

Sweet basil can be grown hydroponically at low phosphorus and high sodium chloride concentration: effect on plant and nutrient solution management

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Abstract

Agronomic inputs in agriculture easily exceed crop needs causing environment pollution. Phosphorus (P) is a macronutrient largely used in agriculture as a mineral fertilizer that requires an accurate management because it is a finite and non-renewable resource. Growing plants hydroponically can optimize plant nutrition achieving high crop nutrient use efficiency. However, irrigation water of poor quality (saline) interferes with crop performance. In this study, the effects of different P and NaCl levels in the root zone were evaluated on basil plants grown in a deep liquid culture system in a growth chamber. Two experiments with different P (0.1, 0.25 and 1.0 mol m⁻³) and NaCl (0, 30 and 60 mol m⁻³) concentrations in the nutrient solution were carried out. Plant growth, leaf gas exchanges and fluorescence, uptake of minerals and water, and leaf quality (content of minerals and pigments, and antioxidant capacity) were analysed. The most relevant morphological plant response to the low P (i.e., roughly 0.1 x of a standard nutrient solution) concentration consisted in a greater root/shoot ratio. However, plants growing at low P concentration did not show any reduction in yield and quality. Sodium chloride did not significantly affect plants yield and quality up to a concentration of 30 mol m⁻³ confirming the tolerance of basil to moderate saline stress. Significant reduction in the total biomass, water uptake and photosynthesis instead occurred at NaCl 60 mol m⁻³. These results confirmed the hypotheses that basil can be grown hydroponically at low P and high NaCl concentration with proper management of the nutrient solution. The absence of significant interactions between P and NaCl suggests that these two factors can be managed independently with potential benefits for crop economic and environmental sustainability.

Keywords: fertigation, leafy vegetables, *Ocimum basilicum* L., root to shoot ratio, salinity, soilless.

1 Introduction

Phosphorous (P) is an essential element for all living systems. Plants take P up from the root zone through the root system that is sensible to P variations, therefore plants adapt their morphology and physiology according to the P availability (Han et al., 2021).

Phosphorous in agriculture is applied as mineral fertilizer, manures, and organic residues. Notably, the principal sources of mineral phosphate are derived from phosphate rock that is a finite and not renewable resource of this element; the rate of P extraction from phosphate rock is indeed largely higher compared to the rate of its replenishment (Han et al., 2021). Furthermore, the overutilization of P and N is responsible for eutrophication of fresh and marine aquatic ecosystem (Zak et al., 2018).

The overapplication in intensive anthropogenic activities despite its increasingly limiting availability is leading to a paradox in the management of P. Therefore, the identification of strategies to increase P use efficiency in intensive cropping system is considered a priority for sustainable productions in terms of reduced inputs and environmental impact (Freiling et al., 2022; Garlnad et al., 2018). In agroecosystems, P availability for crops can significantly vary according to environmental boundary conditions. For this reason, farmers commonly apply P fertilizers in excess, although this increases the risk for P losses through leaching and surface runoff or immobilization by fixation phenomena (Freiling et al., 2022; Garland et al., 2018; Zak et al., 2018).

Considering the complex dynamics of P in soils, the use of hydroponic culture represents a useful pilot system to reduce and optimize the P input directly in the root zone although even in such growing conditions P precipitation may occur if pH is not properly managed (Raviv et al., 2019). However, the high availability of nutrients in hydroponic systems, other than the possibility of monitoring continuously the nutrient solution, makes this growing system suitable for precision plant fertilisation (Khater et al., 2021; Silva et al., 2021). For example, Currey et al. (2020) showed how to

significantly reduce the supply of P without affecting the yield of leafy vegetables grown in soilless substrates.

Salinity is indeed a serious issue that affects agriculture and horticulture in many regions, particularly in the Mediterranean area (Massa et al., 2020; Yasuor et al., 2020). In closed-loop and deep liquid culture systems, mineral elements accumulate in the root zone when their concentration in the irrigation water is higher than the uptake concentration (i.e., the quantity of element absorbed by the crop per litre of water taken up in the same period). The consequent increase in electrical conductivity (EC) will then lead to a partial or total discharge of the nutrient solution, to avoid salinity stress, although this practice implies a waste of water and nutrients (Massa et al., 2020).

Interestingly, in spite of its general detrimental effects on many cultivated species, salinity has also been identified as one of the main multipurpose eustressors to improve some quality characteristics of many vegetable crops (Rouphael et al., 2018). Therefore, the irrigation with saline water opens new perspectives of cultivation if applied to salt-tolerant species cultivated in hydroponic system where the high availability of water and nutrients may limit the adverse effects of salinity.

Ocimum basilicum L. (basil) is the most important species belonging to the genus *Ocimum* in the *Lamiaceae*. Sweet (or common) basil is an aromatic plant rich in essential oil, commonly used in the food, cosmetic and medicinal industry (Ćavar Zeljković et al., 2020; Farsaraei et al., 2020). Common basil is classified as moderately tolerant to salinity (Scagel et al., 2019). Moreover, basil is one of the most popular species cultivated in different soilless growing systems (Khater et al., 2021); therefore, this species could be considered a good candidate to test salinity input in hydroponic conditions.

While the effect of P availability and salinity in the root zone have been individually explored, little information is available on the combined effect of these abiotic factors in the cultivation of vegetable crops. In addition, the relationship between these two factors and their putative synergic plant responses are controversial (Grattan and Grieve, 1999; Talbi Zribi et al., 2015).

Some of the above aspects were earlier discussed in a previous study that reported an improved P use efficiency in basil plants grown with reduced P supply (Massa et al., 2019). Growing plants at low nutrient concentration and with poor (saline) irrigation water may open new perspectives and scenarios for the cultivation in hydroponics, even when combined in aquaponic and cascade cropping systems, with relevant benefits for the so-called circular vegetable production chains (Massa et al., 2020).

This study aimed to assess the effects of different P and NaCl levels in the nutrient solution on some morphological and physiological traits, and crop yield and quality of sweet basil grown in deep water culture. The hypothesis of this study was that basil can be grown hydroponically with P concentration much lower (i.e., roughly 0.1 x) and NaCl concentration higher than those commonly used in commercial cultivation of this and other species (Raviv et al., 2019).

2 Materials and Methods

2.1 Plant material and growing conditions

Experiment 1 (Exp1) and experiment 2 (Exp2) were carried out in a phytotron located at CREA Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics (Pescia, PT, Italy, lat. 43°54' N, long. 10°42' E). The air temperature was 21 ±1 °C at night and 25 ±1 °C during the day; relative humidity ranged from 75% to 80% and the photoperiod was 14 h. Light was supplied by SOX-lamps (Philips®) providing 1050 µmol m⁻² s⁻¹ PAR, on average, at top canopy level (Massa et al., 2019).

Basil (*Ocimum basilicum* L., cv. Tigullio) seeds were germinated in a rockwool plug tray. Three seeds of basil were sown in each plug and thinned to one plant after emergence. Seedlings were fed with 0.5 x concentration of standard Hoagland nutrient solution until they had four true leaves. Plants were then transplanted into 10L black plastic pots filled with 9.5 L of nutrient solution

continuously aerated throughout the cultivation period. Exp1 and Exp2 lasted, respectively, 23 and 25 days after transplanting.

A basic nutrient solution, used for the preparation of the different treatments, was prepared with deionized water; sodium bicarbonate (NaHCO_3) (for pH buffer) and potassium chloride (KCl) were also added at a rate of 2 mol m^{-3} , which were assumed to cause a negligible accumulation of Na and Cl (Fig. 1S). The final composition was as follow: $\text{NO}_3\text{-N}$ 12.0 mol m^{-3} , $\text{SO}_4\text{-S}$ 3.0 mol m^{-3} , Cl 2.0 mol m^{-3} , K 7.0 mol m^{-3} , Ca 4.8 mol m^{-3} , Mg 2.0 mol m^{-3} , Na 2.0 mol m^{-3} Fe 40.0 mmol m^{-3} , B 25.0 mmol m^{-3} , Cu 3.0 mmol m^{-3} , Zn 10.0 mmol m^{-3} , Mn 10.0 mmol m^{-3} , Mo 1.0 mmol m^{-3} .

2.2 Experimental design and nutrient solution management

The nutrient solutions were differentiated when the plants had six true leaves. Treatments consisted in the combination of three different levels of P and (NaCl) salinity (S). P- PO_4 was added as monopotassium phosphate (KH_2PO_4) at a concentration of 0.25 (P025), 0.5 (P05) and 1.0 (P1) mol m^{-3} in Exp1 or 0.1 (P01), 0.25 and 1.0 mol m^{-3} in Exp2, in combination with NaCl added at a concentration of 0 (S0), 13.0 (S15), and 28.0 (S30) mol m^{-3} in Exp1 or 0, 28.0 and 58.0 (S60) mol m^{-3} in Exp2, to the basic nutrient solution. A total of nine treatments were therefore obtained in each experiment. Three replicates, each consisting of one pot with four plants (12 plants per treatment), were arranged in a randomized block design. Salinity and P levels were based on specific literature focusing on basil and hydroponic growing conditions in general (Bernstein et al., 2010; Kiferle et al., 2012; Raviv et al., 2019).

Plant water uptake, measured by a gravimetric method, and the P concentration of the nutrient solution, were measured twice per week. Each pot was refilled to the target level (9.5 L) with fresh nutrient solution of appropriate P content to maintain the target concentration as much as possible (Fig. 1S). All other elements were supplied at the same concentration as in the basic nutrient solutions. At each water refill, a sample of nutrient solution was collected for laboratory analyses, and pH and EC were measured before and after the refill. pH was kept in the range of 5.5-6.5 using sulphuric

acid. Actual P, Na and Cl concentration, and EC and pH of the nutrient solutions in the root zone are shown in Figs. 1S and 2S, respectively, for Exp1 and Exp2.

2.3 Nutrient solution analyses

Phosphorus, NO₃-N and Cl were determined by a colorimetric assay as reported, respectively, by Olsen and Sommers (1982), Cataldo et al. (1975) and Massa et al. (2018). Sodium, K, Ca, Mg and trace elements were analysed through atomic absorption spectrophotometry (AAS240 FS Varian, Australia).

The nutrient uptake concentration, the most relevant parameter for the operational management of the nutrient solution in closed loops, was calculated as the ratio between the apparent uptake of each ion – determined using its concentration in the initial (and refill), final and residual nutrient solution in the pot - and the quantity of water absorbed throughout the cultivation period.

2.4 Gas exchange and photosystem efficiency measurements

Leaf gas exchange and fluorescence were measured only in Exp2, between 9:00 am and 1:30 pm one week before the end of the experiment.

The net photosynthesis rate (Pn, $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E, $\text{mmol m}^{-2} \text{s}^{-1}$), and stomatal conductance (Gs, $\text{mmol m}^{-2} \text{s}^{-1}$) were measured using a portable gas analyser (Portable Photosynthesis System Ciras-2, PPSystems, Amesbury, MA 01913 USA). Measurements were performed on the first mature leaf of three different plants in each pot (replicate). During measurements, the gas analyser chamber was set at a constant value of CO₂ (400 ppm) and PAR ($1.000 \mu\text{mol m}^{-2} \text{s}^{-1}$) while temperature and humidity were set at the same level as the growth chamber.

The maximum efficiency of the photosystem PSII (Fv/Fm) was also evaluated by a portable fluorimeter (MiniPam, Heinz Walza GmbH, Germany) on the same leaves sampled for leaf gas exchange measurement. The leaves were kept in the dark for 20 minutes before measurements.

2.5 *Plant growth and tissue analysis*

At the end of both experiments, all plants were sampled for the determination of plant height, leaf area, and fresh (FW) and dry weight (DW, after drying in a forced-air oven at 70 °C until a constant weight) of roots, leaves and stems. Leaf area was measured with a leaf area meter (WinDIAS Image Analysis System, Delta-T Devices, UK) and the specific leaf area (SLA) was derived from the measured area and relative leaf weight. In the treatments S0xP1, S0xP0.25, S30xP1 and S30xP0.25 of Exp1 only, root morphology was analysed using the WinRHIZO® (Régent Instruments, Canada) image analysis system.

The mineral content of dried plant samples was analysed only in Exp2. After nitric-perchloric digestion, K, Ca, Mg, Na, Fe, Mn, Zn and Cu were determined through atomic absorption spectrophotometry (AAS240 FS Varian, Australia) while P was analysed as previously described for nutrient solutions. Organic N was determined by Kjeldahl distillation after sample digestion with H₂SO₄. NitrateN and Cl were analysed in the water extract of each sample by the methods used for nutrient solutions.

Leaf fresh samples were also collected at the end of both experiments for the determination of pigments and antioxidant properties. Each leaf samples consisted of 10 disks (for a total FW of 100 mg) detached from five mature leaves on the upper (1/3) part of the shoots. Total (a+b) chlorophylls (Chl), Chl a/b ratio, carotenoids (Car), anthocyanins (Antho), flavonol glycosides (Fav-glyc) and phenols were determined as reported by Maggini et al. (2018). Ferric reducing antioxidant power (FRAP, $\mu\text{mol Fe (II) g}^{-1} \text{FW}$) and radical scavenging activity (DPPH, % inhibition $\text{g}^{-1} \text{FW}$) were measured according to Maggini et al. (2018). Leaf chlorophyll content was also indirectly assessed using a portable chlorophyll-meter SPAD-502 (Konica Minolta, Chiyoda, Japan); SPAD measurements consisted in the mean values of six values (two basal, median, and apical leaves) taken from each plant per pot for a total of 24 measurements per replicate.

2.6 Statistics

Data were analysed by two-way ANOVA and significant main effects were then separated by the Student-Newman-Keuls (SNK) multiple-range test (P -value = 0.05). Linear and non-linear regression analyses were also performed to assess the relationship between plant growth or water uptake and the concentration of P, Na or Cl in the nutrient solution, respectively. Statistics and graphics were supported by the computer programs Statgraphics Centurion XV (Stat Point, Inc., Herndon, VA, USA) and Prism 6 (GraphPad Software, Inc., La Jolla, California, USA).

3 Results

3.1 Plant biomass accumulation and biometric traits

In Exp1, shoot and total plant FW and DW, leaf area, specific leaf area (SLA), and plant height showed no significant variation in presence of different concentrations of P (0.25, 0.5 and 1.0 mol m⁻³) and NaCl (up to 30.0 mol m⁻³) in the nutrient solution (Table 1S). Table 1 reports the same and other biometric parameters determined at the end of the second experiment (Exp2), in which basil was grown under more limiting growing conditions compared with Exp1. Data collected from Exp2 then confirmed what was observed at the extreme concentrations of 0.25 mol m⁻³ P and 30.0 mol m⁻³ NaCl tested in Exp1. Yet, in Exp2, a further decrease in the level of P to 0.1 mol m⁻³ did not affect total and shoot DW. The increase in the salinity level, up to 60.0 mol m⁻³, instead led to a significant reduction (25% on average) of potential harvestable production, leaf area and plant height. Surprisingly, plant dry matter concentration and SLA were not affected by salinity.

Figure 1 shows a representative visual example of root development using nutrient solution with 1.0 or 0.1 mol m⁻³ P concentration. Furthermore, the relationship between P concentration and root/shoot ratio is reported in Fig. 2. The non-linear regression analysis was performed using data from both experiments; an exponential decay curve significantly fit measured data explaining 99% of the experimental variability. In both experiments, plant root biomass increased with a decrease P

concentration thus leading to higher root/shoot ratio (Table 1). An increase in root biomass at decreasing P concentration was confirmed by the morphological analysis performed through the WinRHIZO® image analysis system in the Exp1 (Table 2). In more detail, root length increased significantly when plants were exposed to the lower P concentration, but no variation was observed in terms of root diameter. However, root length and mean root diameter did not show significant differences under saline conditions. Salinity did not significantly influence the plant root/shoot ratio in any of the experiments (data not shown), but significantly decreased root weight of plants exposed to the highest concentration of NaCl in the second experiment Exp2 (Table1).

3.2 Gas exchange and water relations

Phosphorus variation did not influence leaf gas exchange of mature leaves while plants grown at 60 NaCl mol m⁻³ showed decreased values of Pn, Gs and E (-19%, -29% and -15%, respectively) compared with the S0 treatments (Table 3). Conversely, P01 treatments led to a slight albeit significant decrease in the maximum photosystem efficiency, determined fluorometrically as the Fv/Fm ratio, but all detected values were above 0.8 (data not shown).

Similar to leaf gas exchange, plant water uptake significantly decreased in the S60 treatments (Table 3). Interestingly, the correlation coefficient of the relationship between plant water uptake and root DW ($R=0.87$) was higher than in the relationship with plant leaf area ($R=0.71$) (Fig. 3).

3.3 Leaf pigments and antioxidant properties

Phosphorus concentration did not affect most of the investigated parameters reported in Table 4 except for the anthocyanin content, which increased in P01 plants, and for DPPH, which significantly decreased in P025 and P01 plants compared with those grown in the P1 treatments.

In contrast, NaCl significantly influenced most of leaf pigment parameters. Total chlorophyll content was not influenced by salinity, but the Chl a/b ratio and carotenoid content were both lowered in the S60 treatments (Table 4). For the latter, a significant reduction (32%) was observed compared

with S0 and S30 treatments, on average; however, the ratio between total chlorophyll and carotenoids and SPAD index were higher in plants treated with NaCl.

A significant reduction was found in S60 compared with the control treatments S0 for anthocyanin content (-30%), total phenols (-18%), FRAP (-19%) and DPPH (-17%) (Table 4). The leaf content of flavonol glycosides was also significantly reduced in the presence of NaCl.

3.4 Tissue mineral content

Table 5 reports the main macronutrients and Na and Cl content measured in basil roots and stems (Exp2). Furthermore, for leaves the same macronutrient shown in roots and stem were reported with the addition of the micronutrients Fe, Mn, Zn and Cu (Table 6).

The lowest P level (P01) in the root zone reduced the P content of all plant tissues (Table 5) and resulted in an increase of Cl content in root tissues. In stems, Ca and Mg showed opposite trends each other, with a progressive decrease in Ca concentration and a parallel increase of Mg. Calcium and Mg were instead reduced in leaves of P01 plants and the same reduction, but starting from the P level 0.25 mol m^{-3} , was observed for Fe.

Increasing NaCl in the root zone caused a progressive accumulation of Na and Cl in all plant tissues but at different extents in roots and stems compared with leaves. In roots, Na and Cl increased 7.3 and 3.8 fold, respectively, in S60 compared with S0. These two elements showed a similar trend in the stem (7.7 and 3.5 fold, respectively for Na and Cl). The accumulation of Cl was instead much higher (5.2-fold) while the accumulation of Na was much lower (2.5 fold) in leaves compared with roots and stems. Consequently, the Na/K ratio increased significantly in all plant organs. Interestingly, this increase had detrimental effects on the K content of roots only.

Plants exposed to high salinity levels showed decreasing Mg concentration in roots and increasing Mg concentration in leaves without any significant effect on Ca. In stems, the higher Mg content was concomitant with a lower Ca concentration at higher concentrations of NaCl. Organic

nitrogen was lowered by the presence of NaCl only in stems, starting from the S30 treatment while the same concentration of NaCl significantly nitrate accumulation in stems and leaves of basil plants.

Finally, salinity induced a significantly higher accumulation of all micronutrients, except for Cu, while P shortage led to a progressive reduction of the Fe content in leaves.

3.5 Uptake concentration of phosphorus, sodium and chloride

Fig. 4 shows the relationships between P, Na and Cl uptake concentration and their actual mean concentration in the root zone. The trends of the investigated elements were well represented by power functions in the tested concentration range.

In the case of P uptake concentration, the non-linear regression analysis produced a power exponent of 0.43 explaining 98% of the experimental variability. As highlighted by the fitted equation, a stable equilibrium, between nutrient solution input and nutrient solution concentration in the root zone, could be achieved for the maintenance of 0.1 P mol m⁻³ by replacing the absorbed nutrient solution with nutrient solution at a concentration of 0.61 mol m⁻³.

As observed for P, the proposed model significantly fitted measured data of Na and Cl uptake concentration. However, the shape of the curves for these elements was opposite to P with power exponents >1. For the maintenance of 30 Na or Cl mol m⁻³, in this case, the concentration of this elements in the irrigation water should be 5.75 and 8.85 mol m⁻³, respectively.

4 Discussion

4.1 Plant morphological and physiological response

Data collected on growth parameters (Table 1 and 1S) confirmed the hypothesis that it is possible to grow basil at P concentration much lower than those commonly adopted in hydroponics (Raviv et al., 2019). Currey et al. (2020) reported similar results down to till a P concentration of 0.32 mol m⁻³, when basil plants were grown in a soilless substrate (peat and perlite) with P ranging from

290 0 to roughly 1.3 mol m^{-3} in the fertigation solution. In the study conducted by Currey et al. (2020),
291 the concentration of 0.16 mol m^{-3} significantly affected plant growth probably due to the lower
292 nutrient availability in substrate conditions compared to liquid culture.

293 To the best of our knowledge, this is the first study assessing the influence of low P
294 concentration in the root zone on basil grown in water culture. Conversely, the salinity tolerance of
295 this species has been deeply investigated in previous studies. Basil is considered a salt tolerant species
296 although the mechanisms underlying this tolerance are still partially unknown (Buturi et al., 2021;
297 Scagel et al., 2019). In agreement with our findings, shoot biomass of basil grown hydroponically
298 was not affected by NaCl up to 50 mol m^{-3} although some cultivars were more tolerant than others in
299 previous studies (Farsaraei et al., 2020; Kiferle et al., 2012; Tarchoune et al., 2010).

300 The tolerance of basil to NaCl is mainly associated to its ability to counteract the detrimental
301 effect of Na and is probably due to the control of upward fluxes of Na^+ from roots to leaves (Attia et
302 al., 2009; Attia et al., 2011; Bernstein et al., 2010), as suggested in this study by the different Na
303 accumulation trend observed in plant tissues (Table 5 and 6). In fact, in the present experiment, a
304 general increase of Na and Cl was observed in all the organs of plants treated with NaCl, in agreement
305 with the general plant-salinity response data reported in several species (Grattan and Grieve, 1999;
306 Munns and Tester, 2008; Parida and Das, 2005; Shannon and Grieve, 1999). However, Na
307 accumulated in stems and roots to a greater extent than in leaves. Moreover, a lower content of Mg
308 and K and higher Na/K values were detected in roots compared with shoots (Table 5). Therefore,
309 considering Na^+ homeostasis, basil showed the typical behaviour of glycophytes that accumulate
310 more Na in roots than in stems followed by leaves (Estrada et al., 2021). The opposite trend was
311 observed for Cl that accumulated at a higher rate into the leaves. Other possible mechanisms of
312 tolerance to salinity in basil could be Na^+ compartmentation in leaves, and a desalinization of the
313 xylem vessels with a successive phloem translocation of Na^+ towards roots through (Attia et al., 2009;
314 Attia et al., 2011; Bernstein et al., 2010).

Basil biomass and plant height were reduced above a certain NaCl concentration, likely existing between 30 and 60 mmol m⁻³, as previously reported by Bernstein et al. (2010). This pattern, characterized by the presence of a threshold, and a successive linear decline, represents the most common model for describing yield response to salinity stress in vegetable crops (Shannon and Grieve, 1999). The basil biomass reduction was mainly ascribable to the indirect effects of the osmotic stress that causes multiple plant physiological disorders such as reduced stomatal activity, which in turn limits leaf transpiration and CO₂ intake (Parida and Das, 2005; Massa and Melito, 2019) as found in this work (Table 3).

The decreased stomatal conductance, which is triggered by ABA-dependent signalling mechanisms under salinity (Massa and Melito, 2019; Park et al., 2016), was consistent with the lower transpiration per leaf unit area (Table 3). However, leaf dry matter concentration and SLA were not affected by salinity despite the high Na accumulation observed at the highest salinity level. Furthermore, the maintenance of optimal leaf water status under high salinity is an adaptative mechanism showed by salt-tolerant species, which is in agreement with the absence of any variation in dry matter concentration and SLA (Table 1S and 1), already observed in basil (Attia et al., 2009).

The tolerance of basil to saline environments was also demonstrated by the absence of any significant effect of NaCl concentration on leaf chlorophyll content and (Table 4) and the maximum photosynthetic efficiency response of the PSII (namely measured as Fv/Fm ratio). The decrease in carotenoids provided the evidence that SPAD index is more related to the chlorophyll/carotenoid ratio rather than to total chlorophyll content only. This suggests that the use of SPAD under abiotic stress requires attention (Table 4). Our findings also advocate that salinity stress can be easily reversible in basil even at high level of NaCl concentrations in the root zone. However, these aspects should be better assessed in further studies.

Magnesium and Ca often show reduced accumulation in plants grown in presence of NaCl (Parida and Das, 2005) or opposite trends between each other (Grattan and Grieve, 1999), as shown

in our study. In fact, we found a reduction of Ca in stems only. The reduced water uptake and transpiration rate, observed in S60 plants, probably were the determining factors for such a reduction since the delivery of Ca to aerial organs is related to water fluxes (Atkinson et al., 1992). Similar results were observed in spinach, where Ca was reduced by salinity stress while Mg was more sensitive to K than to Na variations in the root zone (Ferreira et al., 2020).

The absence of any alteration of stomatal conductance and net photosynthesis would support the hypothesis of a generally healthy status of the plants grown in low P concentration (Table 3). The reduced maximum efficiency of the PSII observed in low P plants may be a consequence of the role of P in the photosynthetic machinery – it allows energy transfer outside the thylakoids to drive other reactions in the stroma – related to CO₂ assimilation (Han et al., 2021). However, in all treatments the Fv/Fm ratio was above the typical thresholds of 0.8 for healthy plants (d'Aquino et al., 2022).

The main plant response to the low P input consisted in morphological adaptations, namely an increase of the root/shoot ratio at the lower P concentrations (Fig. 2). The non-linear exponential decay model approaching a minimum threshold was observed for this parameter at increasing P concentration. Such a plant response is coherent with the theory that, above a certain threshold, the net nutrient-ion influx becomes constant (Massa et al., 2009). Although the enhancement of root development is a typical response to reduced P availability in the root zone, it appears fascinating how strong the impact of the nutrient concentration *per se* influences plant growth and development to a similar extent in both hydroponics and soil cultivation (Niu et al., 2013; Shenoy and Kalagudi, 2005).

Plants growing under low P availability indeed adapt their root architecture by increasing root length, volume and surface area, and the number of lateral root hairs, thus facilitating ion interception capacity (Gutierrez-Alanis et al., 2018; Han et al., 2021). This behaviour has been observed in several plant species, both monocotyledons and eudicotyledons, growing in different conditions (Chen et al., 2018; Dissanayaka et al., 2017; Han et al., 2021). The main molecules driving signalling impulses

under P starvation are the DELLA proteins. Enhanced expression of DELLA genes has in fact been associated with enhanced P starvation response mechanisms, which include enhanced root growth, changes in root architectures and anthocyanin accumulation into leaves (He et al., 2021) as also observed in the present study.

Despite the lower P concentration in plant tissue observed in the P01 treatments, the level of sufficiency (roughly 2.5 g kg⁻¹ in the whole plant) for this crop was maintained (Currey et al., 2020) thus supporting plant growth and development adequately. This indicated that the enhanced root development was indeed effective in the uptake of P from the culture solution. Yet, water uptake was not affected by P level. Interestingly, it was observed a stronger correlation between water uptake and root biomass than between water uptake and plant leaf area (Figure 3). Therefore, the negative impact of low P concentration on Ca accumulation into the shoot was probably due to a reduction of its active transport throughout plant tissues mediated by P-type ATPases transporters (McAinsh and Pittman, 2009) and not to a decrease of water uptake as argued for salinity effects (Atkinson et al., 1992). However, the Ca reduction in plant shoots did not significantly affect plant growth in P treatments and the leaf content of this element remained in the typical range or above the values observed for healthy plants of sweet basil (Currey et al., 2020; Walters and Currey, 2018).

4.2 Shoot mineral content, pigments and antioxidant properties

Leafy vegetables and herbs are worldwide appreciated as medicinal and cosmetic plants, and extensively consumed since they contain nutraceutical substances and mineral elements that are functional for human metabolism (e.g., K, Mg, Ca and Fe) (Buturi et al., 2021; Ćavar Zeljković et al., 2020; Farsarasei et al., 2020; Scagel et al., 2019). On the other hand, evaluating the mineral content of leafy vegetables can improve the knowledge on sufficiency levels of nutrient elements and will help in the assessment of plant health for correct fertilization (Walters and Currey, 2018).

The reduced transpiration rate combined with the antagonism of Na⁺ against Ca²⁺ likely were the main factors that limited Ca accumulation into the plant shoot under salinity while the role of P

390 in the active transport of Ca^{2+} is one of the possible causes of such a reduction under low P
391 concentration as above discussed. Currey et al. (2020) reported a similar Ca reduction for sweet basil
392 grown in substrate culture. These authors obtained different trends for Fe and Mg compared with
393 those herein reported, but all the above elements remained in the typical range observed for soilless-
394 grown sweet basil (Walters and Currey 2018; Currey et al., 2020).

395 Magnesium, and Zn tended to increase under salinity showing a kind of “auto-biofortification”
396 action for basil since their concentration in the nutrient solution was not augmented *ad hoc*, as -
397 suggested in many reports to improve the quality of leafy vegetables agronomically (Di Gioia et al.,
398 2019; Giordano et al., 2019). Walters and Currey (2018) reported a similar trend for Zn at increasing
399 EC, but an opposite trend for Mg. However, in liquid culture, most mineral elements show
400 concentrations in the typical range or higher in comparison with those observed for soil and substrate-
401 grown basil and other plants (Currey et al., 2020).

402 Unlike other mineral elements, excessive intake of nitrates should instead be avoided in
403 human diet while leafy vegetables are natural accumulators of such molecules (Rouphael et al., 2018).
404 Increasing salinity induced a significant reduction in the accumulation of nitrates in basil shoots, as
405 expected. This well-known effect, which is due to the antagonism between Cl^- and NO_3^- influx into
406 the root (Massa et al., 2009), would support the idea that salinity is a powerful eustressor to reduce
407 the presence of “anti-nutrients” in basil as reported for many leafy vegetables (Rouphael et al., 2018).
408 In contrast, in the present work, leaf nitrate content was not influenced by P level. Moreover, we did
409 not observe a positive influence of salinity on the accumulation of bioactive antioxidant compounds
410 (Rouphael et al., 2018). Most of the analysed leaf pigments and antioxidant molecules did not increase
411 at 30 mM NaCl while significantly decreased at 60 mol m^{-3} . Similarly, the antioxidant capacity
412 assessed through the FRAP and the DPPH assays was reduced by the highest NaCl level.

413 Decreasing P concentration in the nutrient solution did not induce any significant variation for
414 most of the leaf pigments and antioxidants compared with the control treatments. The higher

415 accumulation of anthocyanins in P-starved plants (with no variation in the content of carotenoids)
416 and changes in root architecture were consistent with the plant response mechanisms to P shortage
417 (Chen et al., 2018).

418 **4.3 Operational implications and benefits**

419 The absence of any variation in SLA, dry matter percentage, and maximum photosynthetic
420 efficiency would confirm that the stressful condition observed at 30 mol m⁻³ of NaCl can be reversible
421 for basil thus allowing large EC variations in the root zone. This is a key factor for safe management
422 of soilless crops under saline conditions. The accumulation of NaCl in the cultivation system is one
423 of the main limiting factors for the virtuous management of soilless cropping in the Mediterranean
424 region (Thompson et al., 2020). However, it has been demonstrated that different strategies can be
425 adopted to improve water and nutrient use efficiency of salt-tolerant vegetables irrigated with saline
426 water, which include allowing EC oscillations in the root zone, due to the accumulation of saline ions
427 combined with the discharge of exhausted nutrient solution at very low nutrient concentrations
428 (Massa et al., 2020). The waste and related environmental impact of nutrient losses into the
429 environment indeed is due to the quantity of discharged water and its pollutant concentration (Massa
430 et al., 2020).

431 The absence of any significant interaction between P and NaCl concentrations in the root zone
432 for basil, similar to other crops (Talbi Zribi et al., 2011), allows the management of these two input
433 parameters separately without the risk for additive plant stress effects. Therefore, for this crop, the
434 idea of growing plants with saline water and very low nutrient concentrations appears worthy of
435 exploring for saving fertilisers and good quality water without significant effects on crop yield and
436 quality.

437 The perspective of using saline water for crop irrigation has been highlighted in terms of
438 improved quality of the final product (Neocleous and Savvas, 2016; Rouphael et al., 2018).
439 Accordingly, we observed an improvement in some nutritional properties of basil under salinity

(lower nitrate level and increased content of some mineral elements). However, the highest level of 60 mM NaCl was risky for this crop, therefore the threshold of 30 mM should not be surpassed.

The exponential regression model proposed in Fig. 4 can be helpful for the correct management of irrigation water having NaCl at level higher than plant uptake concentration. This approach has been already proposed to manage the nutrient solution of some fruit vegetables like melon (Neocleous and Savvas, 2016), tomato (Varlagas et al., 2010), bean (Savvas et al., 2007), and pepper (Savvas et al., 2008), while other authors experienced linear relationships (Carmassi et al., 2005). However, to the best of our knowledge this model has not previously been tested and parameterized for leafy vegetables. Following the equations reported in Fig. 4, a concentration of 30 mM NaCl could be maintained in the root zone by compensating for crop evapotranspiration with irrigation water containing 8.9 and 5.8 mM of Cl and Na, respectively.

The same approach but in the opposite way can be adopted for the reduction of the nutrient concentration in the root zone. In fact, the maximum nutrient influx rate in plants is achieved at concentrations much lower than those commonly adopted for commercial purposes (Massa et al., 2009). Moreover, plants exposed to low nutrient concentrations exhibit a greater nutrient use efficiency (Massa et al., 2019). Therefore, growing plants at very low nutrient concentrations is possible if the refill nutrient solution is maintained at the actual uptake concentration following the kinetics proposed in Fig. 4 for P uptake concentration.

The above principles can be applied for operational purposes in hydroponics where plant nutrition can be managed with the highest level of precision in agricultural systems. As proposed for deficit irrigation techniques (Costa et al., 2007), a “minimum nutrient concentration” method could be applied for low-input management of crops grown hydroponically. This strategy would indeed allow nutrient saving with improved environmental and economic sustainability without reducing crop yield (Massa et al., 2020; Thompson et al., 2020).

Finally, the above findings for basil offer insights into the possibility of obtaining more resilient cultivation systems, useful also for other leafy vegetable and other nutrient elements. Examples are aquaponics – basil is one of the most important crops in these systems (Love et al., 2015) – and cascade cropping systems where the final aim is the discharge of water with negligible residues (García-Caparrós et al., 2018). The selection of species and the identification of strategies allowing the maintenance of low nutrient concentration in the root zone indeed represent key factors for many cultivation systems (Massa et al., 2020).

5 Conclusions

The results presented in this report showed that, if well balanced, a significant reduction of P in the nutrient solution of hydroponically-grown leafy vegetables is possible, provided that the nutrient solution is properly managed. This aspect is a key factor for an optimized management of particular cultivation systems such as soilless closed loops. On the other hand, basil did not show any limitation up to NaCl 30 mol m⁻³ even in combination with the lowest P concentration of 0.1 mol m⁻³, which confirmed the general hypotheses of the study. Finally, the absence of interactions between P and salinity suggests that these two factors can be managed independently in hydroponic conditions with potential operational benefits for the exploitation of poor (saline) quality water and high nutrient use efficiency for this crop.

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Tables

Table 1. Shoot and root dry weigh (DW), total dry matter concentration (%DW), leaf area, specific leaf area (SLA), and plant height measured at the end of Experiment 2.

Treatment	Shoot DW (g plant ⁻¹)	Root DW (g plant ⁻¹)	Total %DW (g 100g ⁻¹)	Leaf area (cm ² plant ⁻¹)	SLA (cm ² g ⁻¹ DW)	Plant height (cm plant ⁻¹)
Phosphorus (P)						
P1	7.02±1.60	1.19±0.20 b	6.32±0.22	1525.0±331.3	328.9±25.4	45.3±1.57
P025	6.72±1.13	1.23±0.12 b	6.42±0.21	1441.7±243.9	322.6±26.6	43.9±3.13
P01	6.93±1.61	1.37±0.23 a	6.27±0.34	1488.1±245.6	337.6±40.9	44.6±4.35
Salinity (S)						
S0	7.74±1.35 a	1.45±0.27 a	6.25±0.28	1638.5±253.0 a	322.6±39.3	44.9±3.4 a
S30	7.28±1.16 a	1.36±0.23 a	6.45±0.26	1564.4±220.0 a	326.1±21.0	46.4±2.1 a
S60	5.65±0.78 b	1.07±0.18 a	6.31±0.23	1252.0±162.4 b	340.4±31.0	42.5±2.7 b
ANOVA*						
P	n.s.	**	n.s.	n.s.	n.s.	n.s.
S	***	**	n.s.	***	n.s.	**
PxS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*Two-way ANOVA; n.s. = non-significant or *, **, *** = significant at P -value ≤ 0.05 , 0.01 and 0.001, respectively. Values represent the average of three replicates $\pm$ SD. Different letters within each column and each factor of variability (P or S) indicate significant differences according to the SNK multiple-range test (95%).

Table 2. Root lengths and mean root diameter of plants grown in Experiment 1.

Treatment	Root length (m plant ⁻¹)	Root diameter (mm)
Phosphorus (P)		
P1	40.3±6.6 b	0.49±0.04
P025	56.6±6.9 a	0.49±0.05
Salinity (S)		
S0	53.9±17.9	0.48±0.04
S30	43.0±9.8	0.49±0.05
ANOVA*		
P	**	n.s.
S	n.s.	n.s.
PxS	n.s.	n.s.

*Statistics as reported in Table 1.

Table 3. Gas exchanges and water relations. Net photosynthesis (Pn), stomatal conductance (Gs) and leaf transpiration (E) measured at seven days before the ending of Experiment 2, and mean daily water uptake measured gravimetrically throughout the whole experiment.

Treatment	Pn ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Gs ($\text{mmol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	Water uptake ($\text{ml plant}^{-1} \text{day}^{-1}$)
Phosphorus (P)				
P1	14.9±3.5	412.5±140.1	3.31±0.56	81.8±18.1
P025	15.5±3.1	443.0±182.2	3.31±0.67	77.0±11.9
P01	15.5±2.9	435.3±102.9	3.41±0.43	80.4±18.7
Salinity (S)				
S0	16.4±2.8 a	480.4±106.4 a	3.49±0.39 a	91.0±13.8 a
S30	16.0±2.6 a	473.8±151.4 a	3.52±0.52 a	84.3±11.9 a
S60	13.5±3.2 b	339.0±127.5 b	3.01±0.59 b	64.0±7.2 b
ANOVA*				
P	n.s.	n.s.	n.s.	n.s.
S	***	***	***	***
PxS	n.s.	n.s.	n.s.	n.s.

*Statistics as reported in Table 1.

Table 4. Leaf content of total (a+b) chlorophyll (Tot Chl, mg g⁻¹ FW), carotenoids (Car, µg g⁻¹ FW), anthocyanins (Antho, mg cyanidin-3-glucoside g⁻¹ FW), flavonol glycosides (Flav-clyc, mg quercetin-3-glucoside g⁻¹ FW), total phenols (A_{320nm} g⁻¹ FW), and SPAD index and antioxidant capacity measured through ferric reducing antioxidant power (FRAP, µmol Fe(II) g⁻¹ FW) and radical scavenging activity (DPPH, % inhibition g⁻¹ FW) of fresh biomass (FW) of leaves sampled at the end of Experiment 2.

Treatment	Total Chl	Chl a/b	Car	Tot Chl/Car	SPAD	Antho	Flav-glyc	Total phenols	FRAP	DPPH
Phosphorus (P)										
P1	1.34±1.43	2.36±0.36	128.5±34.5	11.17±2.82	39.6±1.4	27.3±8.8 b	17.5±5.1	2.79±0.59	30.9±7.4	28.6±9.2 a
P025	1.34±1.88	2.38±0.47	127.0±39.7	11.13±3.55	39.9±2.0	28.7±4.7 ab	17.5±3.3	2.78±0.43	33.1±8.0	23.3±7.5 b
P01	1.33±1.56	2.43±0.48	136.3±47.2	10.44±4.18	40.4±2.2	31.9±6.5 a	17.2±2.3	2.67±0.44	29.7±6.2	22.0±5.7 b
Salinity (S)										
S0	1.30±1.61	2.46±0.33 a	145.5±31.1 a	9.25±2.07 b	38.6±1.7 b	33.5±5.7 a	19.4±2.3 a	3.04±0.47 a	35.1±7.0 a	27.7±7.7 a
S30	1.36±1.47	2.53±0.41 a	147.1±42.5 a	9.76±2.47 b	40.2±1.9 a	31.6±5.2 a	16.6±4.0 b	2.81±0.46 a	31.6±4.8 a	24.5±7.5 ab
S60	1.35±1.73	2.18±0.48 b	99.1±26.3 b	13.92±3.81 a	41.1±0.9 a	22.9±5.2 b	16.2±3.8 b	2.40±0.28 b	27.0±7.6 b	21.8±8.1 b
ANOVA*										
P	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*
S	n.s.	*	***	***	***	***	*	***	***	*
PxS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*Statistics as reported in Table 1.

Table 5. Content of macronutrients, Na and Cl (g kg⁻¹ DW) in roots and stems sampled at the end of Experiment 2.

Plant organ	Element	Phosphorus (P)				Salinity (S)		ANOVA*		
		P1	P025	P01	S0	S30	S60	P	S	P*S
Roots	N-NH ₄	34.0±1.8	35±1.9	33.8±1.6	34.8±2.0	34.9±1.5	33.1±1.3	n.s.	n.s.	n.s.
	N-NO ₃	19.3±2.1	19.6±3.1	18.8±2.1	20.0±2.1	19.1±2.6	18.6±2.6	n.s.	n.s.	n.s.
	P	6.97±0.52 a	6.66±0.56 a	4.89±1.26 b	5.98±1.14	6.22±0.96	6.32±1.64	***	n.s.	n.s.
	K	32.9±5.5	31.9±6.9	33.3±7.4	39.9±2.7 a	31.9±2.7 b	26.2±3.7 c	n.s.	***	n.s.
	Ca	7.11±0.74	7.70±1.05	7.62±0.83	7.77±1.00	7.38±0.89	7.28±0.80	n.s.	n.s.	n.s.
	Mg	10.41±1.42	10.85±2.58	9.55±1.71	12.14±1.49 a	10.06±1.58 b	8.62±0.91 c	n.s.	***	n.s.
	Cl	15.67±8.33 b	14.93±8.02 ab	17.06±8.15 a	6.66±1.38 c	15.74±1.64 b	25.27±1.81 a	*	***	n.s.
	Na	15.71±10.53	16.81±10.70	18.28±11.34	3.88±1.49 c	18.55±2.27 b	28.36±2.96 a	n.s.	***	n.s.
	Na/K	0.53±0.41	0.61±0.47	0.64±0.49	0.10±0.04 c	0.58±0.06 b	1.11±0.24 a	n.s.	***	n.s.
Stems	N-NH ₄	22.7±0.8	22.8±1.0	22.8±1.0	23.4±0.8 a	22.5±0.6 b	22.5±1.0 b	n.s.	*	n.s.
	N-NO ₃	15.6±2.3	14.0±2.1	15.7±2.6	17±2.1 a	14.7±2.1 b	13.6±1.8 b	n.s.	**	n.s.
	P	5.63±1.00 a	4.97±1.24 a	3.37±0.42 b	3.37±1.51 b	4.97±1.45 ab	5.64±0.85 a	***	*	n.s.
	K	43.9±6.75	52.2±4.36	48.2±14.8	52.4±13.8	44.8±8.15	47.1±5.78	n.s.	n.s.	n.s.
	Ca	18.2±3.6 a	13.2±2.3 b	11.7±1.9 c	16.8±4.1 a	14.1±3.3 b	12.3±2.9 c	***	***	n.s.
	Mg	2.73±0.47 b	3.32±0.38 a	3.41±0.66 a	3.03±0.39 b	2.86±0.42 b	3.57±0.68 a	**	**	n.s.
	Cl	12.8±6.05	12.1±6.16	12.9±7.88	5.69±0.58 c	12.5±3.20 b	19.7±4.02 a	n.s.	***	n.s.
	Na	5.57±5.62	6.69±5.34	6.79±6.25	1.62±0.25 c	5.06±1.55 b	12.4±5.49 a	n.s.	***	n.s.

Na/K	0.13±0.13	0.13±0.10	0.15±0.13	0.03±0.01 c	0.11±0.04 b	0.26±0.11 a	n.s.	***	n.s.
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*Statistics as reported in Table 1.

Table 6. Content of macro and micronutrients, Na and Cl (g kg⁻¹ DW) in leaves sampled at the end of Experiment 2.

Element	Phosphorus (P)			Salinity (S)			ANOVA*		
	P1	P025	P01	S0	S30	S60	P	S	P*S
N-NH ₄	39.1±1.9	37.9±2.5	39.4±2.1	38.1±2.4	38.1±1.7	40.1±1.8	n.s.	n.s.	n.s.
N-NO ₃	6.47±2.25	8.89±1.81	7.36±2.27	9.18±2.78 a	6.71±1.57 b	6.84±1.52 b	n.s.	*	n.s.
P	6.27±1.22 a	5.60±1.55 a	4.14±0.51 b	5.69±1.70	5.51±1.35	4.80±1.26	**	n.s.	n.s.
K	40.2±5.08	38.4±5.40	40.1±6.41	39.5±4.84	36.6±3.24	42.5±6.71	n.s.	n.s.	n.s.
Ca	20.4±1.69 a	21.8±1.97 a	18.5±2.42 b	21.2±2.63	20.0±2.11	19.5±2.40	**	n.s.	n.s.
Mg	6.82±1.34 ab	7.21±1.00 a	6.40±1.22 b	5.81±0.70 c	6.43±0.50 b	8.20±0.68 a	*	***	n.s.
Cl	16.5±10.47	16.4±10.51	17.0±10.83	5.06±0.46 c	15.4±0.75 b	29.3±1.79 a	n.s.	***	n.s.
Na	2.02±0.89	2.07±0.87	1.87±0.96	1.18±0.12 c	1.81±0.23 b	2.98±0.74 a	n.s.	***	n.s.
Na/K	0.05±0.01	0.06±0.03	0.05±0.03	0.03±0.01 c	0.05±0.01 b	0.07±0.02 a	n.s.	***	n.s.
Fe	0.13±0.02 a	0.12±0.01 b	0.11±0.01 b	0.12±0.02 ab	0.11±0.01 b	0.13±0.02 a	**	*	n.s.
Mn	0.18±0.03	0.18±0.02	0.17±0.02	0.16±0.02 b	0.17±0.01 b	0.20±0.02 a	n.s.	**	n.s.
Zn	0.15±0.03	0.17±0.04	0.16±0.03	0.13±0.02 b	0.16±0.04 a	0.18±0.02 a	n.s.	**	n.s.
Cu	0.009±0.011	0.009±0.001	0.014±0.007	0.010±0.007	0.008±0.001	0.014±0.01	n.s.	n.s.	n.s.

*Statistics as reported in Table 1.

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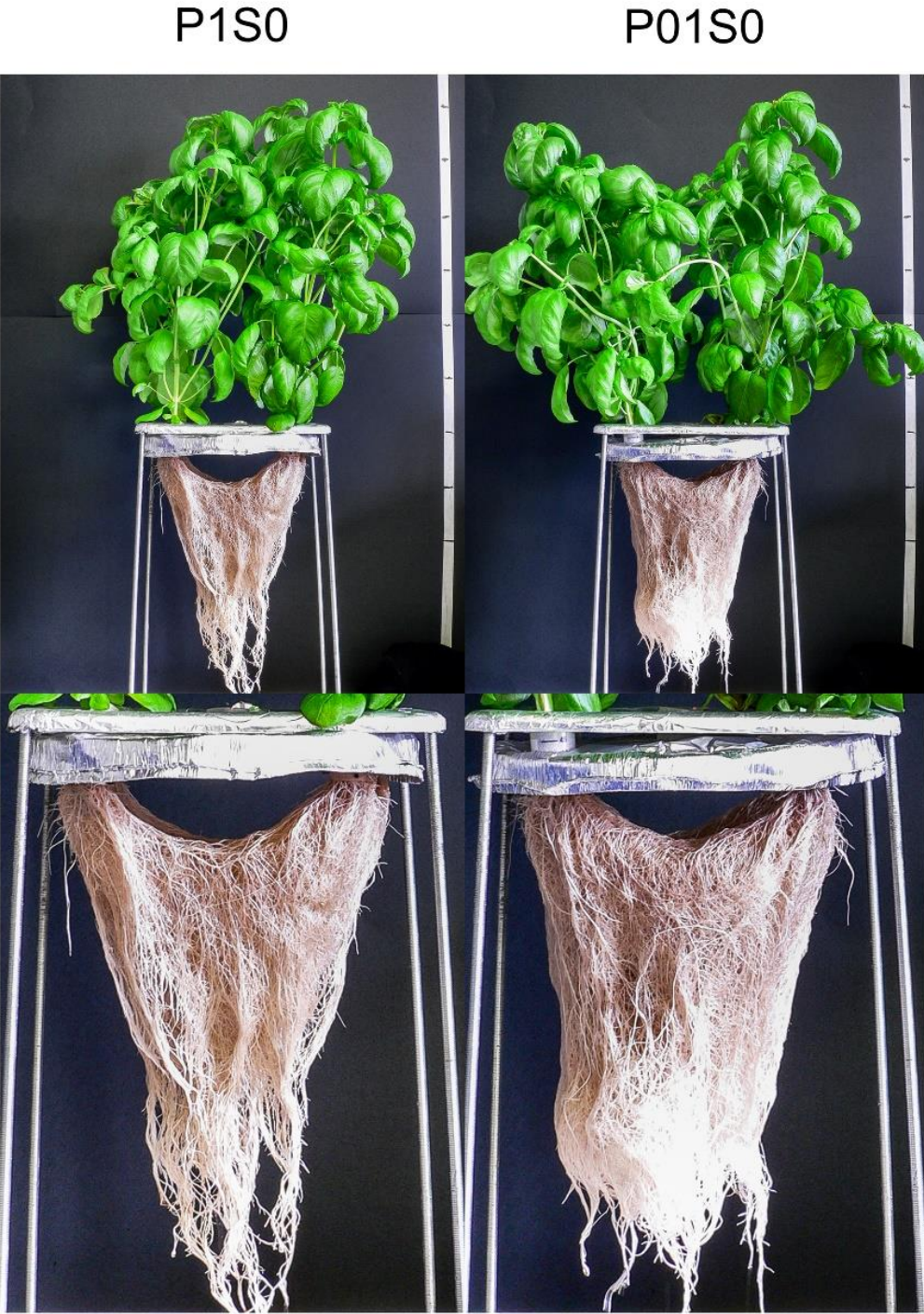
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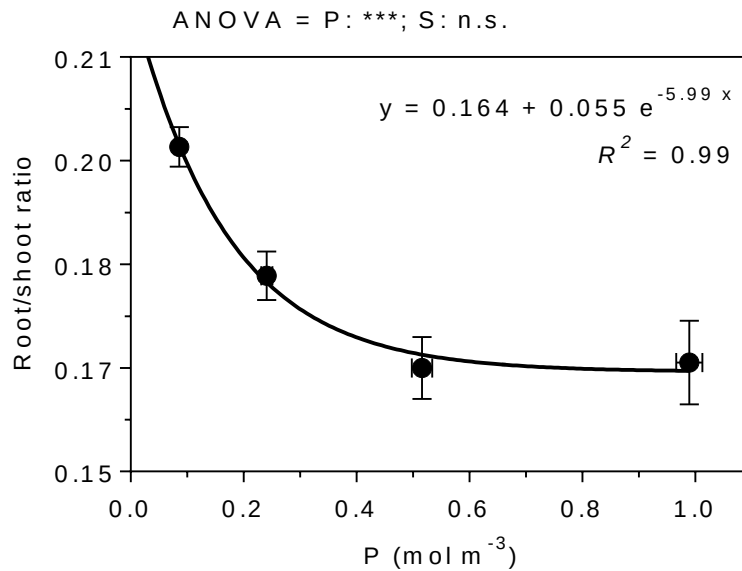
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695 **Figures**

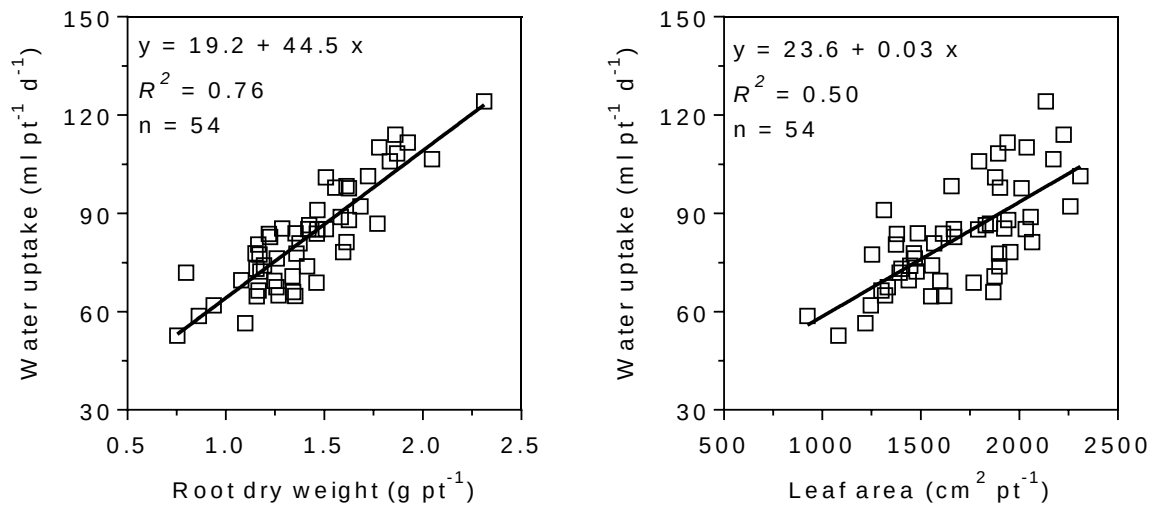


697 Fig 1. Shoots and roots of basil plants grown at the same lowest NaCl concentration (S0) in
 698 combination with the standard (P1, 1 mol m⁻³) and lowest P concentration (P01, 0.1 mol m⁻³); images
 699 are taken from mature plants of Experiment 2.



700
 701 Fig 2. Non-linear regression of root/shoot ratio versus the actual P concentration in the root zone;
 702 symbols represent the mean values (number of replicates depending on the treatment, see
 703 methodology) of data collected from Experiment 1 and 2 $\pm$ SE.

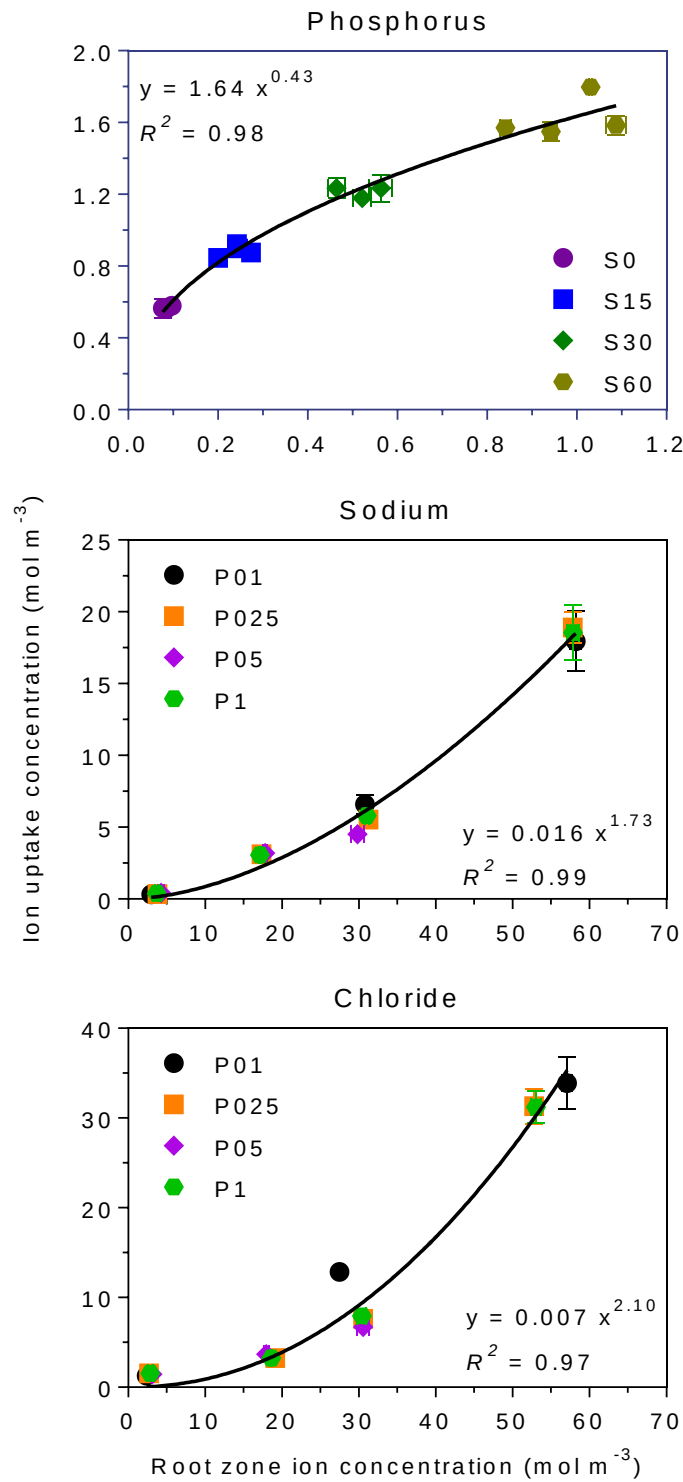
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706 Fig. 3 Linear regressions of the mean daily water uptake versus root dry weight or leaf area measured

707 in the Experiment 1 and 2.



709 Fig. 4. Non-linear regressions of the uptake concentration of P, Na and Cl versus the respective ionic
710 concentrations in the root zone. Values represent the mean value (number of replicates depending on
711 the treatment, see methodology) of data collected from Experiment 1 and $2 \pm \text{SE}$.