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# Novel Topological-Architectural Parameters of Root Growth in Soybean (Glycine max (L.) Merrill) to Determine the Presence of Soil Mechanical Impedance

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## Abstract

**Objectives:** Mechanical impedance causes structural changes in roots. Nevertheless, little is known about the changes in soybean root systems grown under compacted soils (mechanical impedance). The aim of this work was to understand the morphological and topological-architectural changes occurred in plants root system of soybean grown under soil compaction. **Methods/Analysis:** Three experiments were carried out and three mechanical impedance levels were tested. Silt loam soil passed through a 2-mm-mesh sieve (Typic Argiudol Esperanza series) was used. Three soil compaction levels were determined: 1.1 $g.cm^{-3}$, null mechanical resistance (NR); 1.3 $g.cm^{-3}$, low mechanical resistance (LR); and 1.5 $g.cm^{-3}$, corresponding to high mechanical resistance (HR). Three soil resistances were consequent determine: < 0.1 MPA, 0.5 MPa and 3.5 MPa, respectively. Morphological, geometrical and topological-architectural roots parameters were measured. **Findings:** Plants grown in HR conditions had a root system confined to the first centimeters of the ground and showed shorter total root length, less number of lateral roots, higher diameter and low specific length. Growth form of root systems was sensitive to soil mechanical impedance even at resistance levels lower than 1 MPa. As soil impedance increases, lateral root growth occurs via the principal root rather than via the secondary roots and there were higher numbers of lateral roots on the principal root in the area from the proximal zone to the stem base. **Novelty/Improvement:** The main differences among NR, LR and HR plants were due to changes in the characteristics of the root system rather than in the shoot system, particularly in the root growth zone. As a conclusion, the present research demonstrates that there are morphological parameters that can be used to determine if crops have been exposed to soil compaction.

**Keywords:** Architectural Parameters, Compacted Soil, Glycine Max, Morphological Parameters, Root Growth, Root System, Topological Parameters

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# 1. Introduction

Abiotic stress like water deficit and salinity have been considered as a major environmental stresses affecting the performance of many crops[1]. These abiotic stress are commonly related with an increase of soil resistance[1]. Soil compaction is a major threat to arable land, especially in regions where mechanized agriculture dominates[2,3]. It is estimated that 68 million ha on the world[2] of arable land are degraded by soil compaction that is mainly caused by heavy agricultural machinery[4]. In particular, macro- and mesopores disappear during soil compaction resulting in decreased soil porosity and pore connectivity[5] and increased soil bulk density[6,7]. These initial effects of soil compaction on soil physical properties cause a set of subsequently altered properties affecting plant productivity[8]. On the one hand, increased levels of soil compaction cause increased mechanical impedance to roots[6,9]. Soil compaction causes slower water infiltration rates[10] and, subsequently, water deficit[11] or reduced gas diffusivity[12] resulting in a higher risk of water logging and anaerobic conditions[4]. Both increased penetration resistance and the risk of anaerobic conditions decrease root growth and, therefore, the agricultural productivity in production systems that are affected by soil compaction[2–4,9,13,14].

The soil bulk density from which the soil strength becomes so high that reduces or prevents root growth is denominated critical bulk density[15] and its value depends mainly on soil textural class. In[16] proposed critical bulk density for some textural classes: 1.30-1.40, 1.40-1.50 and 1.70-1.80 g cm$^{-3}$ for clayey soils, clay loam soils and sandy loam soils, respectively. However, some crops may grow even in compacted soils, depending on the plant characteristics[17]. Plasticity changes in plants that improve growth under high soil resistance conditions would be an important trait for crop selection or soil management practices.

High soil resistance produces structural changes in roots which contribute to characterizing those root systems growing in compact soil[18]. Nonetheless, no definition has so far been agreed to assess the degree of similarity or difference between the soybean root systems grown under contrasting impedance conditions.

Several studies agree in that the main changes in soybean root system consist of a low root elongation rate and an increase in root diameter[18–24]. Besides, further morphological changes also occurred as a result of soil mechanical resistance, such as changes in the transverse section of roots from a circular shape to an oval shape[20], an increase in the density of radical hairs[25] and an increase in the root angle[26].

It has also been observed that morphological changes in roots growing in compact soil are so prominent that they could be used as tools to identify soil layers with high mechanical resistance[18]. The latter varies both horizontally and vertically in a scale from meters to centimeters[27].

In[28] measured the taproot growth of cotton plants in compacted layers of different soils. They found that root growth was reduced as mechanical resistance approached 2 MPa. Primary roots are generally more sensitive to soil mechanical impedance increase than lateral roots[22]. This behavior has, nonetheless, not been observed in wheat in which the length of both its principal and lateral roots was similarly reduced[22].

Soybean is considered a highly important crop product in Argentina, particularly in the Humid Pampa and Brazil[29]. Previous research has demonstrated that soybean production has notoriously decreased as resistance to penetration increased due to farm machinery traffic[30].

The purpose of the present study was to determine changes in soybean root systems growing at three mechanical impedance levels which are the most important morphological alterations in root systems growing in compact soil. These morphological characters could be considered in genetic improvement programs as a novel parameters to determine if a determine soil is compacted.

# 2. Materials and Methods

Three consecutive experiments were performed at the experimental field in the Facultad de Ciencias Agrarias of the Universidad Nacional del Litoral (UNL) in Esperanza, Argentina (31º26´S, 60º56´W, altitude 40.5 meters above sea level). Ten plants for each treatment of soybean cv. RA 518 was grown in 10 L pots. Silt loam soil passed through a 2-mm-mesh sieve (Typic Argiudol Esperanza series) was utilised. Three levels of soil density were used, namely 1.1 g.cm$^{-3}$, 1.3 g.cm$^{-3}$ and 1.5 g.cm$^{-3}$.

Soil mechanical resistance was determined using an electronic penetrometer with a cone top 4 mm in diameter[31]. The resistances measured were < 0.1 MPa; 0.5 MPa and 3.5 MPa which corresponded to the densities 1.1 g.cm$^{-3}$, 1.3 g.cm$^{-3}$ and 1.5 g.cm$^{-3}$, respectively. The following levels of soil mechanical resistance were determined: Null mechanical resistance (NR), Low mechanical resistance (LR) and High mechanical resistance (HR) corresponding to 1.1 g.cm$^{-3}$, 1.3 g.cm$^{-3}$ and 1.5 g.cm$^{-3}$, respectively.

## 2.1 Soil Densification

In order to reach the above-mentioned densities, the amount of dry soil corresponding to each pot was firstly weighed. The soil was sprayed with Hoagland solution for a proper densification. The volume of Hoagland solution used to wet soil reached 15% of the dry soil used. After that soil was gradually densified every 4 cm o soil layers using a hydraulic press Pegasus. For a homogeneous densification, the compaction pressures 2 MPa; 7.5 MPa and 14.0 MPa were determined for the treatments 1.1; 1.3 and 1.5 g $cm^{-3}$, respectively.

## 2.2 Culture Conditions

Soybean seeds (cultivar RA 518) were sterilised superficially in 0.05% sodium hypochlorite solution for 10 min and then washed in distilled water. They were placed in Petri dishes with wet tissue paper in a growth chamber at 24° C during 2 days according to Schroeder[32]. Seedlings were transplanted in 10 L pots (26 cm height, 22 cm internal diameter) and were grown in growth chamber at 22.5° C with 13 h light/9 h dark during 18 days. Plants were watered with nutritive solution[33] at the beginning of the assay. The volume used was the necessary one to free 15% of the total porosity of compacted soil. In order to prevent nutritive solution from evaporating directly from the pots, these ones were protected with plastic bags having a small hole to leave the stem uncovered. Root systems were harvested once the plants had the first trifoliate leaf fully expanded, gently showed with water. They were fixed 48 h in a solution of formaldehyde, acetic acid and ethanol (F.A.A.)[34] and then conserved in ethanol 70 v/v.

## 2.3 Parameters Measured

### *2.3.1 Weight parameters*

Samples were put in a heater at 75° C until constant weight for the determination of the total dry weight of shoot system (LW) and the total dry weight of the root system (W). The root:shoot ratio (R:S) was calculated with these values.

### *2.3.2 Morphological Parameters*

#### *2.3.2.1 Shoot System*

Images were digitalized with a Nikon Coolpix 990 camera (Japan). The surface corresponding to the Total Leaf Area (TLA) was measured using Image Pro Plus software. The Specific Leaf Area (SLA) of the shoot system was subsequently calculated with the ratio TLA and LW.

#### *2.3.2.2 Root system*

The fixed roots were colored using a 0.1% neutral red solution[35] and they were digitalized with a Nikon Coolpix 990 camera (Japan). Total Length (TL) was measured whit computer on the digital images with 600 dpi of the root systems following the line intersection method[36]. Specific Length (SL) of root systems was calculated with TL and W. The following parameters were measured on the apexes of the secondary roots using Image Pro Plus software: Distance from the apex to the first tertiary root (l) and diameter from 1.5 cm of the secondary root apex (d) and the quotient of both variables (l/d named Nd1) was thus obtained.

Magnitude (M) of root systems, branching ratio (Rb) and relation between 1st order external and internal roots (EE/EI) were determined[37,38].

## 2.4 Statistical Analysis

A fully randomized design whit 10 plants per treatment was used. Both normality tests (Shapiro-Wilks) and variance homogeneity tests were carried out. Variance analysis and F test were also carried out for the determination of significant differences among the variables analysed. LSD test was used to compare the mean among treatments. Morphological and topological-architectural responses were analysed by mean of a Principal component analysis.

Info stat software version p2 was used for the statistical analysis[39].

# 3. Results

## 3.1 Shoot System Morphology

Soybean shoot system demonstrated not to be affected by soil densification under the experimental conditions of the present research (Table 1). NR, LR and HR plants evidenced no significant differences in TLA, LW and R:S ratio at the three mechanical impedance levels analysed (Table 1). However R:S ratio tend to increase at the highest mechanical impedance level (HR). Only SLA was significantly higher in LR plants.

**Table 1.** Morphological features of the root system and shoot system in soybean plants grown under three mechanical impedance levels

LW: Dry weight of the shoot system; R:S: Root stem ratio; SL: Specific length of the root system; SLA: Specific leaf area; TL: Total length of the root system; TLA: Total Leaf Area; W: Dry weight of the root systems. Plants were harvested after 20 days of transplant. Three experiments were conducted during 20 days in growth chamber under controlled conditions

| Soil mechanical impedance levels | Null | | | Low | | | High | | |
|---|---|---|---|---|---|---|---|---|---|
| *(g cm$^{-3}$)* | 1.1 | | | 1.3 | | | 1.5 | | |
| *(MPa)* | < 0.1 | | | 0.5 | | | 3.5 | | |
| | m | S.D. | | m | S.D. | | m | S.D. | |
| TL *(m)* | 2.7 | 0.5 | a | 3.8 | 0.7 | b | 2.1 | 0.5 | a |
| W *(g)* | 0.03 | 0.01 | a | 0.03 | 0.01 | a | 0.04 | 0.01 | b |
| SL *(m g$^{-1}$)* | 96.0 | 27.8 | a | 111.1 | 13.1 | a | 49.6 | 9.8 | b |
| TLA *(cm$^2$)* | 23.3 | 7.4 | a | 26.8 | 4.3 | a | 27.2 | 2.2 | a |
| LW *(g)* | 0.10 | 0.02 | a | 0.11 | 0.01 | a | 0.13 | 0.02 | a |
| SLA *(cm$^2$ g$^{-1}$)* | 217.7 | 32.6 | a | 251.7 | 21.1 | b | 217.2 | 20.4 | a |
| R:S | 0.3 | 0.1 | a | 0.3 | 0.03 | a | 0.3 | 0.04 | a |

In each row, different letters indicate significant differences among means at $P < 5\%$

## 3.2 Root System Morphology

At each impedance level, soybean root systems morphology and architecture evidenced variability in form (Figure 1) resulting from the root length, the distribution of lateral roots and the branching order in each treatment (Figure 1, Table 1).

Roots of NR and LR plants (Figure 1 A-F) grew all over the pots whereas those of HR plants (Figure 1 G-I) only explored the upper first 10 cm of the pots. In addition, as soil densification increased, the presence of secondary roots in the first centimetres of the pots also increased (Figure 1). In NR plants, the primary root showed a prominent vertical, longitudinal growth (Figure 1, G-I). The highest number of secondary roots was articulated on the primary root of NR plants and the presence of secondary roots close to the stem base was low. Root system development in LR plants was opposite to that in NR plants. Also, and although the principal root grew all over the pot, the secondary roots concentrated themselves in the proximal area of the stem base. The latter were also longer and they exhibited tertiary roots (Figure 1 A-C).

Both primary and secondary roots in HR plants (Figure 1 G-I) were shorter than those in NH and LR plants. The articulation of secondary roots with primary roots was superficial and their orientation was perpendicular to the axis of the primary root which also changed its growth direction after reaching a depth close to 10 cm.

Soil densification significantly modified the morphological variables of root systems (Table 1). Mean TL of LR plants was 3.809 m, this being a value notoriously higher than that evidenced by NR plants (2.645 m) and HR plants (2.144 m) between which, there were no significant differences.

In contrast to what was observed in TL, root dry weight (W) increased in the high level of mechanical impedance (HR). HR plants exhibited root systems which were significantly heavier (0.043 g) than those of NR plants (0.030 g) and LR plants (0.034 g). There were no differences in specific length of total root system (SL) between NR and LR plants. In contrast, statistically significant differences between NR and HR plants were detected. SL in HR plants (49.63 m g$^{-1}$) was 44% lower than that in LR plants (111.13 m g$^{-1}$).

Analysing the topological-architectural variables, only the Magnitude parameter (M) evidenced significant differences among the treatments (Figure 1). M was reduced with an increase in the soil mechanical impedance (304 and 378 in NR and LR plants respectively and 245 in HR plants (Table 2).

The geometrical dimensions of soybean radical apexes revealed significant changes as a result of soil compaction (Table 3). Distance from the apex to the first primary tertiary root (l) decreased as soil densification increased. In contrast, diameter at 1.5 cm from the apex (d) and the quotient of both variables (Nd1) evidenced a positive relation with soil densification (Table 3). The lowest diameter of secondary roots corresponded to control (NR) plants (0.40 mm). In contrast, the mean value for HR plants was increased up to 0.70 mm. The response of Nd1 as a function of soil densification was opposite to the response of d.

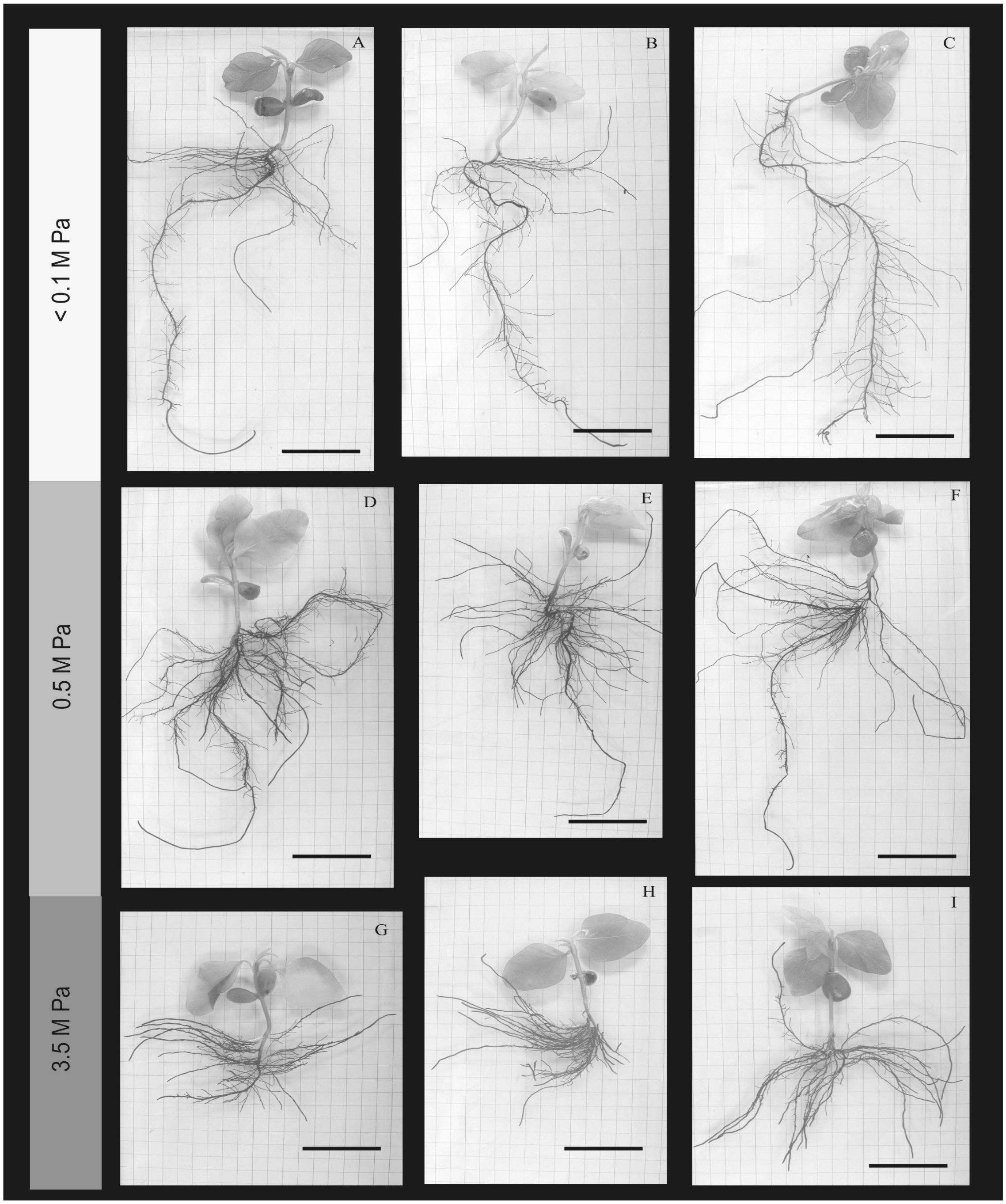


**Figure 1.** Root systems of soybean grown at three mechanical impedance levels.
Three experiments were conducted during 20 days in growth chamber under controlled conditions. AC: control plants under null soil mechanical impedance treatment (NR) D-F: plant grown at low soil mechanical impedance (LR) and G-I: plants grown under high soil mechanical impedance treatment (HR) Scale bar 5 cm.

**Table 2.** Topological-architectural features of the root system in soybean at three mechanical impedance levels
M: Magnitude; EE/EEI: External link-internal link relation and R b: Branching ratio. Three experiments were conducted during 20 days in growth chamber under controlled conditions.

| Soil mechanical impedance levels | Null | | | Low | | | High | | |
|---|---|---|---|---|---|---|---|---|---|
| *($g\ cm^{-3}$)*<br>*(MPA)* | 1.1<br><0.1 | | | 1.3<br>0.5 | | | 1.5<br>3.5 | | |
| | μ | S.D. | | μ | S.D. | | μ | S.D. | |
| M | 304 | 92.2 | ab | 378 | 104 | b | 245 | 62.9 | a |
| EE/EEI | 0.11 | 0.11 | a | 0.13 | 0.02 | a | 0.16 | 0.13 | a |
| Rb | 17.1 | 2.6 | a | 14.2 | 5.6 | a | 14.4 | 4.0 | a |

In each row, different letters indicate significant differences among means at P< 5%.

**Table 3.** Morphological changes of the secondary root apexes in soybean at three mechanical impedance
D: Diameter from 1.5 cm of the secondary root apex; l: Distance from the root apex to the first tertiary root; Nd1: Quotient of the l and d. Three experiments were conducted during 20 days in growth chamber under controlled conditions

| Soil mechanical impedance levels | Null | | | Low | | | High | | |
|---|---|---|---|---|---|---|---|---|---|
| *($gr.cm^{-1}$)*<br>*(MPA)* | 1.1<br>0.1 | | | 1.3<br>0.5 | | | 1.5<br>3.5 | | |
| | μ | S.D. | | μ | S.D. | | μ | S.D. | |
| d *(mm)* | 0.40 | 0.06 | a | 0.45 | 0.10 | a | 0.70 | 0.15 | b |
| l *(mm)* | 37.9 | 8.4 | a | 40.3 | 10.2 | a | 31.7 | 9.0 | b |
| Nd1 *(l/d)* | 93.4 | 19.2 | a | 89.5 | 16.0 | a | 45.9 | 11.9 | b |

In each row, different letters indicate significant differences among means at *P* <5%

## 3.3 Syndrome of Stressed Root Systems

When morpohological, topological and architectural parameter were analyzed together (Tables 1, 2 and 3), the shoot system and root system revealed certain associations among these variables which contribute to describing the response of plants to soil mechanical impedance (Figure 2). This response may contribute to clearly identifying the above-mentioned NR, LR and HR plant architectureal-toplogical types.

Principal Component Analysis (PCA). Reducing the multivariate space two principal component 1 and 2 not reducing the original variability, it was possible to found trends and associations between parameters and plants. Components CP1 and CP2 explained 64.8% of total variability (Figure 2). CP1 explained 39.8% of total variability and separated HR plants from the NR and LR plants. The auto vectors (more important variables at the moment of separate treatments) with highest weight in CP1 were d, l and Nd1 as attributes of roots and SL as an attribute of the root system. On the other hand, CP2 explained 25% of total variability and separated LR plants from the NR ones based on variables TLA, SLA of the shoot system and TL and M of the root system.

The relation between the variables found in the present research indicate that l, Nd1 and SL were positively associated with each other whereas they were negatively associated with d. As to the other variables, only positive associations were observed between W and LW, SLA with M and TL.

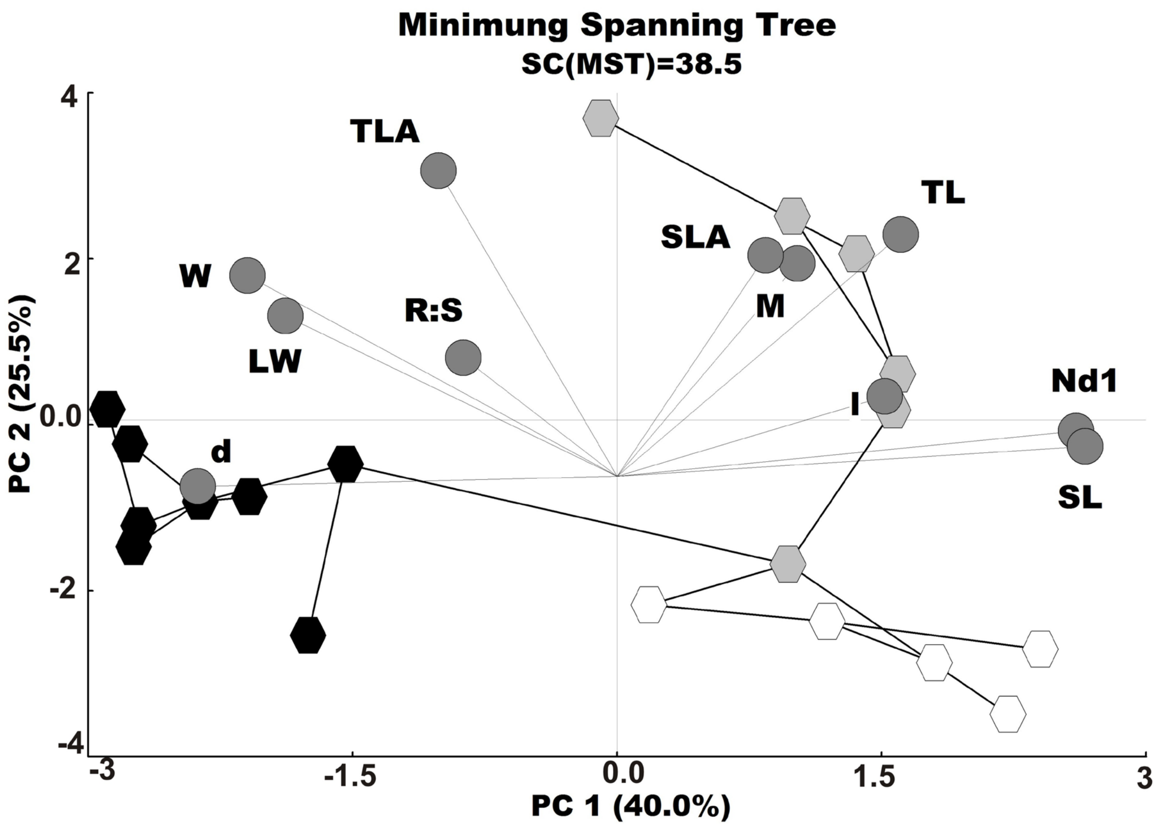


**Figure 2.** Principal components with standardized date and biplot.
D: Diameter from 1.5 cm of the secondary root apex; l: Distance from the root apex to the first tertiary root; Nd1: Quotient of the l and d; LW: Dry weight of the shoot system; M: Magnitude of the root system; R:S: Root stem ratio; SL: Specific length of the root system; SLA: Specific leaf area; TL: Total length of the root system; TLA: Total leaf area; W: Dry weight of the root systems. ●: HR plants. ●: LR plants and ○: NR plants.

## 4. Discussion

### 4.1 Shoot Morphology System

Soils with mechanical impedance have serious problems to agriculture as they reduce root-explored volume, thus restricting the access of roots to water and nutrients. This, in turn, reduces crop yields[40–44]. These restrictions alter the morphological, topological-architectural characters of the shoot system which are crucial over crop production components such grain number, grain weight and total biomass produced[41]. Previous studies had reported a decrease in the foliar elongation rate in TLA and LW in cereals[45,46], oil crops[47] and vegetable crops[42,48] when plants were grown in compacted soils.

Development of the soybean shoot system was not affected by soil densification under experimental conditions. It could be also observed that plants in the tree treatments evidenced no significant differences in TLA and LW. These results are opposite to those above-mentioned although they agree with those reported by in[41], who studied the effect of sub-superficial compaction on the growth of wheat and grain yields under field conditions. These researchers found that both LW and TLA were not affected by soil mechanical resistance. However, they did find a 12-23 % decrease in grain yield and a 9-20 % decrease in stubble production resulting from a lower number of tillers per area unit.

Root:Shoot ratio (R:S) is one the parameters most frequently used to indicate the occurrence of plant responses to the environment in which they grow[44]. A high R:S value is indicative of a higher turnover of belowground organs towards the root system[49]. R:S ratio in NR (0.28 $g.g^{-1}$), LR (0.32 $g.g^{-1}$) and HR (0.33 $g.g^{-1}$) was not significantly different, although it tended to increase in the highest impedance level (HR), suggesting certain capacity of adaptation of soybean plants under mechanical impedance.

Soybean SLA in the first vegetative stages varies from 200 to 250 $cm^2$ $g^{-1}$[50]. This is in agreement with the findings of the present study as in NR, LR and HR plants; the SLA differences recorded were within this interval. Only SLA was significantly higher in LR plants (251 $cm^2$ $g^{-1}$). This could be due to the fact that the shoot response to mechanical impedance is related to a reduction in the size of the mature cells of leaves[51] when plants grow in soils with mechanical impedance.

## 4.2 Root Morphology System

A biotic stress, including salinity, water stress and soil computation, have a large effect over a wide morphological and physiological parameters on plants[1,44,52]. Some effects of theses stresses are an increased difficulty of water absorption, reduced photosynthetic rates and an elevated ROS production[1].

At root level, two responses of root systems to soil mechanical resistance have been characterised to date, namely a decrease in the root elongation rate and an increase in the diameter of roots in the root growth area[53–55]. However, these are not the only responses manifested by root systems growing under mechanical impedance conditions. The similarities found in shoots in the present research are expected to disappear under field conditions if it is taken into account the three root systems types.

Growth form of root systems is genetically determined[56]. However, its expression is conditioned by the environment in which plants grow[57]. Growth forms of root systems in NR, LR and HR plants were different. The differences observed were due to changes in the root length (TL), the distribution of lateral roots (EE/EEI) and the branching order revealed by plants in each treatment (RB).

Bingham and Bengough[22] found that when roots of barley grew in soil uniformly compact (1.4 g $cm^{-3}$ and 1 MPa of mechanical impedance) primary roots were shorter than those in loose soil whereas lateral roots evidenced a 29% average length increase with respect to the plants growing in loose soil (1.1 g $cm^{-3}$ and 0.25 MPa of mechanical impedance). This is accorded with other results reported in previous studies[56,59]. In NR plants (Figure 1 A-C) the primary root evidenced an important development in depth and secondary roots articulated on it all along its length. The development of secondary roots close to the stem base was low. In contrast, in LR plants (Figure 1 D-F) the development of the root system was opposite to that in NR plants. Although the root explored all the profile of its container, the secondary roots concentrated in the proximal area of the stem base. This type of response in soybean evidenced the sensitivity of the branching process to mechanical impedance when NR and LR plants were cultivated with 0.01 MPa and 0.5 MPa, respectively, with no restrictions in nutrients, physical space and porosity.

Three different forms of soil exploration were identified in soybean. The first one shows a pivotal behaviour (control plants, NR). The second form of soil exploration is characterised by the apparition of shorter primary roots and the presence of high quantity of secondary roots. For last, the third type of soil exploration occurred in soybean plants summated to high soil resistance. In these case, roots only explores the upper zone of soil, presented low specific root length, higher values of d (diameter of root at 1.5 cm from the apex) an lower values of l (distance from the root apex to the first tertiary root) and Nd1 (l and d ratio). It is known that roots are in general abundant in the most superficial soil horizons[60,61]. This behaviour is more prominent when soil mechanical resistance restricts in-depth soil exploration. Roots therefore tend to be abundant in the superficial layers[62–64] when they are exposed to compacted soils.

High values of root Specific Length (SL), which are associated with high root growth rates, are indicative of an effective use of resources to maximise the contact with the soil[65]. In contrast, low values of SL are common in environments with physical and chemical restrictions for root growth[66,67]. In soils with mechanical impedance, SL is expected to be low as a result of the direct relation of W with impedance and to the inverse relation with TL[21,22,68]. According to what was describe, soybean plants decreased its Specific root Length (SL) significantly up to 49 m $g^{-1}$ in HR treatment. In general, SL is lower in Dicots than in Monocots. In[69] reported that for pea SL was 37.2 m $g^{-1}$ and 32.9 m $g^{-1}$ when plants grew with 0.5 MPa and 2 Mpa, respectively. In[70] analysing rice cultivars (*Oriza sativa* L.) found that in 21-day-old plants LE varied between 246 m $g^{-1}$ and 360 m $g^{-1}$.

The presence of lateral roots in the pericycle[71] increases the amount of new structures which, in turn, increase the ability of root systems to grow perpendicularly[55]. The magnitude of root systems is a direct parameter of this increase. Mechanical impedance inhibits both perpendicular growth of primary roots[72] and the regular development of lateral roots[18]. In[73] observed that in corn the number of secondary and tertiary roots decreases as soil mechanical impedance increases. In our study it was observed that M value was 304 and 245 in NR plants and in HR plants, respectively. This is in agreement with[73] findings and highlights the fact that the increase in soil mechanical impedance reduces the size of root systems in length as well as in the number of roots.

The branching pattern of root systems determines the ability of plants to take resources from the environment in which they grow and brings about consequences on the construction cost[61]. The topological-architectural herringbone-shaped systems are related to a high efficiency in soil exploration although the construction cost of these systems is higher than that of dichotomous systems, which, in turn, are related to a low efficiency in exploration[61].

A root system with low EE/EEi values and high Rb values is related to branching patterns of the herringbone type. NR plants have a tendency to the herringbone type with respect to HR plants. As to LR plants, they fall within an intermediate hierarchy. Although the differences recorded were not significant, soil mechanical impedance contributed to differentiating root systems from the herringbone type.

Because soil mechanical impedance affects cellular expansion[72,74–76] and consequently, both the growth and form of roots[26,77], when length and diameter of secondary root apexes were analysed, it could be observed that geometrical dimensions in soybean changed significantly with soil densification (Table 2). In addition, the roots grown under impedance conditions were shorter and wider. In NR and LR plants, l was lower than in HR plants whereas for d this effect was the other way around. This agrees with results collected by other researchers[26,68,72,76,78]. As a result of these changes, Nd1 had a negative relation with soil impedance level.

## 4.3 Syndrome of Stressed Root Systems

Studies about mechanical impedance on plants have, in general, been carried out taking into account root responses, which are eventually accompanied by responses of the shoot system[45–47,51]. In spite of their interdependence[55], the treatment of responses is in general univariate[49].

The decrease in crop yields reported to be the final consequence of the effects of mechanical impedance on plants[40–43], begins with the formation of different plants according to the level of mechanical impedance observed. Morphological differences in soybean plants cultivated with contrasting mechanical impedance (<0.1 MPa vs 3.5 MPa)22,[58,59] seem to be a logical consequence. However, the sensitivity of the changes is striking on account of the fact that between 0.1 MPa and 0.5 MPa there are also morphological differences which are enough as to identify NR, LR and HR (Figure 2).

The main differences among control plants (NR), plants under low soil mechanical resistance (LR) and under high soil mechanical resistance (HR) were due to changes in the characteristics of the root system rather than in the shoot system, particularly in the growth zone (d, l, SL and Nd1). In[79] demonstrated the interdependence among root elongation rate, root diameter and branching density. Soybean plants growing in soils with mechanical impedance (3.5 MPa) and with no nutrient-, water-, air-restrictions nor with physical space, are expected to have shorter roots with higher diameter and low Specific root Length (SL)[21,22]. As to diameter and because roots become flattened and their transverse section acquires an oval shape, the diameter measured is then the highest. In contrast, if plants grow in soil with a low level of mechanical impedance (<0.1 MPa-0.5 MPa) the main differences with respect to those growing in soil with high impedance level, are observed in the shoot system (total leaf area, TLA; and specific leaf area, SLA) and in the root system (TL, M). When mechanical impedance is lowest (0.1 MPa), plants are expected to have the smallest size in the foliar area, in length and in the number of roots. The way roots explore the soil was also different (Figure 1). At <0,1 MPa soil exploration is possible via an important growth of the primary root and of the secondary roots distributed all along the primary root length. When impedance is 0.5 MPa, root exploration occurs after the development of the secondary roots located in the area from the proximal zone to the stem base.

## 5. Conclusions

Soybean shoot system demonstrated not to be affected by soil densification under the experimental conditions of the present research. Contrary, soybean root systems under increased level of mechanical impedance evidenced variability in form resulting from the root length, the distribution of lateral roots and the branching order in each treatment.

Plants grown under the highest mechanical impedance treatment (HR) only explored the upper first 10 cm of the pots. As soil impedance increases, lateral root growth occurs via the principal root rather than via the secondary roots, and there were higher numbers of lateral roots on the principal root in the area from the proximal zone to the stem base.

The parameters distance from the root apex to the first tertiary root (l), the diameter from 1.5 cm of the secondary root apex (Nd1) and specific root length system (SL) contributed significantly to separate plants from different treatments.

It was possible to identify correlations between the different parameters studied. The relation between the variables found in the present research indicate that distance from the root apex to the first tertiary root (l), the ratio between l and the diameter from 1.5 cm of the secondary root apex (Nd1) and specific length of the root system (SL) were positively associated. These three parameters were negatively associated with the diameter from 1.5 cm of the secondary root apex (d). As to the other variables, only positive associations were observed between W and LW, SLA with M and TL.

Finally, three different forms of soil exploration were identified in soybean. The first one shows a pivotal behaviour (control plants, NR). The second form of soil exploration is characterised by the apparition of shorter primary roots and the presence of high quantity of secondary roots. For last, the third type of soil exploration occurred in soybean plants summated to high soil resistance. In these case, roots only explores the upper zone of soil, presented low specific root length, higher values of d (diameter of root at 1.5 cm from the apex) an lower values of l (distance from the root apex to the first tertiary root) and Nd1 (l and d ratio).

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