Analysis of root traits of watermelon under phosphate fertilization using minirhizotrons

Análise de características radiculares da melancieira submetida ao manejo da adubação fosfatada utilizando minirhizotrons

Análisis de las características de la raíz de la sandía sometida al manejo de la fertilización con fosfato usando minirizotrones

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Abstract

The objective of the present study was to assess the effects of phosphate fertilization management on root traits of irrigated watermelon crop by minirhizotron observation. The experiment was conducted in an area with acrisol soil in Mossoró-RN using a completely randomized block design with six replicates corresponding to the assessment times. The evaluated parameters were four doses of P (34, 80, 137, and 206 kg ha^{-1} de P_2O_5) and two methods of fertilization: basal fertilization (F0) and basal + top-dressing fertilization (F1). Triple superphosphate was used for basal fertilization and monoammonium phosphate was used for top-dressing (34 kg ha^{-1} of P_2O_5). In addition, two control treatments were included: one without the use of P (C1) and another with only top-dressed P at a dose of 103 kg ha^{-1} of P_2O_5 (C2). A portable root scanner was used to collect images through 0.6-m clear tubes (minirhizotrons). The root mean diameter decreased with increasing doses of phosphate in F0; however, fine roots were observed in C2. There was a positive effect of P doses on the number of roots only in F0. In the absence of P, the reduction in the number of roots was greater than the reduction in total length.

Keywords:. *Citrullus lanatus,* minirhizotron, phosphorus.

Resumo

Objetivou-se com este trabalho avaliar os efeitos da adubação fosfatada sobre características das raízes da melancieira irrigada através de minirhizotrons. O experimento foi realizado em um Argissolo, em Mossoró-RN, em delineamento experimental de blocos casualizados e seis repetições, correspondentes às épocas de avaliação. Foram avaliadas quatro doses de P (34, 80, 137 e 206 kg ha^{-1} de P_2O_5) e duas formas de adubação: fundação (F0) e em fundação mais cobertura (F1). Para as doses em fundação utilizou-se o superfosfato triplo e para as doses em cobertura, o fosfato monoamônico (34 kg ha^{-1} de P_2O_5). Foram aplicados ainda dois tratamentos-controle: sem aplicação de P (C1); e aplicação de P somente em cobertura na dose de 103 kg ha^{-1} de P_2O_5 (C2). Com o auxílio de um escâner de raízes portátil, foram capturadas imagens através de tubos transparentes (minirhizotrons) de 0,6 m. O diâmetro médio das raízes diminuiu com o aumento da dose de fosfato na adubação F0, porém raízes mais finas foram observadas no tratamento C2. Houve resposta positiva das doses de P sobre

o número de raízes apenas na adubação F0. Na ausência de P a redução do número de raízes foi mais intensa do que a do comprimento total.

Palavras-chave:. *Citrullus lanatus,* minirhizotron, fósforo

Resumen

El objetivo de este trabajo fue evaluar los efectos de la fertilización con fosfato en las características de las raíces de la sandía regadas a través de minirizotrones. El experimento se realizó en un Argisol, en Mossoró-RN, en un diseño de bloques al azar con seis repeticiones, correspondientes a los períodos de evaluación. Se evaluaron cuatro dosis de P (34, 80, 137 y 206 kg ha-1 de P2O5) y dos formas de fertilización: base (F0) y base más cubierta (F1). Para las dosis de base se utilizó triple superfosfato y para las dosis de cobertura, fosfato monoamónico (34 kg ha-1 de P2O5). También se aplicaron dos tratamientos de control: sin aplicación de P (C1); y la aplicación de P solo en cobertura a una dosis de 103 kg ha-1 de P2O5 (C2). Con la ayuda de un escáner de raíces portátil, las imágenes se capturaron a través de tubos transparentes (minirizotrones) de 0,6 m. El diámetro promedio de las raíces disminuyó con el aumento de la dosis de fosfato en la fertilización F0, pero se observaron raíces más delgadas en el tratamiento C2. Hubo una respuesta positiva de las dosis de P en el número de raíces solo en la fertilización F0. En ausencia de P, la reducción en el número de raíces fue más intensa que la de la longitud total.

Palabras clave: Citrullus lanatus, minirhizotron, fósforo

1. Introdução

Watermelon is a horticultural fruit crop that is best adapted to the levels of radiation and low relative air humidity of the Northeast region of Brazil; however, little is known about the development of this crop below the soil surface, which has an influence on the performance of the crop (Rocha *et al*., 2011).

One factor that affects root growth is the availability of phosphorus (P) for plants, to which the latter respond in the form of adaptations in root system morphology and architecture (Fita *et al*., 2012; Meng *et al*., 2014) as well as in the partition of assimilates between the roots and the aerial part (Marschner, 2012).

Insufficient supply of P to plants occurs frequently in soils with low P availability or high P adsorption capacity. These are the main reasons why large amounts of P are required in

fertilization, especially in tropical soils. Therefore, the general recommendation is the localized application of full dose of P to reduce contact with the soil.

Moreover, considering that under localized irrigation, crops tend to have a restricted root zone volume, frequent replenishment of P in the soil solution is required to meet crop demand (Mohammad *et al.*, 2004). In a study with tomato, Marouelli *et al.* (2015) reported that the application of the total amount of P at pre-planting resulted in lower absorption of this nutrient by the plant in the crop cycle process and suggested that application of P at pre-planting combined with top-dressing (fertigation) is a good strategy to ensure the initial availability of P for plant growth and to maintain adequate levels of P in the soil throughout the crop cycle.

In some cases, the mixture of phosphate fertilizer with larger fractions of soil increases phosphate fertilizer use efficiency, especially in soils with a low content of clay and aluminum and iron oxides, unlike in the typically acidic soils of the tropics (Barreto; Fernandes, 2002).

Thus, to better understand the effects of different methods of phosphate fertilization on plants, it is important to evaluate their effects on root traits. However, this information is still scarce because of the difficulty in measuring roots, especially under field conditions, and the low precision of the results (Linsenmeier *et al*., 2010).

The current techniques of image processing and analysis are well advanced, particularly in the study of plant roots (Jorge; Rodrigues, 2008), and allow recording and processing data on small-diameter roots, which collect most water and nutrients for a plant. According to Brasil *et al*. (2007), the use of the minirhizotron method coupled with a high-resolution camera has great potential for *in vivo* studies of plant roots.

Thus, this type of study may elucidate some aspects of the watermelon crop, a crop that is characterized by the intensive and rapid use of the available water and nutrient resources, thereby requiring a highly efficient production system. Therefore, the aim of the present study was to investigate the effects of phosphate fertilization on root traits of the watermelon crop irrigated under field conditions.

2. Material and Methods

The experiment with watermelon cv. Magnum was conducted in the experimental area of the UFERSA farm in Mossoró-RN, municipality of Alagoinha (5° 3'30.37" S; 37°23'58.21"

O, at an altitude of 72 m). The predominant climate of the region is warm and dry, type BSwh' according to the Köppen climate classification.

The analysis of the superficial layer of the soil in the experimental area showed the following chemical characteristics (0–20 cm of depth) according to EMBRAPA (2009): pH (H_2O) = 5.7; organic matter (g kg^{-1}) = 18.43; Mehlich-P (mg dm^{-3}) = 4; K^+, Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}, and H + Al^{3+} ($mmol_c$ dm^{-3}), and V (%) = 2.30, 4.40, 14.7, 5.0, 0.0, 24.8, and 52, respectively.

A completely randomized block design was used in the experiment in a factorial arrangement, with six replicates corresponding to the different assessment moments (12, 19, 30, 37, 51, and 58 days after emergence).

The treatments consisted of four different doses of P (34, 80, 137, and 206 kg ha^{-1} of P_2O_5) applied as basal fertilization (F0) and as basal + top-dressing fertilization (F1). Triple superphosphate (TSP) was used as the source of P in F0, whereas monoammonium phosphate (MAP) (61% P_2O_5), equivalent to 34 kg ha^{-1} of P_2O_5, was used in the top-dressing applications.

In addition, two control treatments were included in the experiment: C1 (without the use of P) and C2 (with only top-dressed P applied) (103 kg ha^{-1} of P_2O_5), as shown in Table 1.

Table 1 - Doses of P_2O_5 (kg ha^{-1}) corresponding to the two methods of fertilization (F0 and F1) and control treatments (C1 and C2)

| Basal	TD[1]	Total	Basal	TD	Total
--------------F0 --------------------------			--------------F1 --------------------------		
34	-	34	0	34	34
80	-	80	46	34	80
137	-	137	103	34	137
206	-	206	172	34	206
--------------C1 --------------------------			--------------C2 --------------------------		
0	0	0	0	103	103

[1]TD – top-dressing

Soil preparation for the experiment included plowing and harrowing and the creation of ridges of approximately 0.30 m in height and 0.6 m in width, set 2.0 m apart. Watermelon cv. Magnum was planted directly in the field on November 8th 2014.

Before planting, a 0.60-m long tube (minirhizotron) was installed parallel to the irrigation line in the experimental plots with an area of 24 m² (12.0 by 2.0 m).

Each tube was placed 10 cm from the dripper, and sowing was performed between the dripper and the tube. The tubes were placed at an angle of 45º, with the top 10 cm of the tubes remaining above the soil surface and the remaining 50 cm inserted in the soil. The images were obtained using a scanner (CI-600 Root Scanner) (Figure 1).

Figure 1 - Schematic drawing of minirhizotron + portable scanner used to capture images
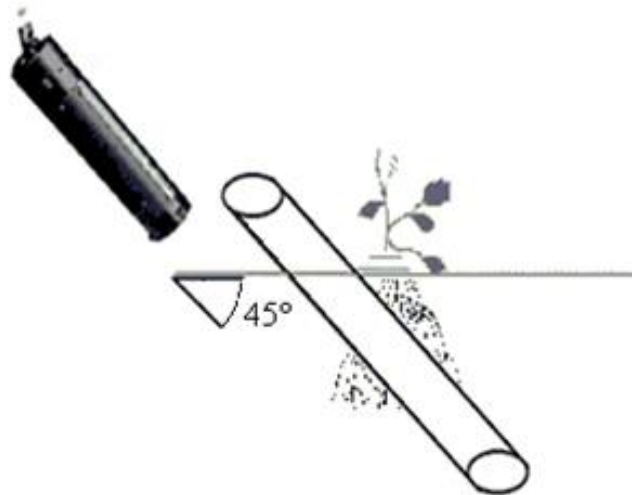
A drip irrigation system was used, with emitters spaced 0.30 m apart. The mean flow was 1.13 L h^{-1} (pressure of 0.65 kgf cm^{-2}) and the emission uniformity coefficient was 91%. Irrigation was managed by monitoring soil moisture using tensiometers installed at depths of 15 cm and 20 cm, and soil matric potential was maintained above −20 kPa.

The water used in the experiment had the following chemical characteristics: pH = 7.9; EC = 0.71 dS m-^{1}; Ca^{2+} = 1.84; Mg^{2+} = 1.36; K^+ = 0.75; Na^+=1.80; Cl^- = 2.40; HCO_3^- = 4.32; CO_3^{2-} = 0.00 (mmolc L^{-1}).

Basal phosphate fertilization was performed manually before planting at every 30 cm, adjacent to each emitter. The top-dressing applications (including the application of other nutrients) were performed through fertigation using fertilizer tanks connected to the irrigation system.

The net requirements of N and K were defined based on a model developed by Paula *et al*. (2011). The fertigation applications were started on the first week after planting, and the total applied amounts were 141, 148, 33, 7, and 28 kg ha^{-1} of N, K_2O, CaO, MgO, and S, respectively.

On the fourth week after planting, approximately 0.44 kg ha^{-1} of B, in the form of boric acid, was applied through fertigation as well as 1.30 kg ha^{-1} of chelated micronutrients

YaraVita Rexolin® (2.1% of B; 0.36% of Cu; 2.66% of Fe; 2.48% of Mn; 3.38% of Zn; and 11.6% of K_2O; 1.28% of S; and 0.86% of S).

Two images were obtained (21.6 cm by 19.6 cm with 100 dpi resolution) by scanner of each measurement along the acrylic tube, which were overlaid to produce a single image of 42.4 cm in length and 19.6 cm in width, corresponding to the perimeter covered by the camera. The images were subsequently edited with the PhotoScape software (version 3.7) and analyzed using the Safira software (version 1.1) according to the method proposed by Jorge and Rodrigues (2008) to obtain the number, volume, area, diameter, and length of the roots.

The root length density (RLD) was calculated using two alternative methods (Johnson *et al.*, 2001): RLD1—relates the number of observed roots (N) to the area of the image (A) (considering a theoretical conversion factor of 1 m m^{-1}) (Equation 1) and RLD2—considers the total length of the roots (TL), assuming that the 2D image of the minirhizotron has a depth of field (DF) of 0.002 m (Equation 2).

$$RLD1 = \frac{N}{A} \quad \text{(Equação 1)}$$

$$RLD2 = \frac{TL}{A \, x \, PC} \quad \text{(Equação 2)}$$

The data were transformed in $(x + 1)^{0.5}$ and subjected to analysis of variance by the F test, with $P < 0.05$, using the SISVAR software (version 5.3; Ferreira, 2010), and regression analysis was subsequently applied using the Table Curve 2D software (Scientific, 1991). The best-fitting models that provided the simplest and most coherent explanation were selected. Significance (* and ** indicate 5% and 1% probability, respectively) of the models was tested by the F test and that of the equations' coefficients was tested by the t-test.

3. Results and Discussion

There were no significant differences in the root system volume between the methods of P fertilization and between doses of P. The overall mean volume was 440.28 mm³ (Table 2).

Table 2 - Summary of variance analysis for the number of roots - NR, volume - Vol (m mm^3), area (mm^2), diameter - Diam (mm), lengths total - CT (mm) and average - CM (mm) Length of roots - DCR (m m^{-3}) of watermelon crop 'Magnum' submitted to the management of phosphate fertilization.

| SV[1]	DF[2]	Vol.	Area	Diameter	TL	ML	NR	RLD$_1$	RLD$_2$
					Mean Squares				
Blocks	5	607.22*,	2837.04*,	4.06E-04*	1488.79*,	0.09^{ns}	108.95*,	11962.11*,	7995.98**
Method	1	14.90^{ns}	96.65^{ns}	1.01E-03*	83.82^{ns}	0.12^{ns}	11.68^{ns}	1234.65^{ns}	449.39^{ns}
Dose	3	44.95^{ns}	196.01^{ns}	6.69E-04*	92.35^{ns}	0.51^{ns}	5.53^{ns}	596.27^{ns}	495.82^{ns}
T x.	3	16.03^{ns}	147.72^{ns}	7.36E-04*,	99.85^{ns}	1.59^{ns}	16.22*	1761.38*	535.80^{ns}
Fact[3]	1	114.41^{ns}	812.27*,	1.13E-03*	523.58*	9.17**,	105.93*,	11587.66*,	2812.19**
Fact vs.	1	2.04^{ns}	3.89^{ns}	5.79E-04^{ns}	3.87^{ns}	0.07^{ns}	2.56^{ns}	284.90^{ns}	20.57^{ns}
Error	45	33.06	144.36	1.66E-04	70.56	0.39	4.55	495.84	378.70
CV[4] (%)		27.37	25.35	1.02	24.40	16.21	22.48	22.83	24.42
Mean		440.28	2224.56	0.60	1184.48	14.32	89.03	9513.7	6349.95

[1]SV=Source of variation; [2]DF=Degrees of freedom; [3]Fact=Factorial: Method × Dose; [4]CV=Coefficient of variation; *Significant at 5%; **Significant at 1%; ^{ns}not significant.The variability of the obtained data may have hindered the detection of significant differences with regard to this characteristic. This problem had already been pointed out by Lisenmeier *et al.* (2010) in reference to studies on plant roots.

Moreover, the frequent supply of water and nutrients near the emitters through fertigation may have reduced the need for root system expansion, thereby decreasing the differences between the factorial treatments. Eloi *et al.* (2007) evaluated the effects of doses of N and K on root distribution in soursop and stated that daily wetting through localized irrigation limited root development of plants in depth.

This may explain the significant difference in root area observed only between the factorial treatments (2,354.67 mm²) and C1 treatment (1,309.14 mm²). These values are useful as indices to compare soil management systems and interpret their effects on fine root dynamics under natural growth conditions (Brasil *et al.*, 2007) but are not comparable with other methods. Rocha *et al.* (2011) observed an area of 16,500 mm² for watermelon cv. Crimson Sweet under a conventional management system, having obtained the images through trenches.

There was a decrease in mean diameter (MD) with increasing doses of P, despite the low explanatory power of the regression equation (R² = 0.548*) (Figure 2). Greater root MD at lower doses in F0 may be a natural response aiming to increase the total absorption surface, despite the decrease in the area/volume ratio.

Figure 2 - Mean diameter of the roots of the watermelon 'Magnum' as a function of doses of P. (EqF0 is the equivalent dose of fertilization F0 for treatment C2)
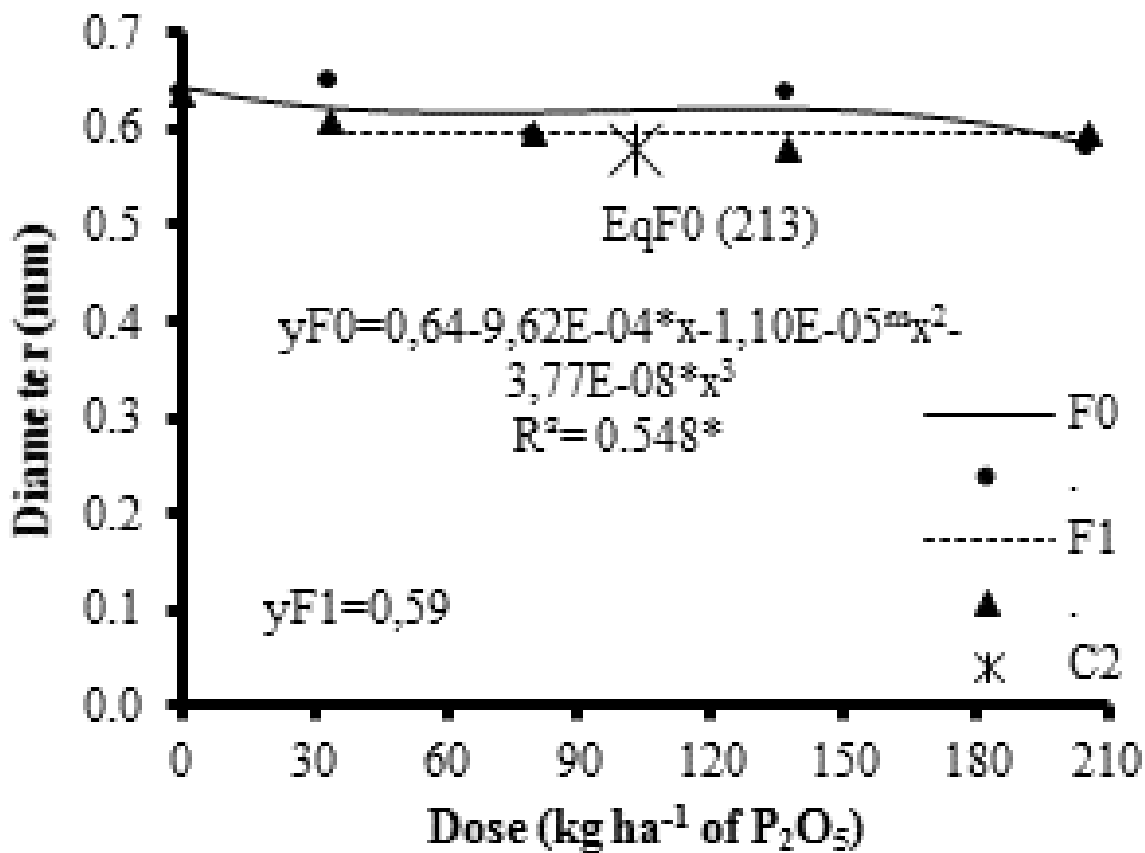
Rosolem *et al*. (2003) obtained the highest value of total root surface at the lowest dose of potassium and explained this fact with the presence of coarse roots. Xu *et al*. (2004) reported a smaller root length per unit of fresh weight of lettuce roots at lower concentration of P in irrigation water, which indicated coarse roots. This is a known response in cases of P deficiency.

Different doses of P in F1 did not have a significant effect on MD. MD in F1 was 0.59 mm, that is, equivalent to that of $F0_{196}$ (196 kg ha^{-1} of P_2O_5 in F0).

MD in C2 was similar to that obtained at the dose of 206 kg ha^{-1} of P_2O_5 in F0 (0.58 mm), which indicated a higher percentage of fine roots with this treatment andtherefore, greater root activity because they are considered to be more efficient in terms of water and nutrient absorption (Coelho *et al*., 2001; Rosolem *et al*., 2003).

In a study with banana seedlings, Pan *et al*. (2011) concluded that fertigation with P created an adequate microenvironment for the plants' roots and influenced their distribution and activity, although in the conventional treatment used as comparison in that study, the

solid fertilizer was incorporated into the soil. Barreto and Fernandes (2002) obtained higher corn root biomass and distribution via top-dressing (broadcasting) fertilization than via pre-planting fertilization.

Total length (TL) is directly related to the frequency of fine roots (ELOI *et al*., 2007). For this trait, only the result obtained in the comparison between the factorial treatments and C1 treatment was statistically significant (Table 1), with the mean in C1 treatment being 645.54 mm, corresponding to 52% of that obtained in the factorial treatments (1,247.62 mm).

Sá *et al*. (2013) observed a direct relationship between corn root growth in direct planting and increasing availability of P; however, the response was low at relatively high levels of the nutrient in the soil.

The higher TL value obtained with the factorial treatment may be associated with a higher number of root segments. The number of roots (NR) per cm of length was calculated to be 0.46 and 0.79 roots in C1 and in the factorial treatments, respectively.

Mean length (ML) of roots in C1 treatment (20.11 mm) was greater than that in the factorial treatments (13.23 mm) by approximately 52%. This result may be explained by changes in root morphology under conditions of P deficiency, namely, the elongation of the root hair (Marschner, 2012).

With regard to the number of roots (NR), different doses of phosphate in F1 did not have a significant effect (mean of 109.2), whereas there was an increase in NR in F0 with increasing phosphate doses, and NR in F0 was higher than NR in F1 at doses equal or higher than 158 kg ha^{-1} (Figure 3).

Figure 3 - Number of roots of the watermelon crop 'Magnum' as a function of doses of P (EqF0 is the equivalent dose of fertilization F0 for treatment C2).
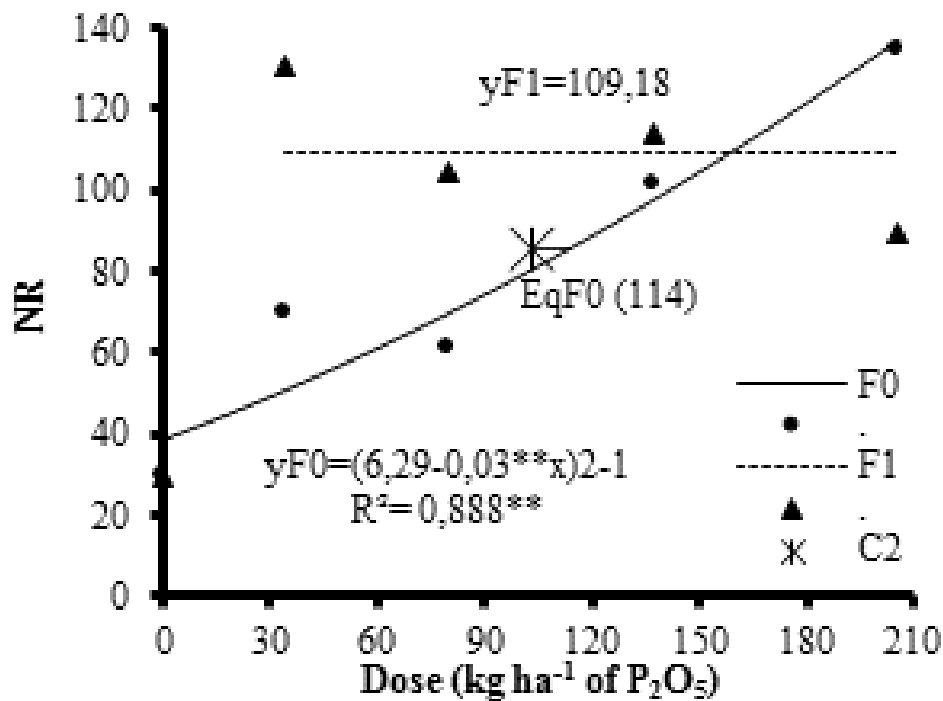
In C2, NR was equivalent to that obtained at the dose of 114 kg ha^{-1} of P_2O_5 in F0 (Figure 3).

It is possible that root turnover was affected by the higher proportion of fine roots both in C2 and F1. According to Tingey *et al*. (2005), despite the role of fine roots in the absorption of water and nutrients, they probably have a significant impact on the plants' carbon economy.

The behavior of RLD estimation using method 1 (RLD1) (Figure 4) was similar to that observed for NR because RLD1 uses the ratio between the number of roots and image area. In general, the values obtained by this method were higher than the mean obtained by method 2 (RLD2), namely, 6,689 m m^{-3} (mean of the factorial treatments).

Regardless of the method used to estimate RLD, the means were lower in C1 treatment than in the factorial treatments, up to 72% lower with RLD1 and 48% lower with RLD2. This is explained by the fact that in the absence of phosphate, the magnitude of the reduction in NR is higher than the reduction in TL.

The underlying cause for this is the elongation of roots in C1 treatment, a possible strategy of adaptation to low availability of P in the soil (Fita *et al*., 2012).

Meng *et al*. (2014) classified seven genotypes of watermelon with regard to responsiveness and P use efficiency and observed that the capacity of P absorption by the plants correlated with the morphological traits of roots.

Crusciol *et al*. (2013) evaluated the effect of different doses of P on rice crop and reported that although there was greater root growth with increasing availability of P, the roots were prioritized for the detriment of the aerial part when the availability of this nutrient was low. Xu *et al*. (2004) also observed this effect in lettuce crop.

Higher values of RLD do not necessarily equal better crop performance, especially in the present study, which was conducted under localized irrigation and in which root development was restricted to the wet volume of the soil, thus leading to a better utilization of P provided by fertigation (Mohammad *et al.*, 2004), particularly in a sandy soil with low P buffer power.

In fact, better performances in terms of yields were obtained with treatments C2, followed by F1 and F0 (data not published). However, caution is advised regarding the use of high doses of P in the fertigation of sandy soils with low P buffer power because of environmental risks. In addition, the initial effects should be considered as well as the long-term benefits of localized phosphate fertilization because of the higher residual effect of P.

In this sense, the combined supply of P in pre-planting and through fertigation appears to be a good strategy to ensure the initial availability of the nutrient for plant growth and to maintain adequate levels of P in the soil throughout the crop cycle (Marouelli *et al.*, 2015).

6. Conclusions

The mean root diameter decreased with increasing doses of phosphate in F0; however, fine roots were detected in treatment C2.

There was an increase in NR in response to increasing doses of P only in F0.

In the absence of P in fertilization, the reduction in NR was greater than the reduction in total length.

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