Influence of water deficit and canopy senescence pattern on sunflower root functionality during the grain-filling phase.

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ABSTRACT

- Root system size and viability are critical for water and nutrient uptake and grain yield realization. In spite of the potential importance of the issue for grain yield determination, root system functionality during the grain-filling phase has received little attention. The study reported here aimed at documenting root system functionality (water uptake capacity and respiration) dynamics during this phase and its responses to water deficit and to contrasting intrinsic patterns of canopy senescence.
- Plots of two sunflower (*Helianthus annuus* L.) hybrids of contrasting canopy senescence dynamics (Aguará 6 "stay-green" [SG, lower leaf senescence rate] and CF 101 "fast dry down" [FDD, higher leaf senescence rate]) were exposed to 2 levels of soil water availability (irrigation [control] and drought) during grain filling. Drought was imposed by suspending irrigation 8 days before anthesis. Measurements of root system variables (length density and viability [capacity to reduce tri-phenyl tetrazolium] in the 0-40 cm soil stratum), root respiration (*in situ*), stem xylem flow and leaf area were carried out.
- At the end of anthesis, control SG live root length density (LRLD) was 24% greater ($p<0.05$) than that of control FDD. After this stage, LRLD values declined in both hybrids at a similar rate (0.007 cm root/cm^3 soil.day). Thus, root senescence overlaps with the embryo filling phase. Water deficit hastened by 9 days the start of root senescence in both hybrids ($p<0.10$), and significantly ($p<0.05$) increased root senescence rate with respect to control values (by 38% in SG and 53 % in FDD).

Root respiration showed a pattern similar to LRLD. Greatest rates (0.65 gCO_2 m $soil^{-2}h^{-1}$) were achieved during anthesis and were similar ($p>0.10$) in both hybrids. Rates of decrease of root respiration in the control treatment were similar in both hybrids ($p>0.10$). Drought significantly ($p<0.10$) increased root respiration decrease rate by 42% in SG (0.0091 vs. 0.00128 g CO_2 m $soil^{-2}h^{-1}$, control vs. drought) and by 57% for FDD (0.0092 vs. 0.0144 gCO_2 m $soil^{-2}h^{-1}$, control vs. drought).

Leaf area index (LAI) values did not differ ($p>0.10$) between hybrids at the beginning of anthesis (BA) (SG = 4.6; FDD = 4.8). Decrease in LAI started to be significant at 14 days after (BA) in control treatments and from (BA) in drought treatments. SG-Control senescence rate was 17 % lower than FDD ($p<0.10$). Under drought treatments, FDD leaf senescence rate was 20% greater than that of SG ($p<0.05$). Changes in transpiration rates (mm/d) correlated with root senescence dynamics. Greatest values of transpiration were measured at (BA) (10 mm/d SG, 8 mm/d FDD). 14 days after BA transpiration flow in control treatments started to decrease at a rate that was similar between hybrids ($p>0.10$. Exposure to water deficit produced significant differences ($p=0.05$) between hybrids in transpiration dynamics. FDD showed a significant decline ($p=0.05$) in transpiration flow beginning 8 days after cessation of irrigation, while in SG this did not occur until 10 days later.

- We conclude that both water deficit and intrinsic canopy senescence dynamics can profoundly affect root functionality during grain-filling. The effects of these factors and their interactions, especially under drought, on yield merit focused attention in future research
- Heretofore there was poor or no evidence about root system behavior during the grain-filling phase. This study documents, for the first time in sunflower and many other crops, the dynamics of root system functionality during this critical development phase. In addition, it has served to highlight the effects of drought, canopy senescence patterns and their interactions on root functioning.

Key words: sunflower – root functionality – drought – canopy senescence

INTRODUCTION

It is well known that crop water uptake is crucially dependent on root system properties. Barley (1970) described two main root system characteristics which are involved in resource uptake, namely, root architecture and root longevity and functionality.

Root architecture has received considerable attention, however there have been only a few attempts to describe its dynamics, particularly during grain-filling. Fukai and Lilley (1994) demonstrated that root growth stops between 54 and 67 days after sowing in rice (*Oryza sativa* L.). In maize (*Zea mays*), Costa et al. (2002) observed that greatest values of root density and depth were achieved at flowering, and similar results were found in sugar beet (Vamerali et al, 2009). In sunflower (*Helianthus annuus* L.), the root system stops growing once flowering takes place (Sadras et al., 1989), coinciding with the timing of the greatest seasonal values for density and depth. Sadras et al. (1989) also noted that root system dynamics exhibit some similarity with those of leaf area. However, the second characteristic described by Barley (1970), has received little attention, and there have been only few attempts to describe root system longevity and functionality and their effect on crop water uptake capacity.

Commencing close to anthesis, grain number and weight per plant are set. Hence, crop water uptake capacity during that phase becomes a very important factor that can affect these yield components, and any reduction of root system functionality during this period will affect grain yield. It has been demonstrated, in many cultivated species, that water deficits during grain-filling phase decrease grain yield due to its effect on grain weight and also on grain number (e.g., Kirkegaard et al., 2007, wheat; Fukai and Lilley, 1994, rice; Chimenti et al., 2006, maize; Chimenti et al., 2004, sunflower; Kashiwagi et al., 2006, chickpea; Hammer et al., 2009, maize) and there are strong evidences which confirm the fact that increasing water uptake capacity during this phase may have important effects on grain yield in water stressed crops (Chimenti et al., 2004, 2006; Kirkegaard et al., 2007, Borrell et al., 2000; Hammer et al., 2009). The robustness of these connections contrast strongly with the poor knowledge of changes in root system behavior during the grain-filling phase. There are some evidences which indicate that root systems senesce during this period (Sadras et al., 1989), lose functionality (Fukai and Lilley, 1994), and their respiration rate falls (Hall et al., 1990). Another important contrast found in the literature is the poor information about root system structure and functionality dynamics during grain-filling phase in comparison to the better information about these issues during the vegetative phases of the crop. Here we report results of experiments aimed filling this knowledge gap using the sunflower crop, under both irrigation and drought, as a model.

Borrell et al. (2000) have shown, in sorghum, that intrinsic canopy senescence patterns can affect yield and water uptake capacity under terminal drought. A stay-green (SG) hybrid with delayed canopy senescence showed a greater grain yield and water uptake capacity under drought than a fast dry down (FDD) hybrid that exhibited rapid canopy senescence. In sunflower, de la Vega et al. (2010) have shown that both SG and FDD types can be distinguished. We have incorporated a SG vs. FDD contrast in our work.

A decidedly non-trivial aspect of most root functionality and water uptake studies is the duration of the intervals between measurements. In the work of Mengel and Barber (1974); Gregory et al., (1978); Fukai and Lilley (1994); Costa et al. (2002); Vamerali et al. (2009) and Dardanelli et al., (1997); observations often were carried out at long intervals, lasting as much as 12 to 30 days. While these first approximations have proved useful, it is evident that shorter intervals between observations would be advantageous, especially when focusing on the grain-filling phase, which may last 30-45 days in sunflower. This becomes even more evident if we consider that sunflower root respiration rates declined 50% during grain-filling (Hall et al., 1990) and root length declined 60% during the same period (Sadras et al., 1989).

The present study aimed at carrying out a spatial and temporal monitoring of root system functionality during the grain-filling phase of sunflower using, wherever possible, non-destructive techniques capable of providing data at short intervals between observations. Particular objectives included the detection of the moment of initiation and the rate of senescence of root system, as well the effects on these variables, under both drought and irrigation, of contrasting intrinsic patterns of canopy senescence.

MATERIALS AND METHODS

Experiments were carried out at the Facultad de Agronomía, Universidad de Buenos Aires (34º35´S, 58º29´W) during two growing seasons. However, in the second year of experiment, the transpiration flow could not be measured during the whole of the grain filling phase. Here we show data from the first year, noting that the patterns found in the second year were fully consistent with those of the first year. Two sunflower hybrids of contrasting canopy senescence dynamics were used: Aguará 6, identified as "stay-green" (SG), and CF 101, identified as "fast-dry-down" (FDD) (Sposaro, pers. comm.). Crops were sown at 7.5 pls/m^2 in plots specially designed to impose water deficit conditions. The whole of the experimental area could be protected from rain with a moveable rain-out shelter, every plot was isolated from its neighbors to prevent sub-surface lateral water flow, and every plot fitted with an independent drip-irrigation system. A split plot experimental design with three replicates was used, with irrigation level as the main plot and hybrid as the sub-plot. Each hybrid was exposed at two irrigation levels, defining four treatments: 1) SG-Irrigation, 2) SG-drought, 3) FDD-Irrigation and 4) FDD-Drought. In drought treatments, irrigation was suspended 8 days before the beginning of anthesis, and in control treatments, plots were irrigated until grain physiological maturity to maintain soil water at close to field capacity status.

Measured variables were:

A) Live root length density (LRLD): From the beginning of anthesis to physiological maturity, samples of soil between 0-40 cm [i.e., layers containing ca. 90% of the root system (Sadras et al., 1989, Angadi and Entz, 2002)] were extracted and roots washed free of soil. To distinguish between live and dead roots, roots were incubated in 2,3,5 triphenyl tetrazolium chloride (TTC), which produces a red stain if root mitochondrial respiration is operative (Sturite et al. ,2005). Treated roots were scanned and analyzed with WinRHIZO software (Winrhizo version Pro V 2008, Regent Instruments INC, Canada) to measure lengths of red (live) and other (dead) roots present in each sample. 5 samples were taken between 10 days before the beginning of anthesis (BA) and physiological maturity with 3 replicates for each sampling moment.

B) Apparent root growth dynamics: 2-m long by 50-mm diameter transparent acrylic tubes were inserted into the soil under the row at a 60º angle with respect to the surface, reaching a depth of 1.7m. Four tubes were inserted for each treatment. A sensor head, connected to a notebook computer and carrying a photographic camera could be inserted in the tubes. This technique not only serves to document root growth but changes in root color serve as a qualitative indicator of root functionality. Photographic images at 20-cm intervals were recorded every four days.

C) Sap flow: Xylem sap flow was measured using sensors (Dynagauge Model SGB19, Dynamax Inc, Houston, TX, USA) installed at the base of the shoot following manufacturer indications. This technique is fully detailed by Steiberg et al. (1989). Every 60 seconds, readings were taken and averaged every 15 minutes using a data logger Cambell CR10X (Campbell Scientific, Logan, UT, USA). 3 plants per treatment were daily measured.

D) Root respiration: Soil plus root and soil-only respiration was measured using a CO_2 soil production device (EGM-4 Environment gas monitor for CO_2 PP System Version 4.15). Measurements were taken over the row. Root respiration was estimated by subtracting soil respiration (after adjusting for effects of soil water content and temperature) from soil plus root respiration. Values reported here and normalized to a temperature of 25 ºC (Hall et al. ,1990). Measurements taken twice weekly during grain-filling with 3 replicates for each sampling moment

E) Leaf area: During the whole growing season, leaf area per plant was estimated from leaf width measurements. A leaf was considered senescent when at least 50% or its area was yellow. 9 plants per treatment were weekly measured.

F) Development: Phenological stages were recorded weekly for 9 plants per treatment following the Schneiter and Miller scale (1981).

RESULTS

Significant differences between genotypes for LRLD were found during the grain-filling phase (Fig. 1). At the end of anthesis (duration = 12 days), control SG values were 24% greater ($p<0.05$) than that of control FDD (0.52 vs 0.42 cm root/cm^3 soil respectively). From this stage until physiological maturity LRLD declined in both hybrids at similar rates (0.006 cm root/cm^3 soil.d) which did not differ significantly ($p>0.10$). Thus, the active root senescence phase overlaps with grain-filling. Using the images recorded in the minirhizotron tubes, we identified the initiation of root senescence as the time at which 10% of total observable root per tube changed color from white to brown. Exposure to water deficit significantly ($p>0.10$) hastened the initiation of root senescence by 9 days in both hybrids. The drought treatment also significantly ($p<0.05$) increased root senescence rate with respect to control values (by 38 % in SG and 53 % in FDD).

Root respiration dynamics showed a pattern similar to that LRLD (Fig. 2). Under control conditions, there was no significant ($p>0.10$) differences between hybrids during the grain-filling phase, both showing similar rates of decrease of root respiration. Greatest rates (0.65 gCO_2 m $soil^{-2}h^{-1}$) were achieved during anthesis and were similar ($p>0.10$) in both hybrids. The effect of drought differed according to the hybrid. Root respiration decrease rate increased significantly ($p<0.10$) by 42% in SG hybrid, which showed values of -0. gCO_2 m $soil^{-2}h^{-1}day^{-1}$ under control treatments and -0.0128 gCO_2 m $soil^{-2}h^{-1}day^{-1}$ under stress condicions. By contrast FDD increased its rate by 57 %, from -0.0092 gCO_2 m $soil^{-2}h^{-1}day^{-1}$ in control to -0.0171 gCO_2 m $soil^{-2}h^{-1}day^{-1}$ under drought.

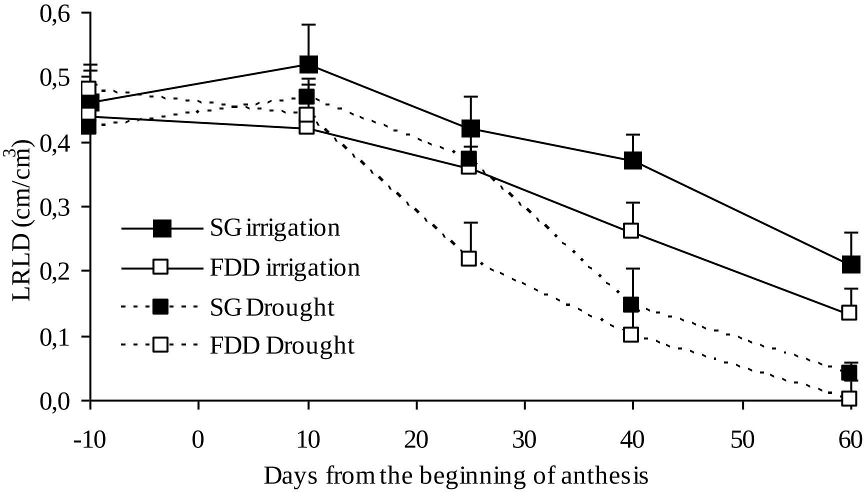

Fig. 1: Dynamics of live root length density (LRLD) (0-40cm) during grain filling for SG and FDD hybrids under 2 post-anthesis irrigation treatments. Full and dotted arrows indicate initiation of root senescence, in drought and irrigation treatments respectively, as judged from minirhizotron images. The broad arrow indicates physiological maturity.

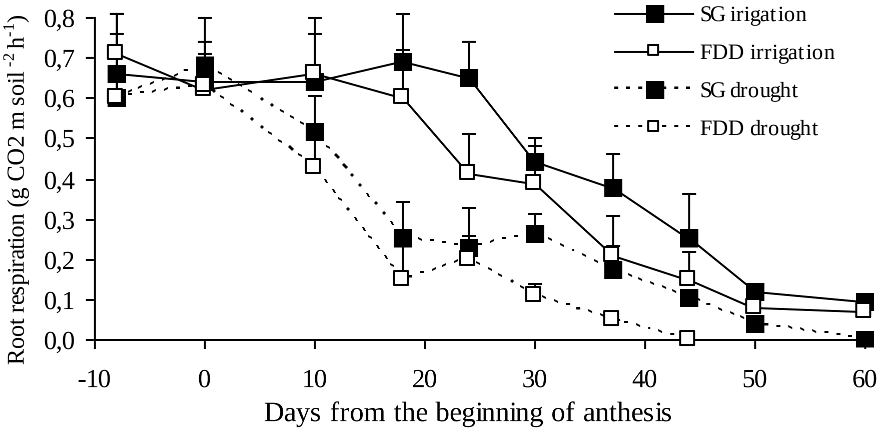

Fig. 2: Dynamics of root respiration during grain-filling in SG and FDD hybrids under 2 post-anthesis irrigation treatments.

At the beginning of anthesis (BA), leaf area index (LAI) values did not differ significantly ($p>0.10$) between hybrids (SG=4.6; FDD=4.8) (Fig. 3). Thus, all differences observed during grain-filling phase were not biased by differences in LAI established during the vegetative phase. Under control treatment, decreases on LAI were observed starting 14 days after BA in both hybrids. After that moment, significant ($p < 0.05$) differences between hybrids became evident. The SG hybrid showed a senescence rate 17% lower than FDD. Just as with the LRLD pattern, drought hastened the beginning of leaf senescence by 14 days in both hybrids and also significantly affected ($p<0.05$) its decrease rate, mainly in FDD, which leaf senescence rate was 20% greater than that of SG hybrid ($p<0.05$).

The greatest values of transpiration were measured at BA where SG hybrids reached values of 10 mm/day and FDD 8 mm/day. 14 days after BA transpiration flow in control treatments started to decrease at a rate that did not differ significantly ($p>0.10$) between hybrids. Changes in the transpiration flow over time (Fig. 4) appeared to correlate partially with patterns of LRLD, LAI and root respiration. Exposure to water deficit produced significant differences ($p=0.05$) between hybrids in transpiration dynamics at a very early stage. The FDD-drought treatment suffered a significant decline ($p=0.05$) in transpiration flow as from BA (8 days after suspending irrigation), while in the SG-drought treatment, this decline did not occur until 10 days later (Fig. 4). This effect was more marked than those visible in the patterns of LRLD, LAI and respiration (Figs.1, 2 and 3) for this treatment.

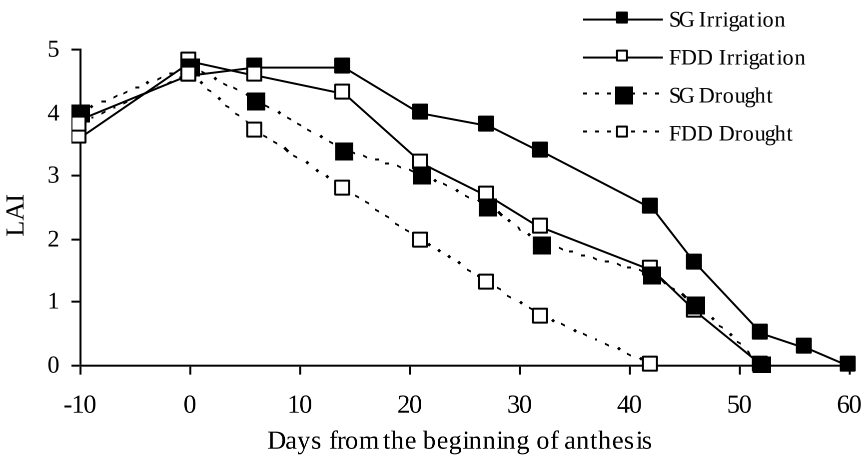

Fig. 3: Dynamics of leaf area index (LAI) during grain-filling for SG and FDD hybrids, under 2 post-anthesis irrigation treatments.

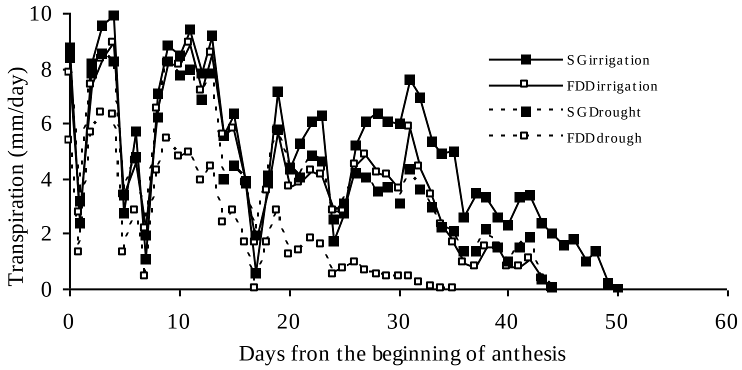

Fig. 4: Dynamics of daily transpiration flow (basal stem xylem flow) for SG and FDD hybrids under 2 post-anthesis irrigation treatments.

DISCUSSION

An important message emerging from this study is that both drought and intrinsic canopy senescence patterns have demonstrable impacts on root functionality during grain-filling in sunflower. Our results have also served to confirm and deepen previous observations (Sadras et al., 1989, Hall et al., 1990) of the loss of root functionality after anthesis in this crop.

While we measured several indicators of root functionality (LRLD, root color change in minirhizotron images, root respiration, and xylem stem flow), xylem stem flow was the variable that responded most rapidly and dramatically to treatments (FDD vs. SG, irrigation vs. drought). It is especially important to note that stem flow in the FDD hybrid under drought decreased much more (in proportion) than LAI (e.g., contrast Figs. 4 and 3 for the periods 8-15 and 20-30 days after BA). These differences are an encouragement to study the effects of intrinsic canopy post-anthesis dynamics on sunflower grain yield under drought. Thus, although these response variables undoubtedly interact, stem xylem flow is evidently the most sensitive and the best integrator of total effect.

To our knowledge, this is the first study to demonstrate an advantage, throughout the grain-filling phase and day by day, of the SG habit over the FDD habit in terms of water capture by the root system. Heretofore, the little information in literature about the SG effects on drought tolerance used, as indicators, water extraction over long periods, grain yield and/or green leaf area (e.g., Borrell et al., 2000).

REFERENCES

Barley, K.P. 1970. The Configuration of the Root System in Relation to Nutrient Uptake. Advances in Agronomy. 22: 59-201.

Borrell, A. K., G. L. Hammer and R. G. Henzell 2000. Does Maintaining Green Leaf Area in Sorghum Improve Yield under Drought? Dry Matter Production and Yield. Crop Science 40: 1037-1048.

Chimenti, C.A., Giuliano, J. and Hall, A. 2004. Osmotic adjustment, its effects on yield maintenance under drought in sunflower. 16th International Sunflower Conference: 261-266.

Chimenti, C.A., Marcantonio, M. and Hall, A.J. 2006. Divergent selection for osmotic adjustment results in improved drought tolerance in maize (Zea mays L.) in both early growth and flowering phases. Field Crops Research. 95: 305-315.

Costa, C., Dwyer, L.M., Zhou, X., Dutilleul, P., Hamel, C., Reid, L.M. and Smith, D.L. 2002. Root morphology of contrasting maize genotypes. Agronomy Journal. 94:96-101.

Dardanelli, J.L., Bachmeier, O.A., Sereno, R., and Gil, R. 1997. Rooting depth and soil water extraction patterns of different crops in a silty loam Haptustoll. Field Crops Research. 54:29-38.

De la Vega, A., Cantore, M., Sposaro, M., Trápani, N., López Pereira, M., and Hall, A. 2010. El papel del stay green en el rompecabezas de los determinantes del rendimiento de en girasol. Taller de girasol-ASAGIR-Mar del Plata.

Fukai, S. and Lilley, J.M. 1994. Effect of timing and severity of water deficit on four diverse rice cultivars I. Rooting pattern and soil water extraction. Field Crops Research. 37:205-213.

Gregory, P.J., McGowan, M., Biscoe, P.V. and Hunter, B. 1978. Water relations of winter wheat. 1. Growth of the root system. Journal of Agricultural Science, Cambridge. 91: 91-102.

Hall, A. J., Connor, D.J. and Whitfield, D.W. 1990. Root respiration during grain filling in sunflower: The effects of water stress. Plant and Soil. 121: 57-66.

Hammer, G, Dong, Z, McLean, G, Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Pszkiewicz, S. and cooper, M. 2009. Can changes in canopy and /or root system architecture explain historical maize yield trends in U.S. corn belt? Crop Science. 49:299-312.

Kashiwagi, J., Krishnamurthy, L., Crouch, J.H. and Serraj, R. 2006. Variability of root length density and its contribution to seed yield in chickpea (Cicer arietinum L.) under terminal drought stress. Field Crops Research. 95:171-181.

Kirkegaard, J.A and Lilley, J.M. 2007. Root penetration rate: A benchmark to identify soil and plant limitation to rooting depth in wheat. Australian Journal Agricultural Research. 47:590-602.

Mengel, D.B and Barber, S.A. 1974. Development and distribution of the corn root system under field conditions. Agronomy Journal. 66: 341-344

Sadras, V.O., Hall, A.J., Trapani, N.T. and Vilella, F. 1989. Dynamics of rooting and root length:leaf area relationships as affected by plant population in sunflower crops. Field Crops Research, 22: 45-57.

Schneiter, A.A. and Miller, J.F., 1981. Description of sunflower growth stages. Crop Science. 21: 901–903.

Steinberg, S, van Bavel, C.H.M. and McFarland, M.J. 1989. A gauge to measure mass flow rate of sap in stems and trunks of woody plants. American Journal Horticulture Science. 114:466-472.

Sturite L, Henriksen TM, Breland TA. 2005. Distinguishing between metabolically active and inactive roots by combined staining with 2,3,5-triphenyltetrazolium chloride and image colour analysis. Plant and Soil. 271: 75-82.

Vamerali, T., Guarise, M., Ganis, A. y Mosca, G. 2009. Effect of water and nitrogen management on fibrous root distribution and turnover in sugar beet. European Journal of Agronomy. 31:69-76.