Defining Optimal Strength of the Nutrient Solution for Soilless Cultivation of Saffron in the Mediterranean

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Abstract: Saffron is traditionally cultivated in soil as a semi-perennial crop, although the feasibility of crop production is today constrained in Europe due to both agronomic and socioeconomic factors. Accordingly, interest has been increasing concerning its possible cultivation within protected environments through adoption of soilless cultivation technologies. The aim of the present study was to optimize nutrient solution features in the soilless cultivation of saffron corms. The trial was conducted in a greenhouse at Almeria University. Saffron was grown in 15-L pots filled with perlite. Three fertigation treatments were used, obtained by a linear increase of all nutrients of one standard in order to reach an electrical conductivity (EC) of 2.0 (control, $EC_{2.0}$), 2.5 ($EC_{2.5}$) and 3.0 ($EC_{3.0}$) dS m^{-1}. Measurements included determinations of shoot length, corm yield, as well as nutrient uptake from the nutrient solution and concentrations within plant tissues. The nutrient solution with the highest EC ($EC_{3.0}$) allowed obtaining three to five times more corms above 25-mm diameter. The increasing EC had a significant effect on the increase of macronutrient uptake, except for NO_3^- and NH_4^+ and resulted in a general increase of nutrient concentrations in tissues, such as corms and roots. Both macronutrient uptake and accumulation in plant tissues were highest under $EC_{3.0}$. Nutrient uptake was significantly correlated with production of larger corms due to higher horizontal diameter.

Keywords: *Crocus sativus* L.; nutrient uptake; tissue analysis; corm yield

1. Introduction

Saffron (*Crocus sativus* L.) is an autumn flowering plant from the Iridaceae family, traditionally cultivated in soil as a semi-perennial crop and renowned worldwide for its red stigmas, which represent the most precious spice in the world [1]. It has been cultivated in the Mediterranean area and Near East since ancient times, used as a condiment for food, as a dye for textiles and in traditional medicinal preparations [2–4]. Interest in its cultivation has been increasing due to its beneficial health effects, including antioxidant, anticancer, anti-inflammatory and anti-depressive properties [5–8].

Saffron is mainly cultivated in Iran, with more than 90% of world production, followed by India, Spain, Morocco, Greece and Italy [9]. Over recent decades, some of the traditional producing countries,

like Spain, Italy or Greece, are facing a decrease in saffron production [1,10], despite the fact that the Mediterranean region is known worldwide as a high-quality saffron producer [11]. This reduction has been highly relevant in Spain, a country that formerly contributed significantly to the global saffron market [12]. According to the information published by the Spanish Ministry of Food, Agriculture and Environment (*Ministerio de Agricultura, Alimentacion y Medio Ambiente*), in 2011, land devoted to saffron comprised 150 hectares, than 6000 hectares in 1971 [13]. The feasibility of saffron crop production is today constrained in Europe due to either agronomical or socioeconomic factors [14]. The requirement for highly skilled labor demanded for just a few weeks per year and the increase of European labor costs in the last 50 years, encouraged farmers to abandon saffron production and move to other crops [12,14,15]. The competition with emerging economies, with lower labor costs, is another incident factor [10,14]. In addition, the seasonal climatic variability and severity of drought due to climate change in recent times have resulted in both fluctuating production and limited saffron yield [14].

Crocus sativus is a sterile triploid plant and it is only vegetatively propagated by corms [16,17]. Every year, each corm produces 2–3 medium corms from the apical buds and numerous small secondary corms, depending on the mother corm dimension and its cropping conditions [10,18]. In the Mediterranean basin, corms of 25–35-mm horizontal diameter [19] are planted in summer, during the dormant period [9]. Flowering then occurs from October to November [14].

Saffron is cultivated with a three to five year cycle in Italy, two to four in Spain or six to seven in Greece [18]. On the other hand, in some regions, like in Abruzzo (Italy)—to overcome the reduction of soil fertility and increase of soilborne diseases—an annual crop cycle with rotations is preferred [18,20].

The cropping techniques have not evolved very much, and overall crop mechanization is not sustainable as it would require high investment to adapt machinery to the crop characteristics, which present very fragile flowers, positioned a few centimeters above the soil, and also as a consequence of the short harvesting period (20–30 days) [10,21]. The main operations—corm planting and lifting, flower harvesting and stigma separation—are therefore performed manually [1], generating a high demand for manual labor. More than 1000 $h \cdot ha^{-1} \cdot y^{-1}$ of labor is required [22], mostly concentrated in autumn for stigma harvesting and separation [23]. This explains the high price of the spice, also considering that to obtain one kilogram of dried stigma, approximately 150,000 to 200,000 flowers are required [24].

To increase saffron yield and quality and to reduce labor cost, a substantial change in cultivation methods is required. Accordingly, both the introduction of new production techniques such as soilless cultivation technology as well as improvements in water, nutritional and pest management, have been suggested as ways to enhance the seed, corm and flower production.

Recently, soilless saffron cultivation was proposed as an alternative system to the open field crop. Maggio et al. (2006), in a cold glasshouse in Naples, yielded 2.2 $g \cdot m^{-2}$ of saffron spice by using different substrates (perlite, peat/perlite mix and vermiculite), overall doubling the common yield achievable in Italian open fields (1 $g \cdot m^{-2}$) [25]. On the other hand, Caser et al. (2019), cultivated saffron in a quartz-sand soilless system within a glasshouse in Turin, achieving a much lower productivity (0.55 $g \cdot m^{-2}$), but with a reported high content of beneficial compounds, including total polyphenols and elevated antioxidant activity [19]. Similarly, Souret et al. (2008), demonstrated that aeroponic and hydroponic systems may allow improvement of the quality of the spice [26]. By controlling the growing conditions, Molina et al. (2004) extended the flowering period to 108 days in a glasshouse [21]. In the Almeria region (Spain), Diaz. et al. (2013), demonstrated that corm density and corm size are determinant factors for flower and seed corm production in a soilless system, thus allowing an increase in corm density of three times that in soil [27]. An advantage of soilless cultivation is that the mechanization becomes significantly easier and sustainable in comparison to the open field. In this regard, Perez-Vidal and Garcia (2020 elaborated a completely automatic system for saffron production inside a greenhouse [23].

In soilless culture, good management of mineral nutrition is essential to obtain high-quality production. The composition of nutrient solution, the electrical conductivity (EC) and pH are key

determinants of yield and quality, therefore species-specific requirements need to be appropriately identified [28]. To date, however, only a few studies have addressed the nutritional requirements of saffron. Within a pot-experiment where plants were kept under a transparent shelter and irrigated with brackish water at different salinities, Sepaskhah and Kamgar-Haghihi (2009) correlated salt stress with reductions in flower production, with the highest yield being associated with EC = 0.5 dS·m^{-1}, and a 49% reduction when the EC was raised to 1.7 dS m^{-1} [29]. Dastranj and Sepaskhah (2019) studied the effects of brackish irrigation water at four different levels of salinity in an open field in Iran, resulting in corms and saffron being negatively affected when the EC of the irrigation water was in the range of 2.0–3.0 dS·m^{-1} [30]. The combined effect of salinity and nutritional supply was studied in a greenhouse experiment where plants were grown on a sand bed [31] and fertigated with a standard Hoagland solution. Conversely, when the Hoagland fertigation solution was enriched with a factorial combination of sodium chloride and potassium, Avarseji et al. (2013) found that by increasing the potassium salinity stress by 50% the symptoms induced by EC up to 9.4 dS·m^{-1} could be alleviated [31].

Currently, the lack of corms—used as means of propagation—primarily affects the establishment of new farm fields [32]. Several studies have also demonstrated a relation between corm size and the production of flowers, stigmas and replanting corms [9]. Mollafilabi et al. (2013) in Iran, obtained the highest yield when using corms with a biomass above 10 g, compared to those between 6–10 g [33]. Corm quality is an important attribute and the environment in which they grow can affect saffron yield [34].

Considering that quality corms are needed for new crops, the limited information available about saffron nutritional requirements and their importance to obtaining a high-quality crop, the present research aimed at enhancing saffron corm production by optimizing the management of mineral nutrition in a soilless culture.

2. Materials and Methods

This trial was carried out on saffron (*Crocus sativus* L.) at the experimental greenhouse of the University of Almeria. The corms were sown in mid- September 2011 and flowered from mid-October to mid-November. Treatments began at the end of flowering. Monitoring of the experiment began with the growth of the shoots at the beginning of January 2012 and ended at the end of March with the beginning of the dormant period (when the leaves withered, and roots dried up). The corms presented a horizontal diameter below 25 mm. Before transplanting, corms were dipped in Propamocarb solution (0.2%) for 20 min and dried in the open air. Corms were then cultivated in trays (0.28 m × 0.48 m and 0.11-m height) filled with perlite (15 L) at a plant density of 45 corms·m^2. The slightly acidic pH (5.5–6.5) standard nutrient solution of Sonneveld and Voogt (2009) [35] was used for fertigation. Three treatments were performed by a linear increase of the nutrient solution strength, in order to reach EC of 2.0 (control, EC$_{2.0}$), 2.5 (EC$_{2.5}$) and 3.0 (EC$_{3.0}$) dS·m^{-1}, respectively. Corresponding fertigation treatments were supplied at 0.33 L plant^{-1} day^{-1}.-Monitoring of pH and EC of the nutrient solution was performed daily using a Crison pH/EC meter MM40 (Crison, Barcelona, Spain). A completely randomized block design was adopted, including three replicates per treatment, with each individual replicate composed of six plants.

Once per week, cation (Na$^+$, NH$_4^+$, Ca^{2+}, Mg^{2+} and K$^+$) and anion (Cl$^-$, NO$_3^-$, PO$_4^-$, SO$_4^-$) concentrations of both the irrigation and drainage solutions were measured for each treatment. Ion concentrations were determined using an ion chromatography system, Metrohm 883 (Metrohm AG, Herisau, Switzerland) equipped with an autosampler processor (863 Compact IC). The instrument was supplied with an anion separation column Metrosep A Supp 4 (250 mm/4.0-mm and particle size of 9 μm) with a guard column (Metrosep A Supp 4 Guard 4.0) and cation separation column Metrosep C4–100 (100 mm/4.0-mm and particle size of 5 μm) with a guard column (Metrosep C 4 Guard/4.0).

Maximum shoot length was measured before aerial part senescence, at 190 days after transplanting (DAT) and expressed as cm from corm neck to the longer blade apex. Simultaneously, two plants per replicate were randomly harvested and separated into roots, corms and leaves, for the determination

of concentration of N, P, K, Ca and Mg in tissues. Finally, the parameters at harvest (total corm weight, total number of corms and corm diameter) were evaluated in four plants per repetition. Corms were classified into three diameter groups (<20; 20–25 and >25 mm). Dry matter (DM) was determined after drying at 70 °C in a JP Selecta oven for 48 h and quantified using an analytical balance with a ±0.01 g precision. Concentration of N, P, K, Ca and Mg in tissues (root, corms and leaves) was performed by wet sulfuric mineralization and expressed as a percentage of DM. Elemental contents were determined using Kjeldahl (N), Olsen (P) and atomic absorption spectrometry (K, Ca and Mg) methods. To assess the mineral nutrition needs, element concentrations were referred to each organ DM weight and expressed as a percentage of crop DM (% DM). The values of N, P_2O_5, K_2O, CaO and MgO were then transformed into the corresponding total nutrient uptake (kg·ha^{-1}) and their distribution in leaves, corms and roots.

All data were statistically analyzed by one-way ANOVA and means were compared according to the LSD test at $p \leq 0.05$. These statistical computations were performed with Statgraphics© Centurion 18.1.08 software.

3. Results and Discussion

3.1. Corm Production

No differences were obtained in maximum shoot length and corm yield (g·m^{-2}) between treatments (Table 1). When saffron plants were irrigated with EC$_{2.5}$, the number of corms increased by 14% and 12% as compared with EC$_{2.0}$ and EC$_{3.0}$, respectively. Concerning small and medium size corms (Ø 20–25 and <20 mm), with EC$_{2.5}$, results showed an increase in corm yield (13% more than EC$_{2.0}$ and 18% more than EC$_{3.0}$) (Table 1). Overall, these results contrast with an open field experiment in drought [30], where deficit irrigation (based on ETc) was applied. When drought was combined with salinity, salt stress symptoms were exacerbated, and a significant decrease in corm yield was experienced when moving from a low (0.45–1.0 dS·m^{-1}) to elevated EC (2.0 and 3.0 dS·m^{-1}) [30]. Based on the hereby presented experiment, it seems however that under conditions of adequate watering, overall yield decay does not occur up to EC$_{3.0}$.

Table 1. Shoot length, corm yield, corm number and diameter-class distribution in soilless grown saffron fertigated with nutrient solutions with electrical conductivity (EC) of 2.0 (EC$_{2.0}$), 2.5 (EC$_{2.5}$) and 3.0 (EC$_{3.0}$) dS·m^{-1}.

	Shoot Length	Corm Yield	Corm Number	Corm Number by Diameter Class		
	(cm)	(g m^{-2})	N m^{-2}	>25 mm	20–25 mm	<20 mm
EC$_{2.0}$	41.9	455.3	245.1 b	4.9 b	39.2 a	201.0
EC$_{2.5}$	42.3	426.4	284.1 a	8.3 b	27.6 ab	248.2
EC$_{3.0}$	43.9	469.3	250.0 b	24.5 a	17.2 b	208.3
	ns	ns	*	*	*	ns

* Different letters within columns indicate significant differences between treatments according to the LSD test ($p \leq 0.05$); ns: not significant.

On the contrary, corms with a larger diameter (Ø > 25 mm) were obtained when the EC$_{3.0}$ was supplied, resulting in a five- and three-fold increase from EC$_{2.0}$ and EC$_{2.5}$, respectively. Moreover, EC$_{3.0}$ produced 19% less corms of medium and small size than EC$_{2.5}$ (Table 1). Yatoo et al. [36] reported that corms with a higher horizontal diameter (>22.5 mm) produced 7.9% more dried saffron, compared with smaller corms (with diameters of 10–22.5 mm). The results demonstrated that by increasing the concentration of supplied mineral nutrients, larger corms may be obtained. An exponential increase (for the evaluated concentration range) in corm dimension (Ø > 25 mm) was observed with the increase in EC of fertigation solution (Figure 1).
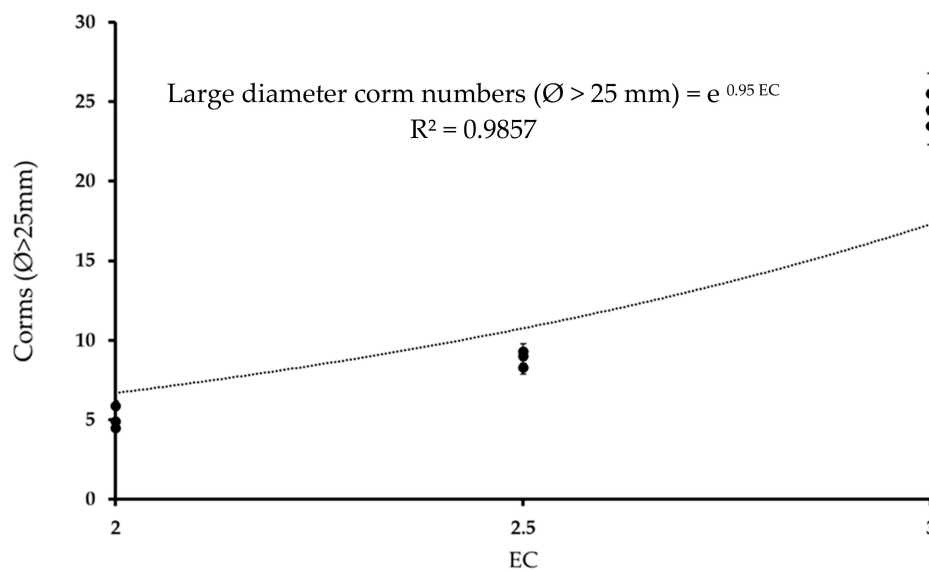
Figure 1. Regression equation between EC nutrient solutions and large diameter corm numbers (Ø > 25 mm) of saffron grown in perlite fertigated with nutrient solutions at three EC levels 2.0, 2.5 and 3.0 dS·m^{-1}.

3.2. Fertigation and Uptake Parameters

Increases in EC (resulting from a linear increase in the concentrations of the mineral elements dissolved in the nutrient solution used for irrigation) were also associated with increased ion concentrations in the drainage solution (Table 2), with the only exception being elements that were not included in the fertigation formulas (Na$^+$ and Cl$^-$). Accordingly, for what concerns anions, increased irrigation EC resulted in increases in the concentrations of NO$_3^-$, H$_2$PO$_4^-$ and SO$_4^{2-}$ in the drainage solution, whereas no changes were observed for Cl$^-$ between EC treatments (Table 2). Among cations, increasing the nutrient solution EC, resulted in increases in the concentrations of K$^+$, Ca^{2+}, Mg^{2+} and NH$_4^+$ also in the drainage solution, whereas no changes were observed in Na$^+$ concentration in response to EC.

Table 2. Average ionic concentration of irrigation and drainage solutions (mg·L^{-1}) and ion uptakes (mg·m^2·day^{-1}) in saffron grown on perlite under EC of 2.0 (EC$_{2.0}$), 2.5 (EC$_{2.5}$) and 3.0 (EC$_{3.0}$) dS·m^{-1}.

		Na$^+$	NH$_4^+$	K$^+$	Ca^{2+}		Mg^{2+}	Cl$^-$	NO$_3^-$	H$_2$PO$_4^-$	SO$_4^{2-}$
Irrigation (mg·L^{-1})	EC$_{2.0}$	94	19 c	92 c	191 c		40 c	188	647 c	120 c	135 c
	EC$_{2.5}$	97	29 b	136 b	262 b		47 b	174	882 b	144 b	155 b
	EC$_{3.0}$	91	37 a	207 a	346 a		54 a	172	1217 a	183 a	196 a
		ns	*	*	*		*	ns	*	*	*
Drainage (mg·L^{-1})	EC$_{2.0}$	133	12 c	115 c	227	c	45 b	243	714 c	114 c	110 c
	EC$_{2.5}$	130	22 b	150 b	273	b	53 a	254	921 b	145 b	142 b
	EC$_{3.0}$	116	28 a	191 a	341	a	54 a	275	1220 a	201 a	169 a
		ns	*	*	*		*	ns	*	*	*
Uptake (mg·m^2·day^{-1})	EC$_{2.0}$	71	68	148 c	328	c	44 b	130 b	863	257 b	368 b
	EC$_{2.5}$	89	84	326 b	427	b	45 b	118 b	826	261 b	335 b
	EC$_{3.0}$	111	86	456 a	586	a	78 a	186 a	838	460 a	499 a
		ns	ns	*	*		*	*	ns	*	*

* Different letters indicate significant differences between treatments according to the LSD test ($p \leq 0.05$); ns: not significant.

The EC increase also had effects on ion uptake (Table 2). As the EC increased from $EC_{2.0}$ to $EC_{2.5}$ and then to $EC_{3.0}$, uptake of K^+ and Ca^{2+} were also augmented. Similarly, an increase of Mg^{2+} uptake was associated with the EC increasing from $EC_{2.5}$ to $EC_{3.0}$, whereas no differences in Mg^{2+} uptake could be observed between $EC_{2.0}$ and $EC_{2.5}$. On the other hand, uptake of both Na^+ and NH_4^+ were not affected by EC treatments. The equilibrium between cations in the root environment was previously shown to affect their content within plant tissues: for instance, Ca^{2+} concentration was shown to strongly affect Mg^{2+} uptake [35]; while the increase in K^+ uptake may be connected to the need for osmotic regulation at higher nutrient concentrations [35]. Conversely, Kempen et al. [37], observed a decrease in Ca^{2+} uptake with the increase of nutrient solution EC in a tomato culture, possibly as a consequence of salt toxicity, highlighting the need for crop-specific indications on the appropriate proportion of cations in the applied nutrient solution.

Regarding anions, $H_2PO_4^-$, SO_4^{2-} and Cl^- uptake were only increased when $EC_{3.0}$ was supplied, whereas no differences were observed between $EC_{2.0}$ and $EC_{2.5}$ (Table 2). Increase of Cl^- uptake may be associated with the increase of Ca_2^+ and K^+ uptake [38], considering that no changes in Cl^- concentration in the irrigation solution between treatments were applied. Uptake of NO_3^- was, on the other hand, not affected by EC, thus possibly meaning that N concentration, in both forms (NO_3^- and NH_4^+) was already sufficient in $EC_{2.0}$.

3.3. Plant Analysis and Total Plant Uptake

Ca^{2+}, K^+ and Mg^{2+} uptake increased with the higher nutrient solution EC (Table 2). This increase also occurred in tissue concentration for K and Ca (Table 3). Increases in the concentration of Ca and Mg in leaves were also observed in response to $EC_{3.0}$, while K concentration reached the highest values in roots.

Table 3. Total contents of elements (% DM^{-1}) in roots, corms and leaves of saffron grown in perlite, with three EC levels in fertigation solutions 2.0 ($EC_{2.0}$), 2.5 ($EC_{2.5}$) and 3.0 ($EC_{3.0}$) $dS \cdot m^{-1}$.

		N	P^-	K	Ca	Mg
Whole plant	$EC_{2.0}$	0.81	0.19 a	0.98 b	0.23 b	0.15
	$EC_{2.5}$	0.76	0.17 b	0.96 b	0.27 a	0.13
	$EC_{3.0}$	0.83	0.19 a	1.06 a	0.28 a	0.14
		ns	*	*	*	ns
Roots	$EC_{2.0}$	0.77	0.19 a	1.39 b	0.32 ab	0.25 a
	$EC_{2.5}$	0.74	0.15 b	1.12 c	0.36 a	0.19 b
	$EC_{3.0}$	0.81	0.17 ab	1.57 a	0.30 b	0.19 b
		ns	*	*	*	*
Corms	$EC_{2.0}$	0.88	0.22 b	0.68	0.07	0.06
	$EC_{2.5}$	0.81	0.22 b	0.65	0.07	0.06
	$EC_{3.0}$	0.91	0.24 a	0.70	0.08	0.07
		ns	*	ns	ns	ns
Leaves	$EC_{2.0}$	0.78	0.15 a	0.88 b	0.31 c	0.15 b
	$EC_{2.5}$	0.74	0.13 b	1.12 a	0.38 b	0.17 ab
	$EC_{3.0}$	0.77	0.15 a	0.92 b	0.47 a	0.18 a

* Different letters indicate significant differences between treatments according to the LSD test ($p \leq 0.05$); ns: not significant.

No differences were observed in N content between any plant organ or between treatments indicating no effect of fertigation solution concentration and a uniform redistribution of N between plant organs (Table 3). This also confirms the hypothesis that N concentration in the nutrient solution is already adequate in $EC_{2.0}$. Furthermore, there were also no differences in the concentration of most elements in the corms in response to fertigation. Only a significantly higher concentration of P was

detected in corms obtained from $EC_{3.0}$. The foliar values obtained for macronutrients (Table 3) for saffron can be used as a starting point for future research for the definition of optimal fertigation management in soilless cultivation.

Moving to the total nutrient uptake (kg·ha^{-1}), differences among plant organs were highly evident (Figure 2). Due to their lower biomass, roots, in general, are the plant organs that demand the least amount of nutrients. Roots accumulated similar amounts of nutrients as compared with leaves for all analyzed ions, except for CaO, which was about double in leaves compared with roots. Corms were the organ associated with the highest uptake of N, P_2O_5 and K_2O macronutrients (Figure 2). Moreover, an increase in total macronutrient uptake was associated with the increase in the concentration of nutrients in the nutrient solution, with the exclusion of MgO, where no statistically significant differences among EC treatments were observed. Increases of EC from 2 to 3 dS·m^{-1} resulted in a statistically significant increase of total macronutrient uptake (Figure 1) for N, P_2O_5, K_2O and CaO. The highest mineral uptake was associated with N and K_2O, and their accumulation was highest in corms.
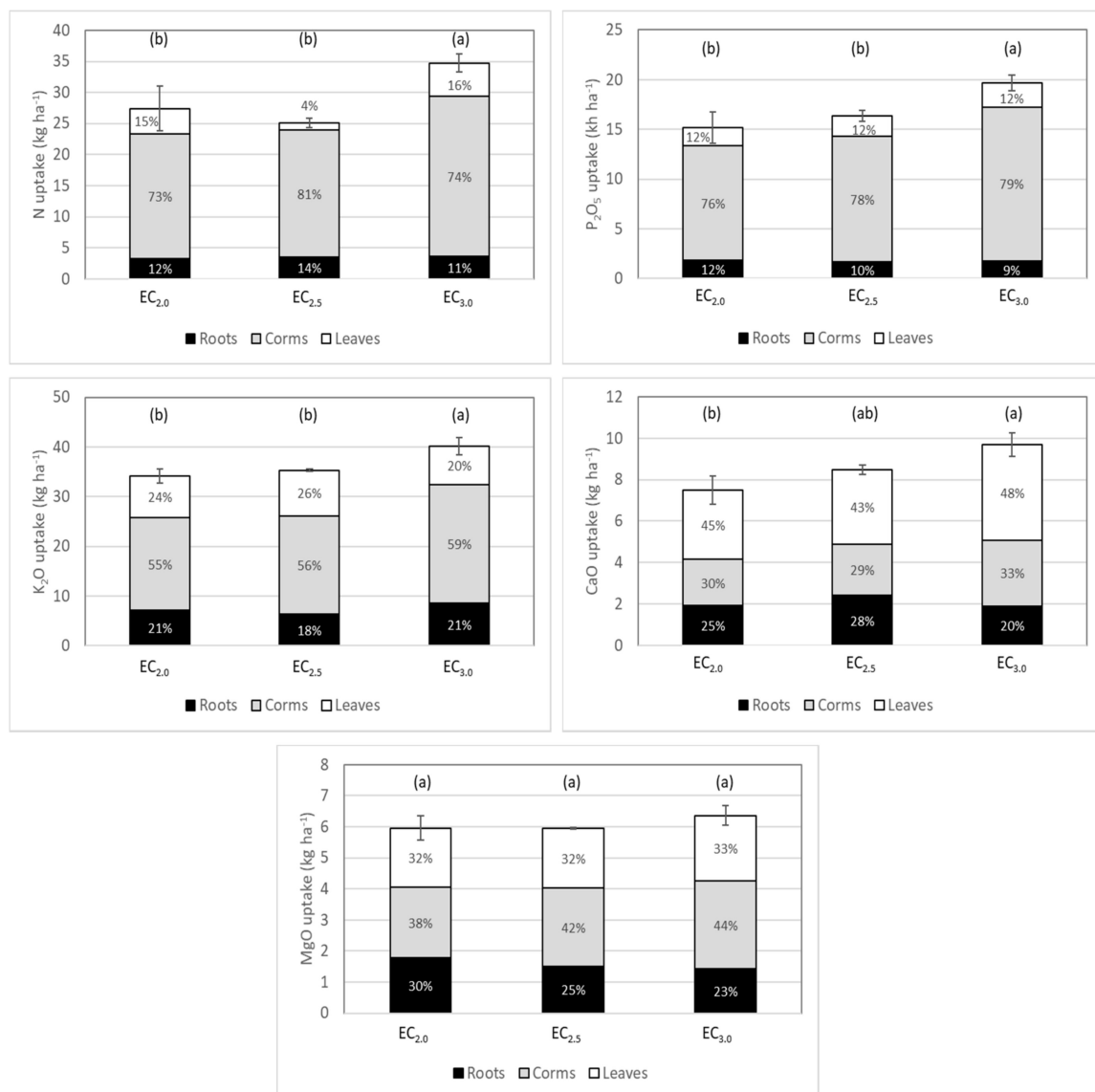
Figure 2. Total nutrient uptake (kg·ha^{-1}) and distribution of nutrients (%) in plant tissues (leaves, corms and roots) of saffron grown in perlite fertigated with nutrient solutions at three EC levels 2.0, 2.5 and 3.0 dS·m^{-1}. Different letters indicate significant differences between treatments according to the LSD test ($p \leq 0.05$).

Interestingly, positive and significant correlations were found between the number of corms with a diameter above 25 mm and the uptake of specific minerals (Figure 3). The calculated linear function expresses the total nutrient uptake ($kg·ha^{-1}$) (Fertilizers Unit) needed to obtain corms with a diameter greater than Ø > 25 mm (number). Such a significant correlation resulted from the significant increase in response to growing EC that resulted in both an increased number of corms above 25 mm and uptake of specific nutrients, following the order $K_2O > P_2O_5 > CaO > N > MgO$. Specific indications on the existing relationship between corm size and use efficiency of specific nutrients (e.g., N and P) were recently provided [39]. Accordingly, this shall be considered when designing a fertilization plan specifically targeting the production of reproductive corms.
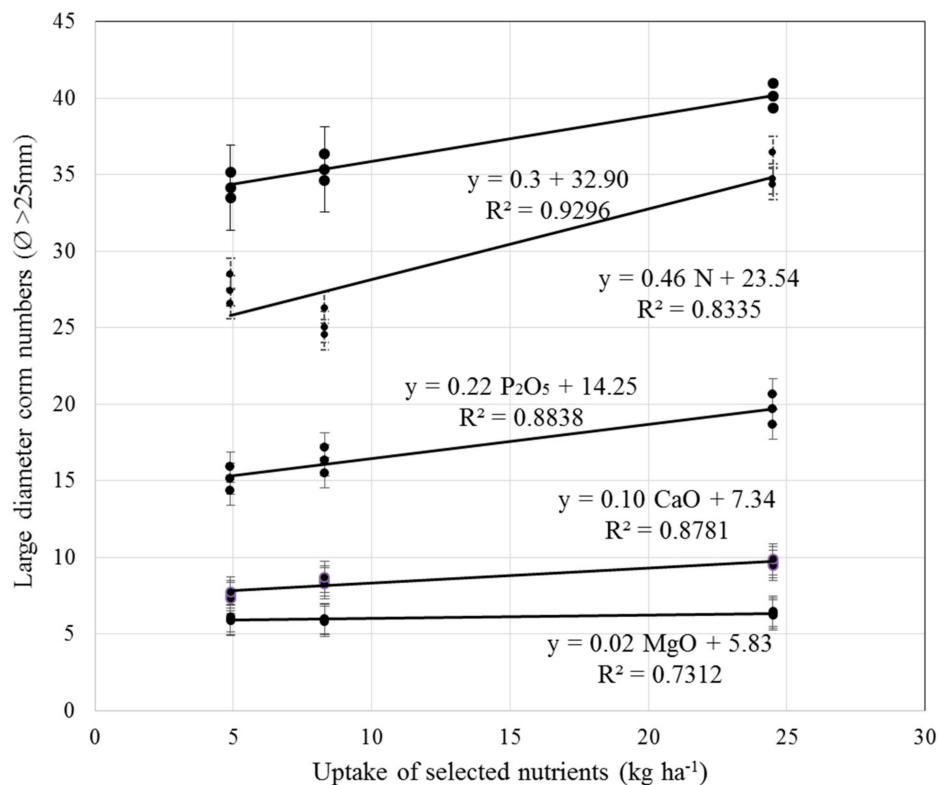
Figure 3. Linear relationship between uptake of selected nutrients ($kg· ha^{-1}$) and number of corms with a diameter above 25 mm (y).

4. Conclusions

This study demonstrated that the management of a nutrient solution is an important tool to optimize corm production. When corms were produced using nutrient solutions with a different EC ($EC_{2.0}$, $EC_{2.5}$ and $EC_{3.0}$), the best quality corms were generated at the highest concentration of supplied mineral nutrients, enabling a yield of three to five times more corms above 25-mm diameter. The highest number of $corms·m^{-2}$ was associated with an EC of 2.5 $dS·m^{-2}$, which also resulted in an enhanced corm yield by a 20%. Increasing nutrient concentration in the fertigation solution also resulted in a significant increase of nutrient uptake. The hereby presented figures also allow predefinition of fertilizer demand as a function of the predicted corm yield. Further tests should be conducted to understand possible effects of nutrient solutions with higher concentrations of nutrients.

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