ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 07, pp.34528-34532, July, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EPOCHS OF LEAF TISSUE SAMPLING FOR EVALUATION OF PRIMARY MACRONUTRIENTS CONTENT IN FERTIGATED TOMATO AT COARSE SAND

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ARTICLE INFO

Article History:
Received 24th April, 2016
Received in revised form
20th May, 2016
Accepted 06th June, 2016
Published online 31st July, 2016

Key words:

Lycopersiconesculentum Mill.,
Index leaf,
Fertigation.

ABSTRACT

The study aimed to evaluate the magnitude of correlation between tomato yield and primary macronutrients content at leaf tissue sampled in different epochs. It was used the hybrid Paronset, cultivated in greenhouse in the fall/winter season, in a fertigation system in pots with coarse sand. Electrical conductivities of 0.8; 1.6; 2.4 and 3.2 d Sm^{-1} were used in a randomized block design with ten replications to induce variation of contents of primary macronutrients in the leaves and tomato fruit yield. It was collected the index leaf in three different epochs of the cycle, at 60, 85 and 110 days after transplantation (DAT), respectively above the first, second and third cluster emitted (chronological order) in the main stem, determining the N, P and K contents (g kg^{-1}). It was evaluated the fruit absolute yield (kg $plant^{-1}$) and relative yield (%), correlating the last one with the nutrients contents by the Spearman coefficient. It was observed higher and more significant correlation, for all nutrients evaluated, with the leaf collected above the second cluster, at 85 DAT, demonstrating to be the one, in these conditions, that most reflects the plant nutritional status regarding to N, P and K.

Citation: Marita Di Loreto y Sampaio, Renan Ribeiro Barzan, Luiz Henrique Campos de Almeida, Gustavo Adolfo de Freitas Fregonezi and Hideaki Wilson Takahashi, 2016. "Epochs of leaf tissue sampling for evaluation of primary macronutrients content in Fertigated tomato at coarse sand", *International Journal of Current Research*, 8, (07), 34528-34532.

INTRODUCTION

The tomato is cultivated in all Brazilian territory, in different regions with several climatic characteristics (Salustiano *et al.*, 2006). Due to sensibility to climate and diseases, responsible for yield drop, the greenhouse cultivation has been increasing in the last decades (Gruda, 2009). Furthermore, the occurrence of soil salinization by excess fertilization (Veduim and Bartz, 1998), as well as root diseases (Vida *et al.*, 2004), stimulate the use of fertigation in substrates (Fontes *et al.*, 2004). The evaluation of plant nutritional status is an important tool for proper fertilization (Martinez *et al.*, 1999), which is even more relevant in hydroponic systems, where the adjustment in nutrient supply along the plant cycle is easier. The nutrient content in the leaf is the main parameter used for nutritional diagnosis, because this organ is the most important compartment of plant metabolism. This way, there is a relation between nutrients concentration in the leaf tissue and plant yield (Marschner, 2011). Some factors as age, genotype and interaction with the environment can influence the concentration of mineral elements in plant tissues (Smith, 1986) and, thus, interfere with its ability to represent the nutritional status. According to Malavolta *et al.* (1997), the tomato index leaf must be the forth below the apex, when the plant is at full bloom or with the first mature fruit. Campbell (2013) affirms that, in protected cultivation, the more recently mature or completely expanded leaf is the best indicator of the nutrition in all stages of development, being generally the third or forth leaf below the growing point. Not always, although, the same leaf better represents the nutritional status for all

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elements, or yet, for all cultivation conditions (Takahashi and Andrade, 2010). In that sense, correlation studies between nutrients contents and yield must be developed in different production systems, genotypes, and, especially, diverse sampling characteristics, as the position and epoch of tissue collection. With that, the hypothesis of this study is that, for tomato growing in fertigation at sand, the contents and representativeness of nutritional status for primary macronutrients are influenced by epochs of leaf tissue sampling. The aim was, this way, to evaluate the N, P and K contents at leaves sampled in three different epochs and its correlation with tomato fruit yield.

MATERIALS AND METHODS

The experiment was carried in arched ceiling greenhouse, covered with transparent polyethylene with 150 µm of thickness, between May and September, 2014, in the Agrarian Sciences Center of Londrina State University, Londrina, PR, Brazil (23º 23' S; 51º 11' W and 560 m high). It was grown the tomato hybrid Paronset, with indeterminate growth habit. The plants were obtained in certified nursery and transplanted in plastic pots, with 9.0 dm^3, filled with sand substrate (844 gkg^{-1} coarse sand) and spaced 0.80 x 0.30 m, providing a population density of 4.16 plants m^{-2}. The fertigation system was constituted by submerged pumps (Atman® AT 203, with 18.6k Pa and 38 W) in tanks with 250 L of nutrient solution. The distribution of nutrient solution was made by plastic hoses, micro tubes and micro sprinklers, which were regulated to 0.3±0.05 L min^{-1}. The fertigation was programmed, using a timer for control the pumps activation, according to water requirements of the plants (Camargo *et al*., 1999). At the beginning, the plants were fertigated with a 1.2 d Sm^{-1} nutrient solution, until they have reached 15 days after transplantation (DAT). After that period, nutrient solutions with electrical conductivities of 0.8; 1.6; 2.4 and 3.2 d Sm^{-1}, with proportional modifications on the nutrients concentrations (Table 1), were used in a randomized block design with ten replications, totaling 40 plots. The fertilizer sources were NH_4NO_3, $NH_4H_2PO_4$, Ca $(NO_3)_2$, MgSO4, KCl, Rexolin BRA® and Rexolin M48®.

The pruning, sprout thinning, pests and diseases control was made periodically. The plants were conducted with two stems, the secondary being emitted in the axillary bud of the leaf below the first cluster in the main stem. It was maintained three clusters in the main stem and two clusters in the secondary stem. For the correlation study, it was sampled the leaves with petioles emitted immediately above the insertion of the first, second and third cluster of the main stem (chronological order of emission), when the first fruits of each reached 15 mm of diameter, respectively at 60, 85 and 110 DAT. After the sampling, the leaves were washed with deionized water, dried in a ventilated oven at 55º Cuntil constant mass and ground with Wiley mill. The tissues were digested with sulfuric acid for nitrogen (N) analysis and nitric-perchloric acid for phosphorus (P) and potassium (K) analysis. The concentrations of these elements were determined in the extracts by Kjedahl method, for N, spectrophotometry, for P, and flame photometry, for K, as described by Malavolta *et al.* (1997). The leaves contents of N, P and K in the three sampling epochs were compared by the non-parametric test of Friedman for *k* related samples. It was evaluated the absolute yield (kg $plant^{-1}$) by harvesting and weighing the fresh fruits. The relative yield (%) of each plot was obtained dividing its yield by the maximum yield obtained among all the plots, multiplying by 100 for conversion to percentage. With that, it was possible to correlate each nutrient content, in each epoch of index leaf sampling, with the relative yield, by the non-parametric Spearman coefficient (ρ) (Lira and Neto, 2006), chosen because the data did not attend the parametric presupposing of error normality by Shapiro Wilk test (n > 10) (Torman *et al*., 2012). The software used for all statistical analysis was BioEstat® v. 5.0.

RESULTS AND DISCUSSION

The general mean yield obtained in the experiment was 1.26± 0.055[1] $kgplant^{-1}$, which corresponds in the population density to 50.54 tha^{-1}, below the mean yield currently reached in Brazil (IBGE, 2015). This, however, is due to the low yields obtained in the plants grown at lower nutrient solution EC conditions.

Table 1. Nutrients concentrations and electrical conductivities (EC) of nutrient solutions used to induce leaf contents and tomato fruit yield variations in fertigated cultivation at coarse sand

EC	N	P	K	Ca	Mg	S	Fe	B	Zn	Mn	Cu	Mo
dSm^{-1}	------------					mgL^{-1}					------------	
0.8	99.0	21.8	76.0	116.5	13.5	19.5	1.38	0.32	0.95	0.03	0.06	0.005
1.6	198.0	43.6	152.0	233.0	27.0	39.0	2.75	0.63	1.95	0.05	0.11	0.010
2.4	297.0	65.4	228.0	349.5	40.5	58.5	4.13	0.95	2.93	0.08	0.17	0.015
3.2	396.0	87.2	304.0	466.0	54.0	78.0	5.50	1.26	3.90	0.10	0.22	0.020

Table 2. Significance level (p-value; n = 40) and Spearman correlation coefficient (ρ) between the primary macronutrients (N, P and K) at index leaves sampled in different epochs and the relative fruit yield of the tomato fertigated at coarse sand

Epoch (DAT)	Nitrogen (N)		Phosphorus (P)		Potassium (K)	
	ρ	p-value	ρ	p-value	ρ	p-value
60	0.4263	0.0061	0.3465	0.0284	0.4523	0.0034
85	0.4467	0.0038	0.6232	0.0001	0.5715	0.0001
110	0.3998	0.0105	0.0320	0.9844	0.3670	0.0198

DAT: Days after transplantation.

[1] Mean standard error.

As it is possible to observe in Table 2, the index leaf sampled above the second cluster, at 85 DAT, presented the higher level of statistical significance for correlation with yield in all primary macronutrients evaluated. For P and K contents, this leaf demonstrated a high significant correlation (p<0.0001). Yet, only for the P content in the leaf sampled above the third cluster, there was no significant correlation with the yield.

Besides the level of significance, according to the Spearman coefficient itself, the magnitude of correlation between all the primary macronutrients and the relative yield was higher for the index leaf sampled above the second cluster (Table 2). Compared to the other nutrients, the nitrogen was the one in which the levels of significance and the correlation coefficients from the different epochs of leaf sampling were more similar, with an amplitude of 0.0067 for p-values and 0.0469 for ρ (Table 2). Locascio *et al*. (1997) observed that the correlation between leaf N and commercial production of tomato fruits drip-irrigated in sandy soils from different locations, was higher when the tissue sampling was made at the sixth week after transplantation. In a study made by Mason and Wilcox (1982), the authors observed higher leaf N contents as a function of increasing age, unlike what was obtained in this work, where the highest N content occurred in the index leaf sampled in mid cycle, at 85 DAT (Table 3).

Table 3. Primary macronutrients (N, P and K) contents in the index leaves sampled at different epochs of tomato Fertigated at sand

Epoch (DAT)	Nitrogen (N)	Phosphorus (P)	Potassium (K)
		(g.kg^{-1})	
60	31.83 b	7.97 ab	26.13 a
85	36.77 a	8.85 a	23.66 a
110	31.07 b	7.00 b	13.54 b

Means followed by same letters are not different by the Friedman test for k related samples in the level of error probability of 5%. DAT: Days after transplantation.

According to Campbell (2013), the sufficiency range for N in tomato grown at protected environment, in most recent mature leaf, is from 35 to 50 g kg^{-1}. This interval is inferior than the obtained in the present study for the index leaf with higher correlation, in which the sufficiency range (80 to 95% of relative yield) is from 47 to 58 g kg^{-1} (Figure 1A). Huett and Rose (1988), growing tomatoes at sand with different concentrations of nitrogen in the nutrient solution, also observed critical values of N content lower than the stipulated in the present study, from 44.5 to 49.0 g kg^{-1}, in index leaves sampled between 4 and 12 weeks after transplantation.

The P content was higher in the leaf sampled at 85 DAT (Table 3), however, in all epochs evaluated, the mean values was above the sufficiency range presented by Campbell (2013), which is 3.0 to 6.5 g kg^{-1}. As for nitrogen, the sufficiency range of P obtained in this study, from 11.84 to 15.0 g kg^{-1} (Figure 1B), comprise higher values than t ******* y the referred author. Pereira and Mello (2002), in a study with application of different foliar fertilizers, obtained for tomato genotype 'Carmem', contents of P also inferior than the ones observed in this experiment, from 3.4 to 4.1 g kg^{-1}, in leaves sampled below and opposed the insertion of the second cluster when the first mature fruits appeared. The potassium was the only macronutrient which presented a great decrease in the mean content of the index leaf from the first to the last sampling epoch (Table 3).

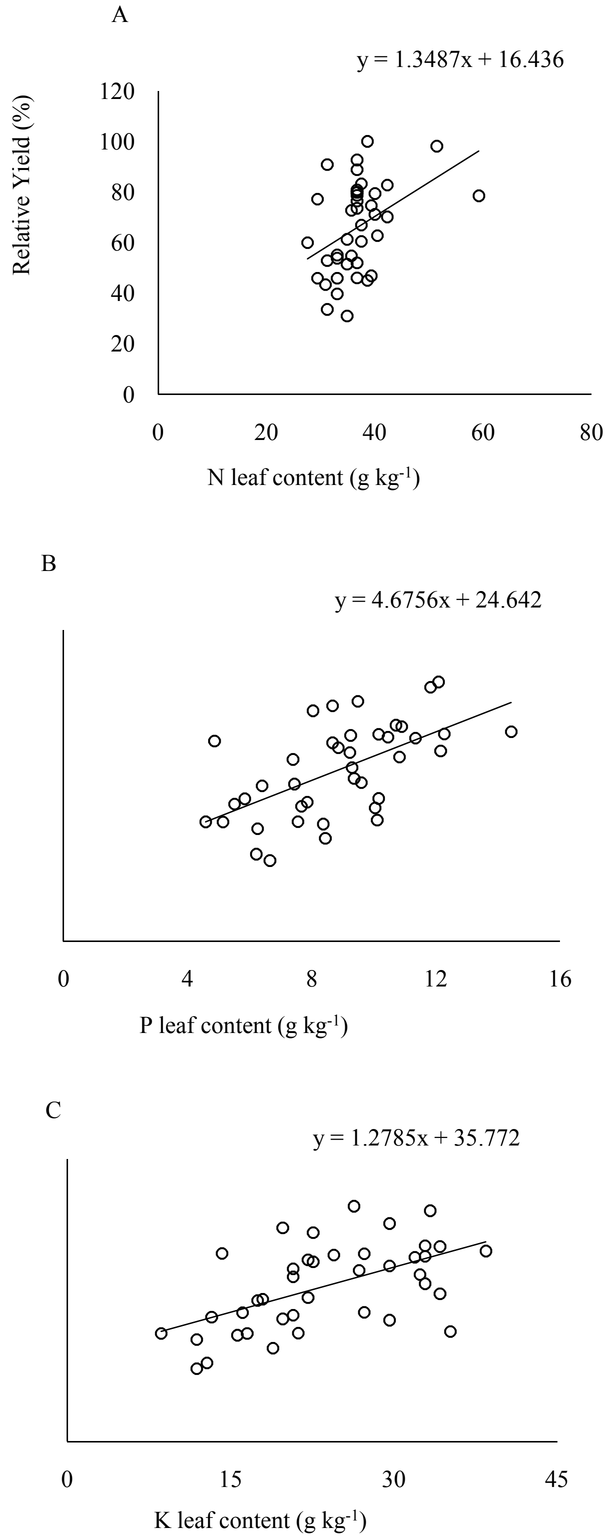
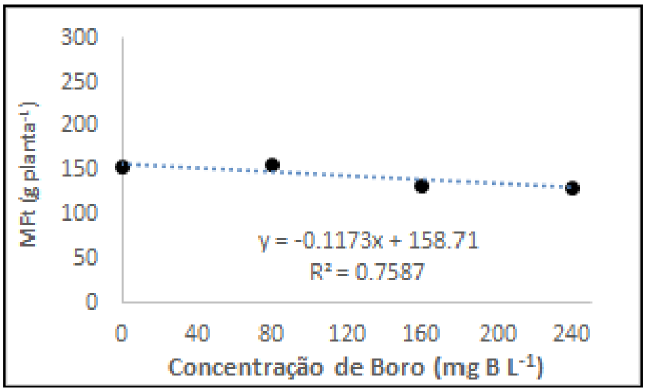


Figure 1. Correlation between fruit relative yield of tomato, Paronset hybrid, and the contents of N (A), P (B) and K (C) in the index leaf sampled above the second cluster emitted in the main stem, 85 days after transplantation, at different concentrations of nutrient solution in fertigated system at coarse sand



Fontes *et al*. (2000) observed a decrease at the critical level of K in the petioles dry matter between sequenced samplings made at the flowering of the second and forth clusters, from 103 to 73 g kg^{-1}, respectively. This reduction may be related to the occurrence of a redistribution process of the nutrient, in a more accentuated way in advanced stages of development, since K is related to translocation of sugars to sink organs (Marschner, 2011), and also due to a reduced contribution of root absorption near to plant senescence. The sufficiency range for K obtained in this study, from 34.6 to 46.33 g kg^{-1} (Figure 1C), was the only one situated very close to the referred by Campbell (2013), which recommends the limits of 35.0to 45.0 g kg^{-1}.

Conclusion

Between the epochs of leaf tissue sampling at 60, 85 and 110 days after transplantation of tomato hybrid Paronset, the second presents higher correlation of primary macronutrients with the fruit relative yield. With that, in fertigated system at coarse sand, the plant nutritional status is better represented, regarding to N, P and K, by the leaf sampled above the second cluster emitted in the main stem in the referred epoch.

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