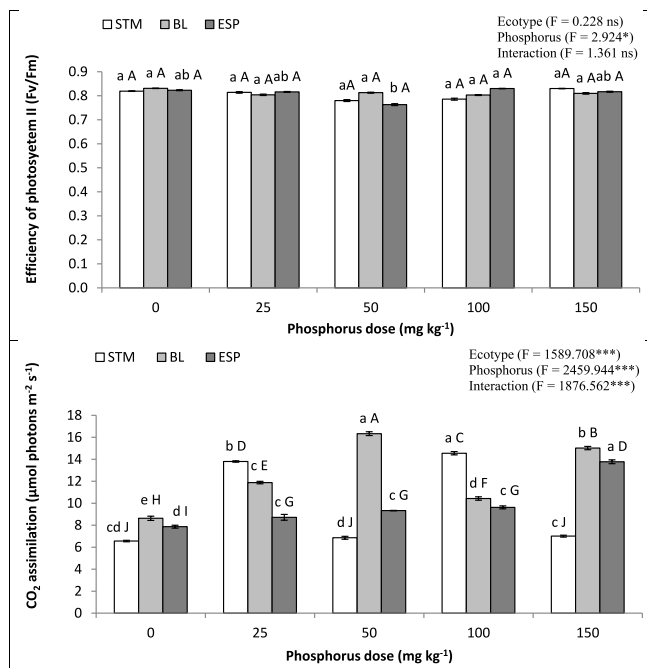


Fig. 4. Efficiency of photosystem II (Fv/Fm: A) and CO₂ assimilation (B) of three giant reed ecotypes grown on different P concentrations (0, 25, 50, 100, and 150 mg kg⁻¹ sand) under greenhouse conditions for 6 months. Different lowercase letters on the same bars of the same ecotype are significant according to the Tukey's test ($p \leq 0.05$) and different uppercase letters are significant regardless of the ecotype according to the Tukey's test ($p \leq 0.05$). Data are Means \pm SD and $n = 3$. *** and \times denote significance at $p \leq 0.001$ and 0.05 , respectively. ns denotes insignificant differences at $p \leq 0.05$.

protein content (19.9 mg g⁻¹), while the BL ecotype clarified the highest buffer-soluble protein content in the culm (42.1 mg g⁻¹) and leaf blade (51.0 mg g⁻¹). The buffer-soluble protein content of the root, culm, and leaf blade of control (received no P) was almost 2-fold higher than the P-

treated plants. For example, root buffer-soluble protein content at the treatment of 100 mg P kg⁻¹ was 15.8 mg g⁻¹ (the highest buffer-soluble protein content among treated plants with P), while control plants exhibited a root buffer-protein content of 31.5 mg g⁻¹. Treated plants with 150 mg P kg⁻¹ had a culm buffer-soluble protein content of 38.7 mg g⁻¹, whereas control plants possessed 48.9 mg g⁻¹. Likewise, the leaf blade buffer-soluble protein content of control plants was 67.9 mg g⁻¹, while the highest content of treated plants with P was 44.4 mg g⁻¹ and corresponded to the treatment of 100 mg P kg⁻¹. All three giant reed ecotypes showed their highest buffer-soluble protein content of root, culm, and leaf blade when they received no P (control plants). Applying P to giant reed plants significantly reduced the amount of buffer-soluble protein in the root, culm, and leaf blade tissues compared to the control.

3.4.2. POD activity

The activity of peroxidase (POD, EC 1.11.1.7) in different plant tissues, i.e., root, culm, and leaf blade, significantly varied among giant reed ecotypes and P treatments. The STM ecotype displayed significantly higher root POD activity (2.27 U mL⁻¹ min⁻¹ g⁻¹ DM) than the BL (2.12 U mL⁻¹ min⁻¹ g⁻¹ DM) and ESP (1.35 U mL⁻¹ min⁻¹ g⁻¹ DM) ecotypes. Treating plants with P significantly induced the root POD activity, where all P-treated plants exhibited higher POD activity in the root system than control plants. Applying P at the rate of 50 mg kg⁻¹ resulted in the highest POD activity (2.84 U mL⁻¹ min⁻¹ g⁻¹ DM) in the root system (Table 3). The STM and BL ecotypes exhibited their highest POD activity in the root system when they received 50 mg P kg⁻¹, recording 3.86 and 3.36 U mL⁻¹ min⁻¹ g⁻¹ DM, respectively, while the ESP ecotype revealed the highest root POD activity (1.98 U mL⁻¹ min⁻¹ g⁻¹ DM) at the treatment of 150 mg P kg⁻¹. The STM ecotype also showed the highest significant POD activity in culm (1.41 U mL⁻¹ min⁻¹ g⁻¹ DM) compared to 1.25 U mL⁻¹ min⁻¹ g⁻¹ DM for the ESP and 1.12 U mL⁻¹ min⁻¹ g⁻¹ dry mass for the BL ecotype.

Similarly, the highest POD activity in the leaf blade of giant reed ecotypes (1.34 U mL⁻¹ min⁻¹ g⁻¹ DM) corresponded to the control plants and those treated with the lowest P concentration (25 mg kg⁻¹). High P concentrations significantly reduced POD activity. The ESP ecotype exhibited the highest POD activity (1.23 U mL⁻¹ min⁻¹ g⁻¹ DM) in the culm tissue compared to the STM and BL ecotypes. The STM and

Table 3

Peroxidase activity and buffer-soluble protein content in different tissues of three giant reed ecotypes (STM, BL, and ESP) under elevated P concentrations (0, 25, 50, 100, and 150 mg kg⁻¹).

	POD activity (U mL ⁻¹ min ⁻¹ g ⁻¹ dry mass)			Buffer-soluble protein content (mg g ⁻¹)		
	Root	Culm	Leaf blade	Root	Culm	Leaf blade
Ecotype						
STM	2.27 ± 1.21 a	1.41 ± 0.43 a	1.09 ± 0.26 b	15.6 ± 10.60 b	38.0 ± 8.94 b	41.7 ± 18.39 c
BL	2.12 ± 0.93 b	1.12 ± 0.22 c	0.85 ± 0.31 c	15.8 ± 7.83 b	42.1 ± 6.37 a	51.0 ± 13.80 a
ESP	1.35 ± 0.36 c	1.25 ± 0.30 b	1.23 ± 0.61 a	19.9 ± 7.12 a	33.7 ± 10.80 c	42.6 ± 14.63 b
Phosphorus (mg kg⁻¹ sand)						
0	0.93 ± 0.19 e	1.34 ± 0.27 a	1.34 ± 0.27 a	31.5 ± 2.66 a	48.9 ± 2.61 a	67.9 ± 5.86 a
25	2.05 ± 0.74 c	1.41 ± 0.29 a	1.34 ± 0.62 a	15.0 ± 5.58 c	34.0 ± 6.47 d	33.9 ± 8.76 e
50	2.84 ± 1.36 a	1.31 ± 0.67 a	1.04 ± 0.48 b	11.9 ± 2.68 d	35.3 ± 10.72 c	37.8 ± 16.37 d
100	1.43 ± 0.17 d	1.15 ± 0.13 b	0.78 ± 0.10 c	15.8 ± 3.08 b	32.8 ± 6.70 e	44.4 ± 3.85 b
150	2.31 ± 0.53 b	1.08 ± 0.15 b	0.78 ± 0.26 c	11.1 ± 2.65 e	38.7 ± 9.99 b	41.6 ± 12.28 c
Interaction						
STM						
0	0.71 ± 0.03 e H	1.64 ± 0.11 b B	1.17 ± 0.03 b CDE	34.3 ± 0.14 a	51.6 ± 0.47 a	70.7 ± 1.07 a
25	2.25 ± 0.11 c D	1.28 ± 0.15 c	0.84 ± 0.04 c EFG	11.4 ± 0.46 b I	38.7 ± 0.58 b E	41.7 ± 0.82 c G
50	3.86 ± 0.20 a	2.04 ± 0.04 a	1.48 ± 0.18 a BC	11.5 ± 0.39 b I	33.9 ± 0.75 c G	23.6 ± 0.23 e K
100	1.59 ± 0.15 d E	1.06 ± 0.04 c CDE	0.89 ± 0.03 c DEF	12.4 ± 0.27 b HI	38.7 ± 0.26 b E	44.3 ± 0.26 b F
150	2.93 ± 0.13 b C	1.01 ± 0.17 c DE	1.08 ± 0.09 bc DE	8.2 ± 0.56 c K	27.2 ± 0.30 d H	28.4 ± 0.39 d J
BL						
0	1.05 ± 0.06 e GH	1.11 ± 0.05 a CDE	1.20 ± 0.33 a CD	29.0 ± 0.42 a C	48.6 ± 0.67 a B	71.9 ± 0.66 a
25	2.68 ± 0.26 b C	1.21 ± 0.06 a CDE	1.15 ± 0.07 a CDE	12.1 ± 0.17 c I	36.6 ± 0.22 d F	35.5 ± 0.31 e H
50	3.36 ± 0.15 a B	0.74 ± 0.03 b F	0.53 ± 0.02 c G	9.5 ± 0.35 d J	46.6 ± 0.33 b C	55.7 ± 0.16 b C
100	1.45 ± 0.08 d EF	1.29 ± 0.08 a C	0.74 ± 0.06 bc FG	16.6 ± 0.27 b F	34.1 ± 0.17 e G	48.2 ± 0.24 c E
150	2.04 ± 0.02 c D	1.25 ± 0.10 a CD	0.63 ± 0.03 c FG	11.7 ± 0.21 c I	44.5 ± 0.26 c D	43.6 ± 0.11 d F
ESP						
0	1.02 ± 0.03 c GH	1.29 ± 0.06 b C	1.65 ± 0.11 b B	31.3 ± 0.48 a B	46.4 ± 0.36 a C	61.2 ± 0.36 a B
25	1.23 ± 0.03 b FG	1.75 ± 0.01 a B	2.04 ± 0.08 a	21.4 ± 0.48 b D	26.6 ± 0.37 c HI	24.4 ± 0.27 e K
50	1.29 ± 0.04 b EFG	1.14 ± 0.07 c CDE	1.12 ± 0.07 c DE	14.8 ± 0.08 d G	25.3 ± 0.21 d J	34.1 ± 0.19 d I
100	1.26 ± 0.04 b EFG	1.09 ± 0.06 cd CDE	0.71 ± 0.06 d FG	18.4 ± 0.35 c E	25.5 ± 0.35 d IJ	40.5 ± 0.41 c G
150	1.98 ± 0.14 a D	0.98 ± 0.02 d EF	0.63 ± 0.02 d FG	13.4 ± 0.24 e H	44.5 ± 0.46 b D	52.7 ± 0.15 b D
Two-way ANOVA (F-value)						
Ecotype	249.200***	44.590***	44.030***	705.135***	1503.135***	1914.986***
Phosphorus	347.328***	25.378***	56.616***	4997.367***	2137.152***	7822.740***
Interaction	81.442***	58.766***	34.572***	197.917***	926.574***	1513.919***

Means in the same column within the same ecotype followed by the different lowercase letters are significant according to the Tukey's test ($p \leq 0.05$) and different uppercase letters are significant regardless of the ecotype according to the Tukey's test ($p \leq 0.05$). Data are Means ± SD and n = 3. *** denotes significance at $p \leq 0.001$.

ESP ecotypes showed their highest POD activity in the culm tissues (1.48 and 2.04 U mL⁻¹ min⁻¹ g⁻¹ DM, respectively) at the treatments of 50 and 25 mg P kg⁻¹, while the control plants of the BL ecotype had the highest culm POD activity (1.20 U mL⁻¹ min⁻¹ g⁻¹ DM).

4. Discussion

Recently, with great interest in expanding the cultivation of biomass crops, the underlying aim is to combine high yield with low production inputs, especially nutrients. Therefore, determining the appropriate fertilization management practices is a cornerstone in the success and sustainability of biomass crops cultivation in long-term plantations.

Deprivation of P in the growth medium leads to noticeable changes in root architecture and a higher root/shoot ratio. Upon the depletion of P in the rhizosphere region, the root system expands, scavenging the soil for P [1]. Our results revealed that the root structure of the giant reed markedly changed according to the P level in the growth medium. Control plants (received no P) possessed a root system distinguished with long axial roots with very few lateral branches. In contrast, fertilized plants with P had a typical fibrous root system characterized by many lateral roots (Fig. 3). The three giant reed ecotypes showed almost similar root architecture. The ESP ecotype, however, displayed the longest root system (25.4 cm), while the highest volume of the root system (8.5 cm³) corresponded to the STM ecotype (Table 2). Plants growing under P deficiency conditions tend to rationalize energy and carbon expenditures by reducing the number of lateral roots and diminishing the secondary growth of stele and aerenchyma in the cortex layer, producing thinner and more elongated axial roots [49]. Another possible interpretation of thinner roots of plants grown in P-deficient soils is the greater growth rate of the root system that aims at exploring more P [1]. On the contrary, in soils replete with P, monocots such as

giant reed possess many lateral roots in the upper part of the root system where higher P concentration is found in the upper layer of the soil profile; consequently, a decline in primary root development occurs [50]. Maize plants that received no or low P fertilization possess thinner and more elongated root systems [49]. Changes in root traits according to the P application are supported by other results of the present study. For instance, all P-treated plants had shorter root systems and three times higher root volume than control plants. The treatment of 50 mg P kg⁻¹ showed the shortest root length and highest root volume, regardless of the giant reed ecotype. Similarly, Richardson et al. [51] documented an increase in root length and a decline in the number of lateral roots of maize and common bean grown under P-depleted growth conditions. Moreover, a positive correlation (Table S2; supplementary materials) between the density of soybean root hair and soil P concentration was documented by Wang et al. [52].

Control plants of the STM, BL and ESP ecotypes showed higher root/shoot ratios than P-treated plants, regardless of the concentration. This finding supports the earlier results of Vance et al. [53], who cited a typical increase in root/shoot ratio of plants grown under P-depleted conditions. Two-times increase in the root/shoot ratio was reported by Aziz et al. [54] when rice plants were fertilized with 26 μM P compared with control plants (260 μM). The root/shoot ratios of the STM, BL, and ESP control plants were 2.0, 2.1, and 2.0, respectively, and they are the highest ratios among all treatments. Higher P levels above 50 mg kg⁻¹ (i.e., 100 and 150 mg kg⁻¹) exhibited the lowest root/shoot ratios; however, the lowest root/shoot ratio in all three ecotypes corresponded to the treatment of 100 mg P kg⁻¹. The increased root/shoot ratio of giant reed plants grown under P-deficiency conditions may be ascribed to the diminished growth of the aboveground part, which was distinguished by short culm and fewer leaves [1]. Moreover, starch synthesis in leaves increases in response to low P levels in the rhizosphere area, and it is

translocated via phloem into the root system, leading to a higher root/shoot ratio [55]. Supporting results were cited by Cocozza et al. [56] in their study on the impact of P on giant reed growth. They reported that starch content in leaves of plants that grew in the absence of P was $4.8 \text{ mg g}^{-1} \text{ DW}$, while in the presence of $8.0 \text{ mM KH}_2\text{PO}_4$, the starch content decreased to $1.8 \text{ mg g}^{-1} \text{ DW}$. Another suggested mechanism for the increased root/shoot ratio is the suppressed cytokinin production under low P conditions leading to improved root growth and declined shoot development [57]. Also, strigolactones, secreted hormones/phytohormones from plant roots, were reported to reduce the branching mechanism of the shoot part under P stress upon the translocation from root to shoot part [58]. The findings of the present study supported these mechanisms. Control plants displayed a 50% reduction in the length of culm compared with the P-fertilized plants (Table 2). Also, the volume of the aboveground part of control plants is at least four times lower than the treated plants with P; however, plants that received 25 mg P kg^{-1} revealed about a 5-fold higher increase in shoot volume (Table 2). An increase of 20 cm in shoot height of giant reed fertilized with N in pots was documented by Quinn et al. [59]. Likewise, giant reed plants that grew under open field conditions and received 200, 80, and 200 kg ha^{-1} of N, P, and K, respectively, revealed an increase of 8.3% in plant height compared to control plants. Control STM, BL, and ESP plants also exhibited the lowest number of leaves, internodes, and new tillers compared to P-treated plants (Table 2).

Sagehashi et al. [37], in their outdoor hydroponic experiments about P uptake by giant reed using radioactive P (P-32), documented that absorbed P accumulates first in the giant reed rhizomes and later transports to the aboveground tissues according to plant needs. Moreover, they noticed that P content in leaves varies according to the position of the leaf on the plant culm, where basal leaves showed lower P content than the topmost leaves. In the present study, P uptake showed a significant dependence on the plant ecotype and the concentration of applied P, in addition to different plant parts. The leaf blade displayed the highest P content, followed by the culm tissue, while the root system (no rhizomes) revealed the lowest P content. Also, P content in plants, regardless of plant ecotype, significantly increased upon applying P to giant reed plants up to 50 mg P kg^{-1} and then gradually declined with increasing the concentration of applied P. These results are in contrast to those reported earlier by Kawazoe et al. [60], who cited that 60% or more of absorbed P by giant reed plants accumulated in the rhizomes. This may be attributed to the absence of rhizomes, where giant reed plants used in the current study were somatic embryo-derived seedlings with fibrous root systems. This may facilitate the direct and active transportation of absorbed P from the root to the aboveground part, where root hairs, root tips, and the outer layer of root cells are the main gates where P enters the root system [1]. The BL ecotype showed the highest P content (375.6 mg kg^{-1}) in the aboveground part (culm and leaf blade), while the ESP ecotype displayed the highest P content (77.5 mg kg^{-1}) in the root system (Table 1). The STM plants exhibited the lowest P content in the root (61.9 mg kg^{-1}) and aboveground (330 mg kg^{-1}) parts. Kawazoe et al. [60] revealed that the uptake and accumulation of P largely depended on the age and weight of giant reed seedlings. Old seedlings (several-year-old) displayed lower P content than young ones (one-year-old). The explanation is that old seedlings possess large rhizomes that contain more P than young seedlings, and thus it affects the uptake of P. Also, seedlings with a weight of $100\text{--}200 \text{ g plant}^{-1}$ revealed higher P content compared to those with $300\text{--}400 \text{ g plant}^{-1}$. This may explain the high P content in giant reed plants despite the experiment continuing for four months.

The establishment of giant reed farms is critical because the seeds of giant reed are infertile (not viable) in most countries. Therefore, vegetative propagation emerged as an effective method for establishing reed farms due to the high sprouting ability of the stem and rhizome of the giant reed [61]. Somatic embryogenesis proved its efficiency as a new biotechnological method for the vegetative propagation of giant reed. Micro-propagated seedlings (somatic embryo-derived plantlets) have

many advantages, including a fast supply of cost-effectively priced propagules, pathogen-free, low production cost, precise control of plant density in the farm, ease of mechanized plantation, ease of mechanized harvesting due to the uniformity of the plants [39], in addition to being ideal for studying nutrient uptake dynamics due to its fibrous roots. Consequently, the present study aimed to highlight the response of somatic embryo-derived plantlets of giant reed to P fertilization, especially in the first year of cultivation, since it has advantages over rhizome-derived seedlings.

Phosphorus content in crops generally ranges between 0.1% and 0.5% [1]. The uptake of P in the present study showed heavy dependence on the rate of applied P, where plants grown at higher P concentrations (i.e., 50 mg L^{-1}) exhibited higher P accumulation. Phosphorus uptake and accumulation in giant reed tissues showed high dependence on the rate of N fertilization, where fertilized plants with $60 \text{ kg N ha}^{-1} \text{ year}^{-1}$ showed higher P content (on average of two years, 535 mg kg^{-1}) than control plants (on average of two years, 398 mg kg^{-1}). However, increasing the N fertilization rate to $120 \text{ kg ha}^{-1} \text{ year}^{-1}$ displayed a lower P content of 427 mg kg^{-1} [38]. The content of nutrients in giant reed tissues (stem and leaf) usually fluctuates during the season, particularly in perennial biomass crops, to remobilize the nutrients into the rhizomes to be utilized in the next season [62]. Dragoni et al. [63] revealed a reduction in the P content of the whole aboveground part of giant reed plants over the growing season of giant reed plants from 1.8 to 0.8 g kg^{-1} . Moreover, they reported that the harvest time considerably affects the P content in dry biomass, where the double harvest of biomass in summer/autumn or summer/winter displayed noticeably higher P content than a single harvest in summer, autumn, or winter. They also confirmed that P content in the plant peaked in June, whereas it reached the minimum content in either autumn or winter harvests. Similar findings were reported for other biomass crops such as switchgrass (*Panicum virgatum* L.) [16], miscanthus (*Miscanthus x giganteus* Greef et Deuter) [64], and reed canarygrass (*Phalaris arundinacea* L.) [65].

As a result of low mobility and uneven distribution of P in soil, compensation of absorbed P from the rhizosphere area through diffusion and mobilization is very limited [1]. Therefore, frequent P fertilization is necessary for an adequate P supply to the growing crops. Overall, in most agricultural systems, the efficiency of P uptake by plant crops is about 15–30% of the applied P fertilization under optimum fertilization management [66]. More than 80% of P in soil converts into immobile form and consequently becomes unavailable for plant uptake due to adsorption, precipitation, or conversion to the organic form [67]. As a result of the low concentration of available P in the soil, the root architecture plays a vital role in the replenishment of soil solution with P ensuring the adequate supply of plants with P. Thus, the higher ratio of root surface area to volume is the desired character for maximizing the P uptake. However, the formation of root hairs largely depends on several factors, such as nutrient supply, particularly N, and it varies according to plant species [68]. Both soil total and extractable P contents increased linearly with increasing the rate of applied P. The highest total P content (39.5 mg kg^{-1}) was measured below the BL ecotype, whereas the highest extractable P content (22.4 mg kg^{-1}) corresponded to the ESP ecotype (Table 1). The efficiency of P uptake in the present study reached 55.6% when P was applied at the rate of 25 mg kg^{-1} ; however, increasing the P rate to 50 mg kg^{-1} showed lower efficiency of 36.2%. Regarding the residual P in the growth medium and its various forms at the end of the experiment, giant reed ecotypes showed different responses. The ESP ecotype displayed different behavior than the STM and BL ecotypes exhibiting the lowest total P content and the highest extractable P content. These results prove the importance of establishing precise fertilization management practices not only according to plant species but also the ecotype/cultivar.

Phosphorus can be redistributed within the plant tissues, where plants translocate P from old leaves and inactive tissues to the new leaves, which require P for their growth and activity. Plants store excess

P, in the form of orthophosphate, in vacuoles and reuse it when needed [69]. Akhtar et al. [69] cited that about 85–95% of accumulated P in the aboveground part is stored in vacuoles. For instance, Brassica varieties showed ability to remobilize P from mature parts to non-mature ones [69]. Nevertheless, mobilization of the stored P is limited under P deficiency conditions due to the inhibited photosynthesis process [51].

In the present study, most of the absorbed P accumulated in the aboveground part, as confirmed by the values of TF of P. The STM, BL, and ESP ecotypes showed TF higher than 4.0; however, the STM and BL ecotypes exhibited higher TF than the ESP ecotype. Moreover, all P treatments displayed higher TF than control plants. While control plants had a TF of 1.8, the lowest TF of P-treated plants was 4.5 when plants received 25 mg P kg⁻¹ (Fig. 1). Similarly, 64–90% of absorbed P by rice plants was translocated in the aerial part [54]. Giant reed plants showed a higher ability to bioaccumulate P in their aboveground part from the growth medium, where all the three giant reed ecotypes displayed higher BCF than 5.9. Furthermore, increasing the rate of applied P increased the value of BCF up to 50 mg P kg⁻¹. Higher P treatments revealed a decrease in BCF (Fig. 1). These results demonstrate the ability of giant reed plants to grow in environments replete with P, such as drains. However, higher P levels (i.e., 150 mg P kg⁻¹) did not increase the growth or development of giant reed plants, but at the same time, giant reed plants did not show any visual toxicity symptoms (Fig. 3). Giant reed seedlings grown on 8.0 mM KH₂PO₄ for 43 days in quartz sand showed a two-time increase of P in leaves, causing substantial changes in cell ultrastructure and inducing the production of peroxisomes as indicators for P toxicity. Nevertheless, the growth and photosynthesis efficiency of giant reed did not significantly alter [56].

The giant reed ecotypes showed different PUE, regardless of the concentration of applied P. The BL ecotype displayed the highest PUE of

7.08, while the lowest PUE (5.85) corresponded to the STM ecotype (Fig. 5). Low P levels (25 and 50 mg kg⁻¹) showed higher PUE than higher P levels (100 and 150 mg kg⁻¹). The highest PUE was 13.37 and corresponded to the treatment of 50 mg P kg⁻¹, regardless of giant reed ecotype. Giant reed plants treated with 60 kg N ha⁻¹ year⁻¹ showed nitrogen use efficiency (NUE) of 10.1, while plants that received 120 kg N ha⁻¹ year⁻¹ displayed an NUE of 5.2 [38]. Plants that possess root systems characterized by high root hair density and lateral branching have higher PUE [70]. Plants growing on low P levels adapt to these conditions by replacing the phospholipids in the cell membrane with sulfo- and/or galactolipids increasing PUE [71]. Also, another adaptation strategy to P deficiency is the increased biosynthesis of P-free polysaccharides in the cell walls, like cellulose [72], in addition to avoiding phosphate or ATP-demanding metabolic pathways.

The growth dynamic and development of the three ecotypes of giant reed (STM, BL, and ESP) were significantly altered, according to the manipulation of P in the growth medium. Control plants displayed the lowest values of all the measured plant biometrical traits, including fresh and dry masses and dry matter content of root, culm, and leaf blade (Figs. 2 and 3), culm height, length of the 2nd basal internode, shoot, and root volume, and the number of internodes, leaves, and new tillers (Table 2). However, these results are opposite to those cited by Siri-Prieto et al. [17], who reported that giant reed plants propagated from stem cuttings showed a low response to P fertilization regarding plant growth and biomass yield. This may be due to the high soil P content (≥ 9 g kg⁻¹) at the beginning of the experiment. Nevertheless, in the next years, giant reed showed a higher response to P fertilization; this may be due to the P consumption by plants [17]. Our results were consistent with those earlier documented by Chen et al. [73], maize plants that experienced P deficiency showed a decreased number of leaves. Also, Akinrinde and Gaizer [74] revealed that fertilizing rice plants with 200 mg P kg⁻¹ resulted in the highest shoot dry mass. Cocozza et al. [56], in their study of giant reed tolerance to P deficiency, reported down-regulation of several genes related to chloroplast, membranes, and cell wall structure, despite the P content in leaves did not significantly vary during the experimental period. This illustrates the modifications of the chloroplasts, the system of grana, stroma lamellae [75], and plastoglobules [76] in leaves. Consequently, reduced photosynthesis, shorter culms, and a lower number of leaves were reported by Cocozza et al. [56].

Adequate P supply is needed for the incorporation of gaseous CO₂ into organic compounds during the photosynthesis process [77]. All treated plants in the present study showed higher rates of CO₂ assimilation than control plants. Moreover, increasing the application rate of P caused a gradual increase in CO₂ assimilation rate, regardless of giant reed ecotype. The Fv/Fm ratio did not significantly vary between the three ecotypes and P treatments. In contrast, the rate of CO₂ assimilation showed significant dependence on the ecotypes and P treatments. The BL ecotype displayed the highest CO₂ assimilation rate (12.46 μ mol photons m⁻² s⁻¹), while increasing the rate of applied P increased the rate of CO₂ assimilation (Fig. 4).

Production of reactive oxygen species (ROS), e.g., superoxide (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (*OH), singlet oxygen (¹O₂), and peroxynitrite (ONOO⁻), in plants increases under biotic/abiotic stress. However, plants possess several mechanisms to mitigate and tolerate this stress, mainly via antioxidant enzymes. Giant reed plants that grew in a P-deficient growth medium (sand) showed higher expression of glutathione peroxidase enzyme. These observations were confirmed by the up-regulation of genes involved in glutathione metabolism in leaves [56]. Applying P to giant reed plants significantly reduced the activity of the peroxidase (POD) enzyme in the culm and leaf blade tissues. Control plants showed the highest POD activity in culm and leaf blade, while fertilized plants with 150 mg P kg⁻¹ exhibited the lowest activity. Likewise, control plants showed the highest quantity of buffer-soluble protein in root, culm, and leaf blade tissues compared to treated plants with P (Table 3). The POD oxidizes a wide range of

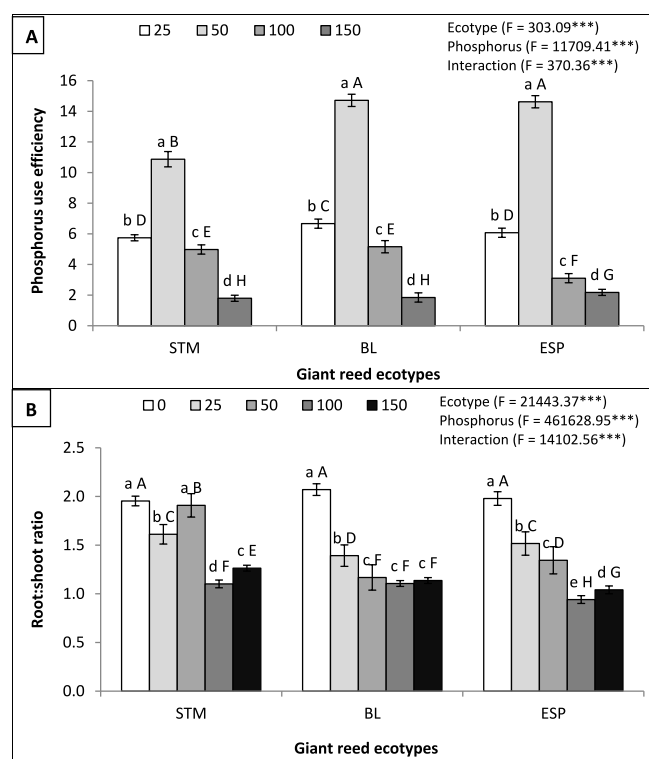


Fig. 5. (A) Phosphorus use efficiency and (B) root:shoot ratio of three giant reed ecotypes grown on different P concentrations (0, 25, 50, 100, and 150 mg kg⁻¹ sand) under greenhouse conditions for 6 months. Different lowercase letters on the same bars of the same ecotype are significant according to the Tukey's test ($p \leq 0.05$) and different uppercase letters are significant regardless of the ecotype according to the Tukey's test ($p \leq 0.05$). Data are Means \pm SD and $n = 3$. *** denotes significance at $p \leq 0.001$.

compounds using H_2O_2 , which converts later to H_2O and O_2 . Consequently, POD plays a vital role in protecting living cells against oxidation by H_2O_2 [78].

The above-discussed results illustrate the importance of fertilization of growing biomass crops to improve productivity. However, appropriate fertilization management practices are needed to determine the optimal rate for each nutrient according to several criteria, including plant species, growth stage, soil condition, interaction with other nutrients, application method, etc. For instance, Cano-Ruiz et al. [38] reported that fertilized giant reed plants with $60 \text{ kg N ha}^{-1} \text{ year}^{-1}$ displayed higher biomass yield, plant height, culm diameter, and photosynthetic pigments than control plants (unfertilized). However, increasing the applied rate of N to $120 \text{ kg ha}^{-1} \text{ year}^{-1}$ did not significantly increase biomass production. In the present study, the plant's response to P fertilization significantly relied on the giant reed ecotype and the application rate of P. Out of 32 parameters measured in the present study, the ESP, BL, and STM ecotypes displayed the highest value for 13, 12, and 8 parameters, respectively. On the other hand, plants that fertilized with 25 and 50 mg kg^{-1} displayed the highest value of 7 and 9 parameters, respectively. High P concentrations (i.e., 100 and 150 mg kg^{-1}) showed the highest value for 6 parameters. Concerning the interaction between the giant reed ecotypes and the concentration of applied P, the STM and ESP ecotypes showed better development at the treatment of 25 mg P kg^{-1} , while the BL ecotype achieved the best growth at the treatment of 50 mg P kg^{-1} .

5. Conclusion

Somatic embryo-derived giant reed seedlings showed their inability to develop well in a P-depleted growth medium (washed sand). Upon the addition of P at the rate of 25 mg kg^{-1} , plants started to grow well without visual P-deficiency symptoms, as all biometrical traits displayed significantly higher values than control plants (no added P). Uptake, accumulation, TF, BCF, and PUE showed a significant dependence on the ecotype of giant reed and the concentration of the applied P. Significant variations among giant reed ecotypes (STM, BL, and ESP) were reported. Applying P at the rate of 50 mg kg^{-1} improved several evaluated properties of giant reed plants compared to the other P treatments. The optimal P fertilization rate varied among giant reed ecotypes. The STM and ESP ecotypes developed well under the 25 mg P kg^{-1} , while the BL ecotype required a higher P rate (50 mg kg^{-1}) for its best growth traits. Despite the valuable findings of the present study, further investigations would be necessary, including long-term experiments, biomass yield evaluation, different types of soils, and different P sources.

Author contribution

Éva Domokos-Szabolcsy and Tarek Alshaal: Conceptualization, József Csajbók, Szilvia Kovács, and Szilvia Veres: Methodology, József Csajbók and Szilvia Veres: Software, Miklós G. Fári: Data curation, Nevien Elhawat and Tarek Alshaal: Writing- Original draft preparation, Éva Domokos-Szabolcsy: Visualization, Szilvia Veres and József Csajbók: Investigation, Miklós G. Fári: Supervision, Tarek Alshaal, Nevien Elhawat: Software, Éva Domokos-Szabolcsy: Validation, Nevien Elhawat, Tarek Alshaal, and Éva Domokos-Szabolcsy: Writing- Reviewing and Editing.

Funding

No fund was received.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was supported by TKP2021-EGA-20 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme." Éva Domokos-Szabolcsy was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biombioe.2023.106805>.

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