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MORPHOLOGY AND BIOMASS VARIATIONS IN ROOT SYSTEM OF YOUNG TOMATO PLANTS (*Solanum* sp.)

Variaciones en la morfología y biomasa del sistema radical de plantas jóvenes de tomate (*Solanum* sp.)

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ABSTRACT. The scarce exploitation of genotypic variability present in plant roots is an attractive breeding choice with regard to abiotic stresses and supports the objective of this work, which is to identify genotypic variation in root system traits of tomato genotypes (*Solanum* sp.). Thus, five tomato genotypes were studied: the commercial hybrid cultivar Jaguar (*S. lycopersicum*), Pera, Volgogradiskij and PE-47 entry (*S. pennellii*), which were collected in Peru, and the interspecific hybrid PeraxPE-47. Plants were grown in hydroponics for 26 days since germination; their roots were extracted and images were digitalized on scanner to evaluate total length, average diameter, the projected area and root length, following the categories per diameter of the whole root system through *software* Win Rhizo Pro 2003. The dry mass of roots and aerial parts was also recorded. Results indicated that genotypes differed in morphology, length according to diameter, root system spatial configuration and biomass, mainly with respect to the wild salinity resistant species PE-47. The interspecific hybrid PxPE-47 could be used as a rootstock to increase salt tolerance of susceptible cultivars.

Key words: roots, morphology, diversity, rootstock

RESUMEN. La escasa explotación de la variabilidad genotípica presente en las raíces de las plantas, es una opción atractiva para la mejora genética ante estrés abiótico y sustenta el objetivo de este trabajo: identificar variaciones genotípicas en caracteres del sistema radical de genotipos de tomate (*Solanum* sp.). Se estudiaron cinco genotipos de tomate: Jaguar, cultivar comercial híbrido Pera, Volgogradiskij (*S. lycopersicum*) y la entrada PE-47 (*S. pennellii*), colectados en Perú y el híbrido interespecífico Pera x PE-47. Las plantas se desarrollaron en hidroponía durante 26 días, desde la germinación, se les extrajo las raíces y se digitalizaron las imágenes sobre escáner para la evaluación de longitud total, diámetro promedio, área proyectada y longitud de las raíces, según categorías por diámetro del sistema radical completo, mediante el programa Win Rhizo Pro 2003. También se obtuvo la masa seca de la raíz y parte aérea. Los resultados indicaron que los genotipos difirieron en morfología, longitud según diámetro, la configuración espacial del sistema radical, así como en la biomasa de las raíces, fundamentalmente, con respecto a la especie silvestre PE-47, resistente a la salinidad. El híbrido interespecífico PxPE-47 podría ser empleado como portainjerto para incrementar la tolerancia a la sal de cultivares susceptibles.

Palabras clave: raíces, morfología, diversidad, portainjertos

INTRODUCTION

Roots not only provide structural support to the aerial part of plants, but also supply nutrients and water. Thus, plant survival depends on its appropriate growth, development and root functions. Early in this century, the need for a second green revolution has been shown, this time leading to increase yields of crops grown on infertile soils of most farmers in the

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third world with little access to fertilizers (1). There are some reports supporting the importance of root system architecture as a development and agronomic trait (2), with implications for the whole plant architecture and growth, resistance to abiotic stress, nutrient acquisition and response to environmental changes (3).

To achieve tolerant cultivars to environmental stresses, the genetic variability of traits related to them is required. According to some authors (4), the understanding of genetic variability and control of root system (RS) architecture could help breeding programs improve crop tolerance to stressful environments caused by high soil salinization.

Moreover, it is known that root system architecture is characteristic of each species and is governed, according to some studies (5), by a genetically-controlled post-embryonic development program that determines high phenotypic plasticity in response to environmental stresses. There are few work reports including root system architecture in crop breeding mainly due to difficulties involved in root recovery of studies on *in situ* root system architecture, as a result of the lack of methods and suitable models for its study. In recent years, the scientific community has focused their interest on studying roots by means of using less difficult systems, such as aeroponics, rhizotrons and hydroponics (6, 7, 8).

Some advances have been reached through the model organism *Arabidopsis thaliana* (L.) Heynh, regarding root response to environmental stimuli, such as gravity and high salt concentrations, with two components: one of signal perception/transmission and another of final translation of these signs that apparently converge on auxin route; however, it has not been determined if environmental signals could also regulate or modulate the sites where lateral roots branch out (9).

Despite tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide, which is affected by environmental stresses, as for instance salinity, heat and drought (10), there is little information on the extent of genetic variation in traits associated with root system architecture and the potential of this variation to improve its tolerance to specific environments. In this case, it has been speculated about the role that root architecture seems to play along with anatomical changes in response to salinity in its ionic phase (11).

In tomato crop, it has been shown that grafting technique could be an alternative to increase cultivar tolerance to high salinity tenors, by using the roots of tolerant cultivars as rootstocks and the aerial part of the susceptible commercial cultivar as grafting (12).

In this crop, there is a great need of basic information about root system genotypic variability and the role it could play in the mechanisms responsible for crop tolerance to abiotic stresses. Consequently, the objective of this work is to identify morphological and architecture differences of tomato genotype root system.

MATERIALS AND METHODS

Plant material. The commercial tomato (*Solanum lycopersicum* L.) hybrid Jaguar that was supplied by Ramiro Arnedo S.A., Pera (P) and Volgogradiskij (V) cultivars as well as PE-47 entry (*Solanum pennellii* L.), which were collected in Peru (13), also including the interspecific hybrid PxPE-47.

Development conditions and experimental design. Seed surface from P, J, V, PE-47 and PxPE-47 genotypes was sterilized with commercial chlorine (sodium hypochlorite, 40 g active $Cl^- L^{-1}$) at 50 % for 10 minutes. After washing, they were placed on a moistened filter paper in sealed *petri* dishes under dark chamber conditions at 25 ± 1 °C. Seeds with radicles (~0,5 cm) were placed on silk cotton in Eppendorf tubes hollowed at the end, to make easy its contact with water. Once cotyledons were fully expanded, 20 plantlets of each genotype were transplanted together with silk cotton to Falcon tubes hollowed at the end; 10 of them were placed in each of two 76x52x13-cm plastic boxes that can hold up to 51,4 L, according to a randomized complete design, where they stayed in Hoagland nutrient solution at 50 % for 26 days, with aeration and weekly solution replacement.

Root image digitization. After 26 days, hydroponic plants were taken out, the aerial part and roots were removed. Roots were placed in an EPSON LA 1600+ (1600x3200 dpi) scanner to digitalize their images. Subsequently, these images were edited by hand (14) through Adobe Photoshop software, version 10.

Evaluated traits. The aerial part and roots were dried in an oven at 60 °C to assess dry mass. Total root length (L) (cm), average diameter (D) (mm) and root length ratio per diameter compared to total length (LD/L) were evaluated on digitalized and edited images (15, 16): LD1, very thin (x<0,5 mm), LD2, thin (0,5<x<2 mm) and LD3, small (>2 mm) whereas the projected root area (A) (cm^2) by Win Rhizo Pro 2003 software.

Statistical analysis. The variance analysis of a factor with randomized complete data was performed and means were compared by Tukey (P≤95 %) using IBM SPSS statistical package, version 20 for Windows (17).

RESULTS

Biomass of the aerial part was higher in every cultivar than in the wild entry PE-47 (*S. pennellii*); in the case of root dry mass, besides all cultivars differed substantially from the low value of PE-47, there were differences among genotypes, since root mass of Volgogradiskij cultivar and the interspecific hybrid PxPE-47 surpassed Pera and Jaguar.

Consequently, P and J cultivars had a lower root mass/aerial part relationship than V, PE-47 and PxPE-47 (Table I).

According to values of root biomass, total length (L) of the complete root system (CRS), average diameter and the projected root area (A), they were lower in PE-47. The average diameter of every cultivar root differed, the greatest corresponding to Volgogradiskij whereas Pera, Jaguar and PxPE-47 hybrid had similar average diameters (Table II).

Root length, following the categories per diameter, enables to know root distribution of distinct diameters in the complete system, recording differences according to the genotype evaluated; Jaguar and Pera cultivars, as well as PxPE-47 hybrid had approximately 80 % thin roots with smaller diameters than 0.5 mm. Instead, root length of this category developed in Volgogradiskij had approximately 70 %, while PE-47 differed from the other genotypes with nearly 90 % roots of this category (Table III).

Table I. Biomass evaluation in roots (RM) and the aerial part (APM), and RM/APM ratio in young plants derived from different tomato genotypes

Genotypes	MR		MPA		MR/MPA	
	mean	DE	mean	DE	mean	DE
Dry mass (mg)						
J	54 b	±7	691 c	±94	0,08 a	±0,01
V	71 c	±8	572 c	±66	0,13 b	±0,01
P	55 b	±9	612 c	±88	0,08 a	±0,01
PE-47	2,0 a	±0,6	21 a	±7	0,10 ab	±0,02
P x PE-47	77 c	±8	532 c	±42	0,14 b	±0,01

Different letters for P≤95 %

Table II. Genotypic effect on CRS morphological characteristics

Genotypes	L (cm)	D (mm)	A (cm^2)	
J	1222 b ±143	0,47 b±,06	182 b	±29
V	1227 b±104	0,57 c±,01	216 b	±17
P	1214 b ±198	0,46 b±,02	178 b	±48
PE-47	133 a±17	0,38 a±,03	15 a	±2
P x PE-47	1236 b ±191	0,49 b±,01	183 b	±21

Different letters for P≤95 % total length (L) average diameter (D) projected area (A)

Table III. Root length ratio per diameter compared to total length (LD/L) evaluated in young roots of different tomato genotypes

Genotypes	L D1		LD2		LD3	
LD/L						
J	0,78 c	±,06	0,20 b	±,02	0,02	±0,00
V	0,69 a	±,01	0,28 c	±,01	0,02	±0,01
P	0,77 c	±,05	0,21 b	±,04	0,02	±0,01
PE-47	0,89 d	±,01	0,10 a	±,01	0,01	±0,00
P x PE-47	0,75 c	±,02	0,23 b	±,03	0,02	±0,01

Different letters for P≤95 %
LD1 (x <0,5 mm)/LT, LD2 (0,5 < x <2 mm)/LT y LD3 (x > 2mm)/LT
longitud total (LD/L)

Likewise, Volgogradiskij recorded the highest root ratio (about 30 %) from 0,5 to 2 mm diameter, unlike PE-47 with only 10 % roots in this category, while the other genotypes had a similar root ratio (0,23-0,28) (Table III). There were few thicker roots (>2 mm) without genotypic differences, which is a characteristic of young 26-day-germinated plants. Such diameter length difference is one of the factors determining root system architecture or spatial configuration, distinguishing PE-47 and V from P, J and PxPE-47.

The figure shows the spatial projection of three related genotypes; PE-47 is notable for its very long and thin roots with few branches along the taproot, unlike P and the interspecific hybrid PxPE-47 had similarities (like V and J, not shown in this paper), which is characterized by plenty lateral roots coming from the taproot that are as long as this one, resulting in a large root mass with a lot of ramifications. Figure. CRS images of Pera cultivar, the wild entry PE-47 (*S. pennellii*) and PeraxPE-47 hybrid.

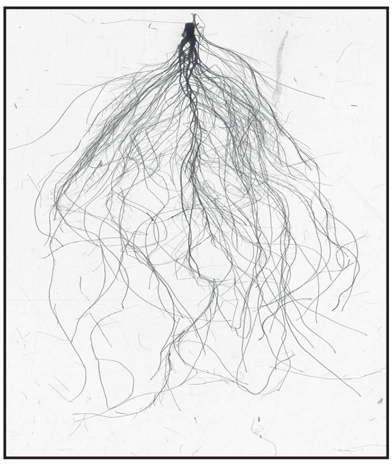
DISCUSSION

The work results at CRS level of young plants grown in hydroponics indicate that there are genotypic variations for several morphological and biomass traits (Tables I, II and III). The use of hydroponics allowed studying CRS without using destructive methods and root modifications caused by substrate barriers, such as soil compaction. This study on CRS in young developing stages has been useful in plants where nodal root traits have been identified, which serve for early screenings of root architecture breeding (18).

Unlike cultivars and the interspecific hybrid PxPE-47, the wild entry PE-47 (*S. pennellii*) has an extremely long root system that branches along the taproot; these roots have smaller diameter so CRS projects a smaller area (Table II, Figure 1) of low biomass; its system has 80 % very thin roots (Table III). These features enable to explore the deepest soil layers searching for water and nutrients, which is consistent with its origin and habitat (wild, Peru) (13), as well as with other studies performed in wild species from other genera (Cucurbita) (19). Root system differences also observed in this work between wild and cultivated species have mostly been recorded during domestication and breeding process, which has led to contrasting spatial arrangements (20).

When analyzing root results of the related genotypes P, PE-47 and PxPE-47, it can be observed that PxPE-47 hybrid has a similar root system to one of its parents, P cultivar, only surpassing it in its dry mass. CRS of PxPE-47 is characterized by plenty lateral roots, as long as the taproot and similar to P in architecture or spatial projection (Figure).

CRS architecture of a plant is determined, among others, by taproot length and lateral root density, besides that it plays a key role in defining whether a genotype is adapted or not to a specific environment (21). The fact that hybrid root system architecture is similar to its cultivated relative makes it attractive to be used as rootstock, because it has an adaptive spatial form to be cultivated, where not so deep roots are needed, but wide branches guaranteeing a greater relatively close area for water and nutrient absorption.



Pera **PeraxPE-47** **PE-47**

Images of SRC from cultivar Pera, wild accession (*S. pennellii*) PE-47 and Pera x PE-47 hybrid

It has been shown that some rootstocks were able to simultaneously increase fruit production and quality of susceptible grafted cultivars when irrigating with saline waters (12, 22, 23), which supports even more the potentialities of having PxPE-47 hybrid as rootstock, as it not only has a strong root but it could also contribute significantly to improve plant support, establishment and survival under biotic and abiotic stresses (24). A parent, like Pera (P), is a tomato cultivar with a semi-halophytic “inclusion” mechanism to be used as rootstock (25) and another one, PE-47 (*S. pennellii*) (13), has a recognized salt tolerance.

Rootstock qualities of PxPE-47 hybrid should be proved under salt stress conditions in future studies. The profitability of genetic variability in wild species, as a natural source of diverse response mechanisms to salt stress, will allow tolerance breeding and to deepen on the knowledge about response processes to that stress (26).

Most genotypes with a greater ratio of very thin roots (Table III), belonging to a tertiary root system, could have some advantages to face water and nutrient deficit or salinity stresses, since they are responsible for a greater portion of quick morphological plasticity responses (initiation, growth and death), the mechanisms by which a plant can adapt to its environment; however, the root system of V, with a higher ratio of thick roots (0,69 very thin and 0,30 thin), mostly lateral ones, belonging to a secondary root system, provides higher strength to anchor and penetrate soils with certain compaction.

CONCLUSIONS

- ♦ Genotypic differences recorded in this paper could have an impact on breeding programs related to adaptation, mainly those related to drought, salinity, nutrient deficit and soil compaction stresses, which must be demonstrated in future works.
- ♦ It is suggested to assess root system changes caused by stress, based on its levels of organization: secondary and tertiary root system separately, since the CRS evaluation provides little information aimed for breeding.
- ♦ Root management when using rootstocks that increase soil tolerance to environmental stresses is a promising choice to improve susceptible cultivar tolerance, but it is good for the market. To advance in this sense, various disciplines have to be integrated, from “omics” technologies, plant physiology, agronomy and breeding to those related with rhizosphere (20).
- ♦ Several studies linking the root traits evaluated at the early stage with the ones determining plant behavior at the adult stage under stressful conditions are required to assess its usefulness for an early selection.

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