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Additional Information

Growth and nutrient absorption in chufa (*Cyperus esculentus* L. var. *sativus* Boeck.) in soilless culture

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## SUMMARY

The efficiency of fertilisation in agriculture is often low, and if one knows the nutrient uptake rate, efficiency can be improved by synchronizing nutrient supply with nutrient demand. Growth and the time-course of nutrient accumulation and its partitioning between the different organs of chufa (*Cyperus esculentus* L. var. *sativus* Boeck.), an under-exploited cultivated plant, were examined. The study was conducted in soilless, open-field conditions, at a planting density equivalent to 55,500 plants ha<sup>-1</sup> in three consecutive seasons. Plants were sampled, fractionated into leaves, roots, and tubers, then dried and weighed. Their macronutrient contents were analysed fortnightly. On average, the yield was 5.0 kg fresh weight tuber m<sup>-2</sup>. Growth of the whole plant until 90 d after planting obeys an exponential function of time; the relative growth rate (RGR) for this period was determined. The highest N and K concentrations were recorded in leaves, and the highest P, Ca and Mg concentrations were found in roots. The highest accumulations of N and P were found in tubers, and of K and Ca in leaves. Nitrogen had the highest nutrient accumulation (58.3 g m<sup>-2</sup>) as well as the highest specific uptake rate.

The cultivated chufa is the botanical var. *sativus* of *Cyperus esculentus* L. The other four botanical vars., *esculentus*, *leptostachyus*, *macrostachyus*, and *hermanii* (Ter Borg and Schippers, 1992), also known as yellow nutsedge or tiger nut, can grow wild or as a weed, and are collectively called “weedy” (de Vries, 1991) .

As a crop in the Spanish Mediterranean Region, chufa tubers are used to produce a beverage called “horchata” or “horchata de chufas” (chufa milk). The milky, aqueous extract has a pleasant flavour, characteristic of vanilla and almonds. Chufa chemical composition and its extraction process have been previously described (Cantalejo, 1996; Alegría and Farré, 2003). The popularity of this drink has recently spread to other countries such as France, the UK, the USA, and Argentina. Chufa tubers are also used to make ice-cream, while fresh chufas can be consumed after soaking. Chufa is cultivated on a small scale in Egypt, Niger, Nigeria, Burkina Faso, Ghana, Togo, Mali, Cameroon, and the Ivory Coast (Omode *et al.*, 1995; Abdel-Nabey, 2001; Djomdi *et al.*, 2007). These countries currently export 3000 t tubers per year to Spain. In Turkey, chufa is also grown on a limited scale but has not been evaluated commercially (Coskuner *et al.*, 2002).

Chufa tubers may be used as a source of starch and dietary fibre in food technology, as a high quality salad oil, as caramel to add body, flavour, or colour to other products, as an antioxidant-containing food, or as biodiesel fuel. Pascual *et al.* (2000), after describing its cultivation in Spain (Pascual *et al.*, 1997), affirmed that these under-exploited products could enhance interest in this crop. More recent studies have reported increasing interest in chufa cultivation, mostly for food technology and biodiesel production, in Brazil, Cameroon, China, Egypt, Hungary, the Republic of Korea, Poland, Turkey, and the USA (Abdel-Nabey, 2001; Coskuner *et al.*, 2002; Djomdi *et al.*, 2007, Matos *et al.*, 2008).

Generally, the efficiency of fertilization is low in horticulture (percolation, volatilisation, and denitrification), but it may be improved by synchronising nutrient supply with demand, after determining the nutrient uptake rate. To promote chufa cultivation, it is essential to determine the levels of accumulation of the different nutrients and to design a rational fertilization program for the crop. Several publications reported on the nutrient uptake of vegetables (Maynard and Hochmuth, 1997); however, the requirements of chufa have not been discussed in the literature due to its limited cultivation.

An earlier study on this subject (Pascual *et al.*, 1997) recommended the addition of 240, 35, and 160 kg ha<sup>-1</sup> of N, P, and K, respectively. In 2004, our horticulture work group initiated studies on the adaptation of chufa plants to a soilless open-field culture and analysed its

nutritional needs (Andrés, 2006). Another experiment was conducted in field in the same season to assess growth and nutrition in response to different frequencies of irrigation (Ballester, 2006).

The present study was designed with two objectives: (i) to evaluate the growth and development of chufa in soilless culture; (ii) to determine the time-course of nutrient accumulation and its partitioning between leaves, roots, and tubers, calculating the corresponding specific nutrient uptake rates; and (iii) to confirm the results obtained in previous research. The results of this study will add relevant information to the existing knowledge of chufa cultivation while providing essential information for the rational fertilization of chufa crop.

## MATERIALS AND METHODS

Experiments were conducted over three consecutive years (2005, 2006, and 2007) on the experimental farm at the Campus of the Universidad Politécnica de Valencia (39° 38' N, 0° 22' W), Spain. The experiments were carried out under open-field conditions using tubers from chufa (*Cyperus esculentus* L. var. *sativus* Boeck.) clone 'Llargueta Alboraia' (Pascual *et al.*, 2003), which produces average-sized oval tubers and is highly valued for "horchata" production. Experimental plants were grown, one plant per 37 l pot, in an open system with grade B12 perlite (0-5 mm in diameter). Pots were placed in four blocks of four rows, each row containing 15 plants. Both blocks and rows were arranged in the East-West orientation, along the radiation gradient, with 0.34 m between blocks, equivalent to 55,500 plants ha<sup>-1</sup>.

The planting dates for experiments 1, 2, and 3, were 18 May 2005, 18 May 2006, and 14 May 2007, respectively. Fertigation was done using drip irrigation with one outlet supplying 1 l h<sup>-1</sup> per pot of a nutrient solution based on Hoagland's No. 2 nutrient solution (Maynard and Hochmuth, 1997). This nutrient solution (EC: 2.31 dS m<sup>-1</sup>; pH adjusted to 6.1) contained the following concentrations of macronutrients (all in mM): NO<sub>3</sub><sup>-</sup>, 14.0; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 1.0; SO<sub>4</sub><sup>2-</sup>, 2.45; K<sup>+</sup>, 6.0; Ca<sup>2+</sup>, 4.0; Mg<sup>2+</sup>, 2.0. Micronutrient concentrations were (all in μM): Fe<sup>2+</sup>, 15; Mn<sup>2+</sup>, 10; Zn<sup>2+</sup>, 5; B<sup>3+</sup>, 30; Cu<sup>2+</sup>, 0.75; Mo<sup>6+</sup>, 0.5. Irrigation doses were established to obtain 10% (v/v) drainage.

Four plants were sampled from each block (one per row and randomly from each row) every 15 d for 180 d after planting (DAP), until harvest time. Plants were divided into three parts and analysed separately: (i) shoots with all their leaves (herein referred to as leaves); (ii) roots and rhizomes as a whole, given the difficulty of separating them (herein referred to as roots); and (iii) tubers. Plant heights were measured, and shoots and tubers were counted at

each sampling. After washing, each sampled plant part (leaves, roots, or tubers) was dried at 65°C in a forced-air oven until constant weight to determine dry weights (Dw), then mineralised according to the analytical methods of the Association of Official Analytical Chemists (AOAC, 1990). The N content was determined using the Kjeldahl (semi-micro) method. Phosphorus content was determined using the phospho-molybdo vanadate colorimetric method at 430 nm, while K, Ca, and Mg levels were calculated with an atomic absorption spectrophotometer, in emission for K (766.5 nm) and in absorption in the cases of Ca (422.7 nm) and Mg (285.2 nm). An acetylene mixture was used as fuel. The nutrients in each sample were analysed in triplicate.

To evaluate the results, the average value of the data corresponding to the four plants sampled from each block was considered as the experimental unit. With the Dw of each part of the plant as well as the corresponding nutrient contents, it was possible to determine: (i) the mean relative growth rate (RGR;  $\text{g g}^{-1} \text{d}^{-1}$ ) between samplings:

$$RGR = \frac{\ln W_2 - \ln W_1}{(t_2 - t_1)}$$

where  $W_2$  and  $W_1$  were the total biomass at sampling times  $t_2$  and  $t_1$ , respectively (Williams, 1946; Radford, 1967; Causton, 1991); (ii) the accumulated nutrient uptake ( $\text{g m}^{-2}$ ); and (iii) the specific nutrient uptake rates ( $I_M$ ) between samplings ( $\text{mg nutrient absorbed g}^{-1}$  dry root weight  $\text{d}^{-1}$ ):

$$I_M = RGR_{\text{root}} \times \frac{(m_2 - m_1)}{(r_2 - r_1)}$$

where  $m_2$  and  $m_1$  are the amounts of a given nutrient at sampling times  $t_2$  and  $t_1$ , respectively, while  $r_2$  and  $r_1$  are the respective dry root weights, and  $RGR_{\text{root}}$  is the mean RGR of the root (Williams, 1946; Bellaloui and Brown, 1998; Zerihun *et al.*, 2000):

$$RGR_{\text{root}} = \frac{(\ln r_2 - \ln r_1)}{(r_2 - r_1)}$$

Data were analysed using SAS Analysis of Variance (SAS Institute Inc., 1993) the year effect was considered random.

## RESULTS

There were no significant effects, at any sampling, of the year or the block (i) on plant height, (ii) on the accumulation and partitioning of Dw, or (iii) on the uptaken nutrients (data not shown). This statistical insignificance meant that the average data of all three experiments could be used.

### *Accumulation and partitioning of dry weight*

The accumulated Dw of the plants increased during the cultivation phases (Figure 1) up to 3.9 kg Dw m<sup>-2</sup> (706 g Dw plant<sup>-1</sup>). At harvest time, being 28% of this amount in leaves, 67% in tubers, and 5% in roots. A yield equivalent to 5.03 kg fresh tuber weight ha<sup>-1</sup> was obtained. Noteworthy is the high rate at which Dw was accumulated in tubers from 75 DAP.

Furthermore, the aboveground biomass accounted for most of the plant fraction during the first half of the cycle, being exceeded by tuber biomass from the beginning of September, due to processes of translocation to the tubers and leaf senescence.

Growth of the whole plant (also that corresponding to different plant organs) until 90 DAP (half of the cycle) obeys a 1<sup>st</sup>-order exponential function of time (Figure 2), which permits to use expressions such as RGR, RGR<sub>root</sub>, and  $I_M$ , each being derived from these relationships. Changes in RGR values during 90 DAP are presented in Figure 3, the highest value (0.09 g g<sup>-1</sup>d<sup>-1</sup>) being found in the first d of the study period.

### *Concentration and accumulation of nutrients*

The nutrient concentration varied differently for each element and plant part (Figure 4 Panels A-E), especially in terms of the high concentrations of N and K in leaves, Ca in roots, and N in tubers. The total nutrient accumulation increased throughout the season; the highest rates of increase coincided (generally) with tuber initiation (75 DAP), since nutrients tend to accumulate in the tubers (Figure 4 Panels F-J). Nitrogen was accumulated gradually until reaching 58.3 g m<sup>-2</sup> at the end of cultivation (Figure 4 Panel F), being 21% of this amount in leaves, 4% in roots, and 75% in tubers. Total P accumulation reached 10.9 g m<sup>-2</sup> (Figure 4 Panel G), 17% being in leaves, 11% in roots, and 72% in tubers. Potassium was the second most absorbed nutrient absorbed in quantity (behind N), equivalent to 35.5 g m<sup>-2</sup> (Figure 4 Panel H), being 67.7% of this amount in leaves, 0.3% in roots, and 32.0% in tubers. Total Ca accumulation was 29.5 g m<sup>-2</sup> (Figure 4 Panel I), 76% of which was in leaves, 18% in roots, and 6% in tubers. Total Mg accumulation reached values of 5.8 g m<sup>-2</sup> (Figure 4 Panel J), 47% of this was in leaves, 6% in roots, and 47% in tubers.

### *Specific nutrient uptake rates ( $I_M$ )*

Changes in the  $I_M$  of all the analysed macronutrients during 90 DAP are indicated in Figure 5. In this 90 d period, the  $I_M$  of both P and Mg was similar, with low and nearly constant values; the  $I_M$  of both N and Ca was also similar, with higher values early in the growing

season, coinciding with the maximum RGR value. Of all the analysed nutrients, and because of its considerable accumulation in leaves, K had the maximum  $I_M$  value, in the early growing season (Figure 4 Panel H).

## DISCUSSION

The yield cited herein, equivalent to  $50.3 \text{ t ha}^{-1}$ , is far higher than that achieved for plants grown in field conditions in the Valencia area ( $20 \text{ t ha}^{-1}$ ) (Pascual *et al.*, 1997); our plants were grown in pots with perlite, fertigated with nutrient solution, and presumably with no nutrient constraint.

The exponential growth is usually short, lasting only a few days because it cannot be maintained once there is competition between plants (for space or nutrient) or internal competition (Gardner *et al.*, 1985). In these experiments, exponential growth continued throughout the first half of the cycle (90 DAP), probably because the space between the plants was larger than under field conditions.

Both the pattern of Dw partitioning and the associated values at harvest time are similar to the results obtained in previous research in soilless and under field conditions (Andrés, 2006; Ballester, 2006). The allocation to root biomass obtained in the present study (12% 70 DAP and 4.5% at harvest time) is lower than those reported for the weedy plants of this genus, yellow nutsedge (*C. esculentus* L.) and purple nutsedge (*C. rotundus* L.) (74% and 75%, respectively, 10 weeks after planting) when grown in pots with drip irrigation (Holt and Orcutt, 1991). The differences in root development suggest that these weeds are better suited to extract nutrients and water and, thus, they have a faster early growth rate (Holt and Orcutt, 1991). Nevertheless, the trend in RGR over time and its maximum value ( $0.09 \text{ d}^{-1}$ ) are in accordance with those reported both for yellow and purple nutsedge weeds ( $0.08 \text{ d}^{-1}$  for both; Holt and Orcutt, 1991), as well as those for cultivated chufa plants ( $0.09 \text{ d}^{-1}$ ; Ballester, 2006).

The highest N and K concentrations were found in leaves while the highest P, Ca, and Mg concentrations were in roots. At harvest, tubers had the highest N, as well as the lowest Ca and Mg concentrations. This partitioning pattern in nutrient concentration also agrees with that reported previously in soilless and under field conditions (Andrés, 2006; Ballester, 2006). Nutrient concentrations in tubers (1.7-0.3-0.4-0.07-0.1% Dw for N-P-K-Ca-Mg, respectively) agree with other reported values for chufa tubers (Abdel-Nabey, 2001; Alegría and Farré, 2003; Andrés, 2006; Ballester, 2006). Values for P, K, and Mg concentrations are similar to those reported for most nuts, such as peanut, almond, pistachio and walnut, whilst Ca content for chufa is similar to peanut but lower than the other abovementioned nuts (Ayadi *et al.*,



2006; Ozcan, 2006; Moddley *et al.*, 2007). Given these concentrations, “horchata” is a beverage with a balanced mineral content, which is one of the reasons why it is a traditional component of the Mediterranean diet (Alegría and Farré, 2003; Bixquert, 2003).

Accumulated N-P-K per unit land area in this experiment, 583-109-355 kg ha<sup>-1</sup>, was quite pronounced, clearly above (most of all, for N and P) those reported for other plants grown for their tubers, such as potato [up to 390-30-340 kg N-P-K ha<sup>-1</sup> (Maynard and Hochmuth, 1997; Cogo *et al.*, 2006, Collins *et al.*, 2007)]. Outputs from the system under field conditions are lower (Pascual *et al.*, 1997), because a significant fraction of the absorbed P (11%) was accumulated in roots and then recovered at harvest, due to the harvesting method. Moreover, the majority of the uptaken K was found in leaves (67.7%) from where it is recovered with the remaining ash mass after straw burning. In this sense, Heard *et al.* (2006) claimed that the loss through straw burning of spring wheat, oat and flax, results in an average recovery of 1-76-65 % of the accumulated N-P-K in the straw.

Finally, despite such high accumulations, the  $I_M$  of nutrients was not particularly high, because the cultivation cycle was long. The maximum  $I_M$  values for each nutrient ranged from 1.02 mg Mg g<sup>-1</sup> d<sup>-1</sup> to 19.35 mg N g<sup>-1</sup> d<sup>-1</sup>, being recorded early in the growing season, coinciding with the maximum RGR value. The maximum  $I_M$  of the N value was lower than that reported for the grassland herbs *Plantago lanceolata* and *P. major* (Freijssen and Otten, 1984).

Research is currently underway to further develop a rational fertilisation system for chufa cultivation and to elaborate a chufa plant analysis guide.

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## LIST OF FIGURE LEGENDS

Fig. 1

Dry matter accumulation and partitioning in whole plants, leaves, roots, or tubers. Vertical bars represent  $\pm$  Standard error. Their absence indicates the bar size was less than that of the symbol used.

Fig. 2

Dry matter accumulation (whole plants), and partitioning in leaves, roots, or tubers (Panels A-D respectively) for 90 d after planting (DAP). The curves are the 1<sup>st</sup>-order exponential equation fitting the corresponding dry weights and DAP. The equations of these curves are as follows:

$$\text{Leaves (kg Dw m}^{-2}\text{)} = 0.059 e^{0.34 d}, R^2 = 0.96, P \leq 0.01$$

$$\text{Roots (kg Dw m}^{-2}\text{)} = 0.011 e^{0.036 d}, R^2 = 0.99, P \leq 0.01$$

$$\text{Tubers (kg Dw m}^{-2}\text{)} = 0.0014 e^{0.073 d}, R^2 = 1.00, P \leq 0.01$$

$$\text{Whole plant (kg Dw m}^{-2}\text{)} = 0.050 e^{0.043 d}, R^2 = 0.99, P \leq 0.01$$

d being the number of DAP.

Fig. 3

Changes in RGR ( $\text{g g}^{-1} \text{d}^{-1}$ ) values for 90 d after planting (DAP). The curve is the second order polynomial equation fitting the RGR and DAP. The equation of this curve is:

$$\text{RGR} = 0.134 - 0.0020 d + 0.000011 d^2, r^2 = 0.95, P \leq 0.05, d \text{ being the number of DAP.}$$

Fig. 4

Seasonal changes in N, P, K, Ca and Mg concentrations in leaves, roots, and tubers (Panels A-E respectively), and total accumulation and partitioning of macronutrients (Panels F-J respectively). Vertical bars represent  $\pm$  Standard error. Their absence indicates the bar size was less than that of the symbol used.

Fig. 5

Changes in mean specific nutrient uptake rates ( $I_M$ ; mg nutrient absorbed  $\text{g}^{-1}$  dry root weight  $\text{d}^{-1}$ ) of N, P, K, Ca, and Mg, for 90 d after planting (DAP). The curves drawn are the best-fit, second order polynomials. The equations of these curves are as follows:

$$I_M \text{ N} = 17.38 - 0.31 \text{ d} + 0.0017 \text{ d}^2, R^2 = 0.96, P \leq 0.05$$

$$I_M \text{ P} = 1.92 - 0.029 \text{ d} + 0.00017 \text{ d}^2, R^2 = 0.76, P \leq 0.05$$

$$I_M \text{ K} = 37.62 - 0.98 \text{ d} + 0.0068 \text{ d}^2, R^2 = 0.97, P \leq 0.05$$

$$I_M \text{ Ca} = 18.36 - 0.47 \text{ d} + 0.034 \text{ d}^2, R^2 = 0.95, P \leq 0.05$$

$$I_M \text{ Mg} = 1.89 - 0.048 \text{ d} + 0.00038 \text{ d}^2, R^2 = 0.97, P \leq 0.05$$

d being the number of DAP.











