

# **Root Analysis of Quinoa Plant**

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#### Abstract

Different *Chenopodium* species have a specialized herringbone root system. They have a deep taproot with intense lateral branches. This root topology helps quinoa species to survive through adverse of situations. A plant's root system depends on both its genetic composition and environmental conditions. These variations in plant root architecture due to soil environmental conditions (physical and biological) are important factors that help plants to sustain through adverse conditions. This factor makes study of root architecture an important aspect of science. Study of root architecture can be done through several methods, one of which being through software such as WinRHIZO (described in this chapter). Further, microbial communities are an important aspect that determines root architecture of plants. To have a better understanding about quinoa's adaptation to adverse climatic conditions, this chapter also reviews some of the previous researches carried out to study microbial communities closely associated with quinoa in natural habitats. These communities are governed on the large part by fungi living in the rhizosphere as epiphytes and endophytes of the roots systems and then a few bacterial species that occur as seed endophytes in majority of quinoa seeds produced. Therefore, we can conclude that specialized root architecture of quinoa along with diverse rhizospheric microbial community is responsible for its sustenance under different abiotic stresses.

#### Keywords

 $\label{eq:Quinoa} Quinoa \cdot \text{Herringbone} \cdot \text{Root system architecture} \cdot \text{WinRHIZO} \cdot \text{Root topology} \cdot \text{Root endophytes} \cdot \text{Seed endophytes} \cdot \text{Rhizosphere} \cdot \text{Abiotic stress} \cdot \text{Bioproducts}$ 

8

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# 8.1 Introduction: Why Do We Study Root System?

Plant roots form the connection between the plant body and nutrient source, which is responsible for the life and being of the plant. They form the base on which all of the major processes and biochemical reactions depend and pave way for several beneficial as well as pathogenic interactions with microorganisms, parasites, arthropods, and nematodes. Roots form the channel through which a plant is able to absorb and assimilate nutrients from its surroundings for preparing its food and releasing gases into the atmosphere. Roots help the plant in the accumulation of water, nutrients, and hormones from the rhizosphere and also provide mechanical support to the plant (Merrill et al. 2002). Roots are also found to contribute in disease resistance, important microbial interactions, supply of C and N, and improving the quality of soil organic matter (Sainju et al. 2005a, b). The root network contributes to about 10-20% of the plant weight. Most of the root growth is genetically controlled: however, external environmental conditions also influence their architecture and their efficiency to trap nutrients. Root endophytic microbial interactions play an important role in plant processes as they import important secondary metabolites, enzymes, ions, and even antibiotics, which in turn help the plant to thrive and overcome harsh environmental conditions and pathogen attacks. This kind of a relationship is often mutualistic as the microorganisms benefit from the plant in terms of favourable habitat, shelter, constant supply of C for energy, water, and enzymes that help it to flourish abundantly. It is important to study the different characteristics of roots to understand the underlying mechanisms on which the life of the vegetation depends. Such studies further help in molecular analysis and biotechnological manipulations that can help in transferring a favourable trait from a donor plant to a lacking receiver plant variety or genus. Such experiments might result in the development of species with the ability to grow under extremely unfavourable conditions such as snow, rainfall, drought, and deserts.

## 8.2 Quinoa Root System

Various studies involving different species of *Chenopodium* have revealed a herringbone topology in root system branching. Quinoa plant consists of an extensive root system with a central taproot. It extends up to 30 cm under field conditions, with several lateral branches. According to this classification, there exist a main axis and primary laterals. This branching system favours plants in efficient acquisition of nutrients from a poor habitat. It has been found theoretically and experimentally that this type topology decreases root competition within the plants and thus increases nutrient use efficiency of plants. These root characteristics make quinoa a promising candidate for agriculture under different abiotic stresses such as water scarcity and sandy soil. Under abiotic stresses, quinoa develops a tendency of profligate root elongation with thicker roots. These thicker roots with extensive network of external links travel deep inside the soil for better exploration (Alvarez-Flores et al. 2014). It has been observed that under scarce nutrient availability, plants tend to develop more herringbone-like root system. However, herringbone root system is considered as an expensive root system. Under nutrient scarcity, this root system limits growth, which compensates with the cost of the root system. Therefore, any other species that have the characteristics to grow on infertile or arid soil should tend to develop herringbone root system. Fitter et al. (1991) performed experiments with different plant species and found that under low nutrient supply, plants tend to develop herringbone system, irrespective of being dicots or grasses. As a characteristic of this root system, plants tend to increase link lengths. This root system limits the competition among the roots of the same plant as well as among roots of the neighbouring plants. This helps to improve exploitation of limited water and nutrients available in soil. Furthermore, quinoa ecotypes growing under dry conditions tend to develop strong and deep main root axis to explore deep soil layers for water availability.

## 8.2.1 Case Study: Difference in Root Architecture of Quinoa Ecotypes Helps to Survive Drought Conditions

Alvarez-Flores (2012) performed an experiment with two contrast ecotypes of quinoa, i.e. Salare ecotype from the southern dry Altiplano of Bolivia with the annual precipitation of 150–300 mm and the coastal ecotype from the humid coastal lowlands of Chile with annual precipitation of more than 1200 mm. Two ecotypes differed in their morpho-physiological traits such as photosynthesis and transpiration, leaf morphology, leaf pigment content, stomatal movement, leaf water potential, and root lengths as well as in their pedoclimatic conditions, which include combined effects of temperature, water content, and aeration.

Both ecotypes were grown under water-limiting and water-available conditions. It was found that both ecotypes revealed a herringbone pattern of root architecture. Indeed, as previously stated, this root pattern helped in minimizing neighbouring competition under scarce water conditions. Constant examination of root system architecture of the two ecotypes led to better understanding of the importance of herringbone pattern under drought conditions. Under non-limiting conditions, Salare ecotype developed deeper primary root faster than the coastal ecotype. It was observed that until the first 6 weeks after seed germination, only primary roots developed in both ecotypes. It was only after the sixth week when growth of primary roots halted and development of other root system was accelerated due to ramification and elongation of the lateral roots (Fig. 8.1). In the same experiment, it was observed that under water-deficit conditions, Salare ecotype developed deeper roots as compared to the coastal ecotype (Zurita-Silva et al. 2015). All these observations were possible due to root system architecture analysis.

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Fig. 8.1 Study of root architecture of two different quinoa ecotypes at different time intervals (Zurita-Silva et al. 2015)

# 8.3 Drought Mechanism in Quinoa

Researchers have found that flowering and milk grain stages in quinoa are most sensitive to water scarcity. In order to withstand drought conditions, plants adopt any of the following three mechanisms: (1) morphological strategy (escape, avoidance, or phenotypic flexibility), (2) physiological strategy (antioxidants, stabilization of cell membrane, osmotic adjustments), and (3) molecular strategy (aquaporins, osmoprotectants).

Among all the different strategies, root system architecture of quinoa has been found to play an important role in drought management. Quinoa roots have been found to exhibit faster root elongation as compared to its closely related relatives. It has more abundant and longer external branching of the roots in order to improve its foraging capacity. Under water scarcity, quinoa tends to develop extended taproots with longer, coarser, and more abundant root segments than it does under wet conditions.

Furthermore, it has been found that quinoa roots inhabit several endophytic fungi and bacteria. In addition to different molecular aspects, these endophytic microorganisms tend to alter root architecture of plants. The alterations in root architecture thus produced support plants indirectly to survive under dry conditions (Hussin et al. 2017).

# 8.4 Root System Architecture

The overall spatial configuration of roots of different order and age comprises of root system architecture (RSA). RSA as a whole describes the different aspects of root structure and shape, collectively. Root structure defines the assembly and features of various segments of roots. Various plant developmental processes control root structure, i.e. its expansion, direction of growth, senescence of old roots, and development of new ones, whereas root shape defines spatial distribution of roots and its functional properties such as nutrient uptake, anchorage, and plant hydraulic. Any small variation in RSA of a plant could be responsible for its adaptation to its normal or extreme environment. That is to say, plant adapts to its environment by causing variations in its RSA, such as promotion or inhibition of primary root growth, through growth of lateral roots or through formation of root hairs (Lynch 1995). Since nutrients are heterogeneously distributed in the soil, variations in RSA lead to a significant difference in uptake. Variation in the spatial arrangement of roots is responsible for differential uptake of nutrients from the rhizosphere.

However, it is important to note that RSA do not directly control the nutrient and water supply to plants. Nutrient uptake is governed by various cellular and mechanical mechanisms such as cellular transporters and apoplastic transport. Similarly, water transport is basically governed by local water potential, xylem diameter, etc., but despite these facts, RSA indirectly affects nutrient and water transport in plants.

# 8.5 Study of Root System Architecture

Earlier, visual and manual methods were employed to study root system architecture of a plant. These methods proved to be very tedious, labour- and time-intensive, and more prone to errors. Sometimes it was not even possible for the researchers to measure root architecture. One of the early developed methods to measure total root length was line intersect (LI) method. This method was developed by Newman in 1966 (Delory et al. 2017). In this method, uprooted and washed plant roots were randomly placed in a tray of known area. Newman developed an equation where he estimated the total root length of a sample by counting the total number of intersections made by the root placed in a tray and randomly oriented straight lines, of known lengths, placed in a tray. Later, 2D image analysis method was developed for studying variability in plant roots. Different softwares were developed for easy computation of scanned root images. One such software developed was WinRHIZO.

#### 8.6 Method for Root Analysis Through WinRHIZO

WinRHIZO is an image analysis software designed for the measurement of root as a whole unit in different forms. It can be used to analyse root morphology and architecture along with its topology and colour. This is a user-friendly and cost-effective computer program which is combined with an image scanner component. The program is currently available in four versions (Basic, Reg, Pro, *Arabidopsis*), differing in the features offered (https://regent.qc.ca/assets/winrhizo\_ about.html).

#### 8.6.1 Image Acquisition by Root Scanner

Freshly uprooted and clean roots are gently placed over the glass scanner or Regent's water-proof tray. If Regent's tray is being used, the roots are placed in a thin layer of water. It is easy to position the roots in tray. The roots may overlap at some points during the scanning process. Image can be directly captured using WinRHIZO software, and can be stored in the desired format. The software then takes few seconds to analyse the image. Some analyses, such as nodule counting, colour assessment, and topology need interaction of the user before proceeding further. The software then saves the data obtained, which can be easily read and used further as desired (Fig. 8.2).

The software helps the user to analyse different aspects of root system in detail. The characteristics of root system can be analysed as follows:

**Root Morphology** The software facilitates automatic measurement of total length, average diameter, total area, number of tips, forks and crossings, and manual counting system for nodules. Further, it divides plant roots into different classes and subclasses based on their root diameter, surface area, projected area, and colour. The software makes it possible for the user to differentiate the entire root as a function of colour. It makes it possible for the user to measure root parameters of a specific colour or group of colours.

Link Analysis Measurements The software provides for the global analysis for the entire root image. It measures the total number of links, average link length,

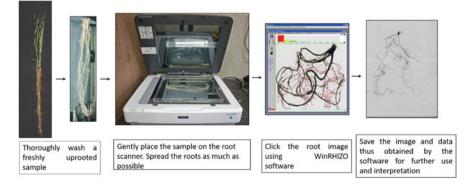


Fig. 8.2 Schematic representation of steps involved in roost system architecture analysis through WinRHIZO

diameter, area, volume, and branching angle. Link analysis is a study of basic morphology and connectivity of root segments. The software can perform this analysis for complete or incomplete root segment.

**Root Topology** The software measures external path length and altitude, globally for the entire root system. It also favours analysis of magnitude, path length, altitude, and structured connectivity for a single link. The user has to make sure that the root system to be analysed is not very dense and can be monitored visually. If not so, the data thus recorded would not be reliable.

#### 8.7 Importance of Root Analysis

Roots are majorly responsible for the acquisition of water and nutrients for plants. In addition, it is important to notice that around half of the food made by the plant is spent in the maintenance of root system. Therefore, it is important to monitor, how efficiently the roots can use water and nutrients in order to be as energy-efficient as possible for the plants.

The study of root functioning in different agricultural plants is necessary to plan sustainable cultivation patterns, maintain soil fertility and composition, and reduce the amount and cost of nutrient and water input without jeopardizing the nutritional quality of the produce. Root analysis helps the cultivators to predict changes in growth patterns, which provide them with sufficient time to adjust their practices to earn maximum benefits and profits. With proper use of resources, financial and external investments can be reduced, which will ultimately lead to more profit and better environmental protection.

About half of the food prepared by the plant through photosynthesis is directed and spent up in the roots for accumulating nutrients and water from the surrounding soil. Efficiency in root functioning directly affects plant yield and the composition of rhizospheric microbiota. Roots are important soil carbon pools and are thus being studied for their effects in various ecosystems. This acts to fight important phenomena to prevent climate change. The carbon added to the soil by the roots remains there for longer durations of time than that obtained from the decomposition of organic matter.

Root analysis helps in studying and reducing both biotic and abiotic stresses that affect plant growth. They give direct indication of plant health and give enough strength to cope up with the adverse situations by adjusting their growth patterns. Roots of the plant affect the soil profile just as much as the soil affects the root growth. Root exudates, composition of microorganisms in the rhizosphere, excretion of secondary metabolites, root respiration and gas exchange, root temperature regulation, water activity, secretion of plant hormones, nutrient regulation, conversion of compounds into easily assimilated forms, etc. are some of the important processes through which plant roots can affect the quality and biomass in the soil.

The following are the root analyses associated with different fields of agriculture and science (Trimble 2019):

- Agronomy: It helps in reducing biotic and abiotic stresses, which in turn helps to improve crop health and productivity. It helps in the identification of different symbiosis, nutrient, and water-use efficiency.
- Soil Science: Roots tend to modify chemical and physical nature of rhizosphere. Roots respire, form symbiotic association with different microorganisms, and secrete different exudates which vary under different circumstances. These root activities tend to change under different biotic and abiotic stress conditions. Therefore, root analysis becomes important.
- Ecophysiology: Under this field, researchers are concerned about the variations in functional, molecular, and physiological mechanisms of root systems when they interact with different microorganisms or with different roots.
- Climatology: Roots are a major source of environmental carbon pool. Root carbon has been found to be very important for the prevention of major changes in climate, and thus root carbon pool should be protected.
- Hydrology: Water availability is an important factor that controls plant root architecture. Root system varies greatly with the status of water in natural ecosystems. Researchers have found major differences in plant root system architectures grown in different ecosystems such as drought or wetlands.

Root analysis can help in identifying crop specific and beneficial microbial species for agro-biochemical formulations. One such example is by Ortuño Castro et al. (2013), in which they designed and performed an experiment where they aimed to isolate microorganisms surrounding the quinoa plants, and then formulated the beneficial ones to be used as bioproducts for increasing the quinoa produce in fields. Many different bacterial and fungal species were isolated, and out of them, several showed plant growth-promoting functions such as phosphate solubilization, nitrogen fixation, nutrient cycling, production of growth hormones, antibiotic production, and activities such as those of biocontrol agents. After proper greenhouse testing and analysis of the effects of these microorganisms, they were first cultivated on a suitable growth media. Then, a large-scale production of these microbial formulations was carried out. The aim was set to use simple inexpensive culture media to manage costs. After thorough testing, plant fields in natural environment were inoculated with these formulations and observed for growth changes (Ortuño Castro et al., 2013). The results showed positive effects in plant productivity. This is an excellent example of industrial microbiological processing for the use of naturally occurring microorganisms as fertilizers and biocontrol agents.

# 8.8 Root Endophytic Fungi of Chenopodium quinoa

A variety of endosymbiotic microorganisms, including mycorrhizae, bacteria, and endophytic microorganisms, are associated with each plant variety and affect its growth and nutrition in a certain direction. Certain endophytic fungi associated with roots are commonly observed to occur in angiosperms for which a comparatively high rate of colonization has been observed (González-Teuber et al. 2017).

Endophytic microorganisms are ubiquitous and colonize different parts and tissues of the plant without any signs of the disease and live as natural inhabitants of the organ.

The plant-microbe interactions are governed by several important ecological aspects, including host colonization patterns, mechanisms of nutrient uptake and transmission, production of secondary metabolites, type of biodiversity, energy source, and waste production. Endophytic fungi have shown to be important in providing Chenopodium quinoa with the ability to survive under stressful conditions. For conducting this study, González-Teuber et al. (2017) performed an experiment to investigate the diversity of fungal endophytes associated with the roots of *Chenopodium quinoa* growing near the salt lake of Atacama Desert, Chile. Although desert conditions provide one of the most challenging habitats for the growth of plants, quinoa still managed to survive in such a condition along with high salt stress. The Atacama Desert is considered to be one of the driest regions of the world with low water availability, high temperature, and irradiance. For this, he collected six quinoa plants from the experimental site, washed the roots thoroughly under running tap water to remove mud and soil debris, and performed surface sterilization. Small sections of the roots were cut and cultivated on PDA plates at room temperature for about 3–4 weeks and were observed for fungal growth. Pure fungal isolates were obtained, classified and grouped based on morphological characteristics. Only three pure isolates of fungi belonging to the same genus were considered for DNA extraction by growing isolated pure cultures and performing molecular extraction and identification techniques. Genomic DNA was then isolated, ITS1-F-KYO1 species identified using the primers were (CTHGGTCATTTAGAGGAASTAA) and ITS4 (TCCTCCGCTTATTGATATGC), and ITS region was amplified using the PCR technique. Purification and sequencing of PCR products were carried out by Macrogen in South Korea. Sequence alignment and preparation of phylogenetic tree were done using various bioinformatics tools and software.

It was observed that the roots of the sample plant were colonized by a large diversity of fungal endophytes and the community was mainly dominated by the genus *Penicillium* (59% of total culturable community), *Fusarium* and *Phoma* (<15%), and others (1–5%). According to the obtained phylogenetic tree, a total of 11 noticeable genera were obtained, which included *Penicillium*, *Alternaria*, *Fusarium*, *Rhinocladiella*, *Cadophora*, *Phoma*, *Bartalinia*, *Neonectria*, *Sarocladium*, *Coniochaeta*, and *Plectosphaerella*.

*Penicillium, Phoma*, and *Fusarium* have earlier been found to be associated with roots of many plant species, and occur to play important beneficial roles in plant's tolerance against abiotic stresses and plant growth (González-Teuber et al. 2017).

In deserted areas, plant survival under conditions of extreme water deficit and high temperature depends upon several integrated morphological and physiological responses that either help in preventing water loss or help the plant to tolerate such extremities. Plant cell architecture, transport mechanisms, root growth and biomass adjustments, water-use efficiency (WUE), decrease in stomatal conductance and transpiration, etc. are some of the factors that help plants survive in arid areas, but one factor that has been extensively studied and has been proven to play very important roles in survival under such conditions is microbial associations. Association of the plant with symbiotic fungi is a beneficial mechanism to regulate water stress tolerance (Malinowski et al. 1997; Redman et al. 2001; Rodriguez et al. 2004; Rodriguez and Redman 2008).

In order to study in details the effects of the root endophytic fungi in response to drought conditions in *Chenopodium quinoa*, González-Teuber et al. (2018) conducted another experiment in which they collected seeds of *Chenopodium quinoa* plants growing around the village of Socaire.

A total of four plant groups were established, in which group 1 was inoculated with laboratory pure culture of *Penicillium minioluteum* (fungal endophyte which was found to be present as a common inhabitant in quinoa roots in his previous experiment) in a formulated solution having the concentration of  $1 \times 10^7$  spores mL<sup>-1</sup> and normal irrigation with sterile water, the second group plants were inoculated with the endophyte without any irrigation intended to induce drought stress conditions, and the other two groups were cultivated without the endophyte inoculation under irrigation and drought conditions, respectively, all under sterile conditions to prevent any contamination, only to study the effects of the fungal endophyte on plant growth in various conditions. All of the plants were supplemented with nutrients in the form of Murashige and Skoog (MS) nutrient solution and sterile vermiculite to support growth. The temperature of the laboratory was kept normal and maintained between 17 and 23 °C.

After careful observations over several weeks, it was found that the fungal endophyte helped the plant to tackle with drought stress conditions by increasing root growth, vigour, and development, and the positive effects were mainly below ground. Root biomass was significantly increased, and root-to-shoot ratio was improved by inoculation of this fungus. Such an increase in root growth enabled the plants to reach and proliferate deep into the soils for nutrition in such an arid climate. Decrease in shoot biomass was also observed in inoculated plants, which ultimately lead to reduction in water loss from leaves through evaporation. It was concluded that the relationship between the plant and the endophyte grew positive only under stress conditions and no such increase or adjustment in biomass took place under normal conditions (González-Teuber et al. 2018).

Urcelay et al. (2010) after experimentation published in their research paper that *Chenopodium quinoa* exhibited no mycorrhizal associations and showed no signs of colonization by dark septate endophytes in their roots. The family of Chenopodiaceae was already considered as non-mycorrhizal, and it was further confirmed by their work. Moreover, they observed that the roots of quinoa plants were infected with significant population of *Olpidium* sp. (*Chytridiomycota*), which are normally considered as harmless root pathogens but have been found to be responsible for the transmission of several plant viruses, which can lead to some of the severe plant diseases.

# 8.9 Root Endophytic Bacteria of Chenopodium quinoa

Noel Ortuño Castro et al. (2014) proceeded with a set of experiments with plants from the Bolivian Altiplano to study much of the bacterial species that were associated with cultivation of quinoa. In the research experiment, molecular identification revealed that *Bacillus aryabhattai*, *Bacillus horikoshii*, *B. megaterium*, *B. pumilus*, *Paenibacillus odorifer*, *Pseudomonas* sp., *B. subtilis*, and *Azotobacter* sp. were present in quinoa roots as endophytes (Fig. 8.3). These endophytes imparted several benefits to the plant, such as activation of jasmonic acid cycle by *B. subtilis* that induces plant resistance against pathogen attack, *Azotobacter* sp., *Pseudomonas* sp., and *Paenibacillus* acting as nitrogen fixers, production of the phytohormone indole acetic acid (IAA) by *Bacillus aryabhattai* and *Bacillus horikoshii*, and solubilisation of phosphorous by *Bacillus pumilus* and *Pseudomonas* sp.

*Bacillus thuringiensis* was also isolated as a root endophyte in quinoa which acted as an entomopathogen against *Lepidoptera* and several other insect species.



**Fig. 8.3** Endophytic bacterial colonies isolated from the roots of *Chenopodium quinoa* (C.F.— Ortuño Castro et al. (2014))

# 8.10 Conclusions and Future Prospects

A plant's root system, along with its rhizospheric community, is responsible for the survival of the plant. Quinoa's herringbone root system accompanied by its versatile microbial community helps the plant to survive through the adverse climatic conditions it grows in. Therefore, the study of both root system architecture and their native microbial community is important to have a better understanding of what helps plants to survive through various biotic and abiotic stresses. Hence, careful observations, detailed study of root system architecture, and microbial interactions are important in order to explore the gap in the present-day understanding of the subject and to delve into new possibilities and sustainable approaches to prevent crop diseases, minimize yield losses, and promise significant profits to the hardworking cultivators all around the globe.

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