Root Phenotyping for Drought Tolerance: A Review

Allah Wasaya 1,2, Xiying Zhang 1,*, Qin Fang 1 and Zongzheng Yan 1

1 Key Laboratory of Agricultural Water Resources, The Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, The Chinese Academy of Sciences, 286 Huai Zhong Road, Shijiazhuang 050021, China; wasayauaf@gmail.com orallah.wasaya@bzu.edu.pk (A.W.); fangqinhebei@163.com (Q.F.); yanzongzheng15@mails.ucas.ac.cn (Z.Y.)
2 College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus, Layyah 31200, Pakistan

* Correspondence: xyzhang@sjziam.ac.cn; Tel.: +86-311-8587-1762

Received: 16 August 2018; Accepted: 29 October 2018; Published: 31 October 2018

Abstract: Plant roots play a significant role in plant growth by exploiting soil resources via the uptake of water and nutrients. Root traits such as fine root diameter, specific root length, specific root area, root angle, and root length density are considered useful traits for improving plant productivity under drought conditions. Therefore, understanding interactions between roots and their surrounding soil environment is important, which can be improved through root phenotyping. With the advancement in technologies, many tools have been developed for root phenotyping. Canopy temperature depression (CTD) has been considered a good technique for field phenotyping of crops under drought and is used to estimate crop yield as well as root traits in relation to drought tolerance. Both laboratory and field-based methods for phenotyping root traits have been developed including soil sampling, mini-rhizotron, rhizotrons, thermography and non-soil techniques. Recently, a non-invasive approach of X-ray computed tomography (CT) has provided a break-through to study the root architecture in three dimensions (3-D). This review summarizes methods for root phenotyping. On the basis of this review, it can be concluded that root traits are useful characters to be included in future breeding programs and for selecting better cultivars to increase crop yield under water-limited environments.

Keywords: root phenotyping; drought tolerance; root traits; X-ray chromatography; canopy temperature depression

1. Introduction

Drought is a major yield-limiting factor throughout the world [1,2], and is a widespread limitation for cereals production especially under dry land conditions [3–6]. Periods of water deficit during critical stages of crop development such as booting and/or grain filling, can greatly impact the yield stability and productivity [7]. Under drought stress, water and nutrient absorption may be reduced in crop plants [8]. Variation in climate alters the hydrologic cycle, and eventually reduces the availability of water due to a change in rainfall patterns, reduction in available water resources and reduction in water supply [9]. Contribution of albedo deserts increases temperature which reduces rainfall. This decrease in rainfall causes soil dryness and reduces vegetation cover [10]. Due to climatic variability, changes in temperature and rainfall patterns affect soil moisture availability. Roots are the main organs to respond, perceive and maintain crop yield under drought conditions. Plants with deeper root systems extract water from deeper soil layers and help the plants to avoid drought stress [11,12].

Plant root systems are essential for adaptation against different types of biotic and abiotic stresses. Apart from genotyping quantitative traits, phenotyping has been a major challenge for plant breeders to improve abiotic stress tolerance in crop plants. It includes genetically complex traits that are extremely difficult to measure, and would be ideal to assist plant breeders for using in breeding program [13]. Roots have been evolved to be responsive and extremely adaptive to the
local environment, their morphology, growth and physiology are closely related with plant genotype and growth medium properties. For example, elongation rate and number of lateral roots can be decreased by high soil water content or soil density and this can also be associated with shoot growth reduction [14]. The type of root distribution required for different crops depends on the target environment, as abiotic stresses experienced by roots have a significant effect on the crop yield [15,16]. Strong root development is essential for survival of seedlings in soils which undergo rapid surface drying, while sufficient moisture remains available in deeper soil layers. Therefore, good understanding about plant responses to abiotic stresses might be helpful in the selection of more resistant crop varieties [17].

The root system architecture (RSA) is affected by various factors such as soil temperature, moisture, nutrients and soil pH [18,19] which greatly affects crop growth and yield [20,21]. Several root characters such as morphological plasticity [22,23], root tip diameter [24], gravitropism [25], and rhizosheaths [26] allow the plants to adapt and respond to various environmental factors and they might be quite useful for improving water use efficiency in crop species [27]. Therefore, it is very important to understand the RSA regulating mechanisms for crop improvement [17,22]. Different types of roots have special features such as primary root length [28,29], length and number of lateral roots [30,31], crown root number [32,33] and cortical cell file number and cell size [34], which help in determining water absorption/uptake ability among various root types as an adaptation strategy under dry conditions [35,36].

Image-based phenotyping of plant roots is based on the non-destructive (where possible) optical analyses of plant traits [37,38], and its main objective is to characterize the plant’s anatomical, biochemical and physiological properties [39]. Root traits are more related to drought tolerance compared with above ground plant parts [20,40] and are key factors to maintain crop yield [4] under drought [41,42]. Root phenotyping is as important as shoot phenotyping, because plant’s ability to uptake moisture and nutrients mainly depends on root architecture and function [43,44]. Therefore, root phenotyping is important for crop breeding, although under field conditions, screening roots by phenotyping is a very difficult task [27].

This review mainly focuses on root traits which are useful for improving crop productivity under drought situation and their phenotyping through non-invasive techniques to save time. Out of the different root phenotyping techniques, X-Ray tomography is a non-invasive and time saving technique to obtain 3-D images of roots to decide which root trait might be useful for obtaining the high crop yield under drought. As single root morphology has scarcely been considered for plant’s adaptation strategy to drought conditions, different types of roots can be characterized by various traits [35] to determine the ability of various root classes for water uptake [36]. It is observed from previous studies that different root traits such as fine root diameter, specific root length and area, root angle, and root length density are useful for improving plant’s productivity under drought conditions. It is, therefore, suggested that all these root traits may be included in breeding programs to improve crop productivity under drought conditions.

2. Root Traits Related to Water and Nutrient Uptake and Drought Tolerance

Plant root systems consist of four different types of roots, i.e., (1) coarse or tap roots (first root to emerge from the seed) [45]; (2) lateral roots (any root branching from another root) [45]; (3) shoot-borne roots (roots which arise from shoot tissues) [45]; and (4) basal roots (roots which develop from the hypocotyl) [46]. Coarse or tap roots give anchorage to plants and establish root system architecture, control root system depth, and thus, determines a plant’s ability to grow under a compacted soil profile [47]. While lateral/fine roots are more active, they constitute a major part of a root system [36,48]. Different types of roots are present in different crop plants. For instance, wheat (Triticum aestivum L.) crop has mainly two types of roots; seminal roots arising from the embryonic seed part and nodal roots arising from the basal part of the tiller [49]. A more vertical angle and higher number of seminal roots
in wheat seedlings have been associated with more compact and deeper roots in wheat [50]. Different root traits have different function/s which are presented in Table 1.

Table 1. Root traits and their role for improving crop productivity under drought.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine roots</td>
<td>Extract water and nutrients from the soil.</td>
<td>[51,52]</td>
</tr>
<tr>
<td>Coarse roots</td>
<td>Support plants in soil, constitute root system architecture, control depth of root system and enhances plant’s ability to grow in compact soil.</td>
<td>[42,47]</td>
</tr>
<tr>
<td>Nodal roots</td>
<td>Harvest late season precipitation.</td>
<td>[42,53]</td>
</tr>
<tr>
<td>Root diameter</td>
<td>Regulates root length, surface area, increase water uptake under drought.</td>
<td>[54–56]</td>
</tr>
<tr>
<td>Root hairs</td>
<td>Assist in root contact with soil particles for uptake of water and nutrients as soil dries.</td>
<td>[55]</td>
</tr>
<tr>
<td>Root angle</td>
<td>Helps in deeper root growth and affects the area from which roots capture water and nutrients.</td>
<td>[45–57]</td>
</tr>
<tr>
<td>Root tissue density</td>
<td>Controls specific root length and specific surface area which increases plant’s performance and carbon economy under water stress.</td>
<td>[58,59]</td>
</tr>
<tr>
<td>Root length density at depth</td>
<td>Involved in efficient extraction of subsoil water.</td>
<td>[60]</td>
</tr>
</tbody>
</table>

Root traits affect the amount of water and nutrient absorption, and are important for maintaining crop yield under water stress conditions [4]. Plants with higher main root diameter have more growth potential as it has direct relation with water absorption [61], and have more ability to explore compact soil [18]. Fine roots are most permeable and thought to have greater ability to absorb water, especially in herbaceous plants [54]. This role becomes even more important in water and nutrient-deficient soils due to an increase in climate variability under current cropping systems [62]. Root architecture also has a significant impact on nitrogen use efficiency [52]. Increased early vigour results in deeper and faster root growth, forming more adventitious roots in the upper soil layer, which increases nutrient and water use and reduces surface soil evaporative losses [63]. In addition to these traits, several morphological root traits such as root tissue density (RTD), specific surface area (SSA) and specific root length (SRL) are correlated with increased crop productivity under drought conditions [64,65]. Root diameter and root tissue density control the root surface area and length; and hence, encapsulate the overall effect in terms of root length per dry biomass allocated to root system [64].

Several studies have reported the significance of a deep root system for uptake of water from deeper soil layers under water-stressed environments in various crops such as sorghum (Sorghum bicolor L.) [66], pulses [67,68], rice (Oryza sativa L.) [55,69], maize (Zea mays L.) [70], and wheat [71,72]. In a study, root length density in the active root zone area (up to 30 cm) improved water and nutrient uptake leading to higher yield of gram (Cicer arietinum L.) grown under water deficit conditions [73,74]. Sufficient water could be extracted by plants having root length density >0.5 cm cm\(^{-3}\) [75]. Wheat genotypes with deeper roots, higher root density at depth, and less root density at the surface have higher yield under rain-fed conditions [76]. Similarly, fine roots increase nutrient and water absorption through increased root surface area per unit mass [77]. The significance of a vigorous and deeper root system for more yield has been documented in soybean (Glycine max L.) [78], bean (Phaseolus vulgaris L.) [40], chickpea [5], wheat [72,76], barley (Hordeum vulgare L.) [6] and maize [9,10,70].

Under drought conditions, plant’s ability to extract water from depth has great relevance in balancing water relation as well as carbon assimilation. It is observed that plants with smaller root diameter and specific root length of fine roots are better adapted to dry conditions [79]. Deep roots are essential for small statured crops, such as wheat, rice and common bean (Phaseolus vulgaris L.) to extract
water from deeper soil layers \cite{47,80,81}. The root architecture gets revamped under drought conditions and facilitates the production of a large number of long lateral roots and root hairs which may enhance total surface area for water acquisition \cite{82}. Different water conservation approaches, such as leaf rolling, stomatal closure, leaf abscission and increased root mass especially at greater depth raise water status in plants, needed for higher yield and biomass production in crop plants. Superior root phenotypes are considered key components for improving drought tolerance characteristics which offer better performance under drought by efficient uptake and utilization of water in crops such as soybean \cite{83,84}. Deep root systems with higher rooting density are better considered to extract soil water mainly from deeper soil layers \cite{85,86}.

Plants may increase root length either by root fineness (RF) or reducing root tissue density (RTD), and/or increasing biomass allocation. For example, in sugar beet, changes in the RTD showed a strong relation to drought tolerance under conditions of drought stress \cite{87}. Similarly, in herbaceous tall grass prairie species, root fineness (RF) is considered a functional trait for drought tolerance \cite{88}. Root architecture as well as root size within the plant community depend on the level of competition for water, and its distribution in soil, which greatly affects the final crop yield \cite{89}. However, selection for deep and fast growing roots may enhance soil water harvesting and help in yield stabilization under water stress conditions.

3. Techniques Used for Root Phenotyping under Controlled and Field Conditions

Roots are an important plant organ and its phenotyping is as important as shoot phenotyping, because plant’s performance mainly depends on the root system \cite{43,44}. For root phenotyping, different techniques are used under laboratory as well as field conditions. The advantages and limitations for growth conditions (Laboratory/field) are presented in Table 2. For ease in the methodology, root phenotyping was first developed in the laboratory and then demonstrated in field to check its applicability \cite{90}. Root phenotyping methodologies typically combine some degree of automation with imaging and image processing. Image analysis approaches have been broadly used as reliable and fast root phenotyping techniques and have become available through different softwares, such as EZ-Rhizo \cite{91}, Smart Root \cite{92}, WinRhizo \cite{93}, Optimas analysis software, Image J \cite{94}, Root Nav \cite{95}, IJ_Rhizo \cite{96}, Root System Analyzer \cite{97} and Root Trace \cite{98}. Commonly-used systems for root observation are based on soil-less growth media. For this purpose, different techniques are used to grow plants, e.g., growing plants in paper rolls \cite{99–101}, gels \cite{50,102,103}, in air regularly sprayed with nutrient solution \cite{104} or in aerated aqueous solutions \cite{105}. Plants are also grown in hydroponics by using transparent plexiglas nail board sandwiches with mechanical resistance. These sandwiches were filled with glass beads of 1.5 mm in size for the circulation of nutrient solution \cite{106}. These systems measure root branching angles, total root length and related root traits manually or through imaging or visual rating. For image processing, high resolution cameras and/or scanners are used for resolving lateral roots, and mostly individual root diameter is used for decision criteria to differentiate between the main and lateral roots \cite{99} by using WinRhizo software \cite{107,108}. RSA can be analyzed through Smart Root software \cite{92} for the measurement of growth kinematics and branching angles of individual roots of a root system \cite{101}. These systems require some manual input for such analyses \cite{101}.

Image processing becomes a more challenging task when soil is used as a growth medium. To make the medium a more natural system or closer to it, soil-filled rhizotrons/columns are used to study soil-root interaction as it is difficult or even impossible to develop soil compaction or drying effects in soil-free systems. For this purpose, soil or any other growth substrate is used for filling of columns or Rhizotrons. Then root assessment along a transparent wall \cite{109} or within a soil column is performed by using X-ray–based computed tomography (CT) \cite{110,111} to visualize 3-D root configuration.

Due to the hidden nature of roots, there are certain limitations for the effective application of current phenomic technologies to assess root system architecture under field conditions for developed root system traits in marker-assisted selection \cite{112}. It is very difficult to assess roots optically in the
field, unless ones need to dig them out or approach them by making a tunnel. The most widely-used conventional methodology for field root study is the trench profile technique, in which soil is removed from the sides of the plant carefully using fine brushes then roots get drawn layer-wise from successive soil profiles [113]. Among the different phenotyping techniques being used in the field, the soil core method and standard excavation method are considered as the best techniques to explore density, depth and angle of root [112,114]. Soil cores are taken for the measurement of vertical root length densities or weights by using excavation techniques [115]. The core break method proposed by Reference [109] is the quickest method used to assess the maximum depth of roots in soil samples, in which about 2 m length soil cores are divided into different portions of 10 cm each for the determination of maximum root depth [100].

Several methodologies/techniques are used for the assessment of root mass as well as root distribution in different soil layers. These methodologies need labor and may cause some destruction to crops during sample collection, as plants are uprooted for the measurement of root architecture and root mass which need vertical pulling strength [110]. Out of these, Shovelomics is a technique widely used for root system analysis for field studies [111,112,116]. In this method, soil is excavated in such a way that one plant should remain in the center of the surface. Then roots are gently washed and the main root branches are analyzed for different root traits like root density and root angles. Different techniques are used to determine the basic root traits such as root dimensions, structure and root branching from simple counting [112] to imaging along with custom image analysis software [116,117].

Mini-rhizotron systems consisting of Plexiglas tubes containing small camera or scanner inserted in the soil to assess the surroundings of the root soil are also used in field studies [118]. Through mini-rhizotrons, limited genotypes may be monitored [119]. Different indirect methods are also used for analyzing RSA, such as root pulling resistance [110] or analysis of abscisic acid (ABA) content in the leaf [120].

An electrical capacitance measurement technique is also used for the measurement of total root mass which inspects the applied current response. One electrode inserted at the stem base and the other inserted in the rooting medium [121]. This technique has been used in the field for high throughput analysis of root mass [122]. However, recent studies have shown that root capacitance may be more associated with root circumference or its cross-sectional area at the soil [123] or solution surface [124]. These observations raised some doubt on its reliability, otherwise it is a good technique to find out the soil-root interactions and root phenotypes based on electrical properties. However, such techniques do not provide detail of root function, architecture, as well root anatomy, e.g., root hair densities under field conditions [27].

The development in non-invasive approaches such as X-ray computed tomography (CT), provides an excellent opportunity to determine 3-D root architecture in undisturbed soil cores in detail. It may be considered as an excellent tool for root phenotyping when compared to other destructive methods. In this method, roots are excavated first from the soil and then washed, imaged and finally analyzed with commercially available softwares [112,117]. CT has various advantages over other destructive methods. Though other different non-invasive 3-D visualization procedures exist, X-ray computed tomography (CT) is considered a good technique for soil-root interaction studies [125]. Different image-based softwares are used for the analysis of root traits, which have some advantages and limitations (Table 3).
Table 2. Advantages/disadvantages of methods used for growing plants for root phenotyping.

<table>
<thead>
<tr>
<th>Growth Environment</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>It allows for easy and non-destructive visualization of RSA; Easy to assess root growth; Non-destructive as it does not require any washing of roots; Time saving as it does not require soil excavation as in the field; Easily repeated under controlled conditions; Requires less resources; It is non-destructive method and gives clear picture of all root types and RSA.</td>
<td>The root system architecture in laboratory-grown plants does not accurately reflect what it is like in the field; Controlled conditions also eliminate possible interaction with beneficial microbes due use of growth media other than soil as in field conditions; As plants are grown in controlled conditions which prevents their exposure to environmental conditions, therefore, physiological relevance of roots need further evaluation.</td>
<td>EZ-Rhizo; RootNav; Root Reader 3D; X-ray computed tomography; SmartRoot.</td>
<td>[18,44,95,126–128]</td>
</tr>
<tr>
<td>Green house/Glass house</td>
<td>Close to the field conditions as the medium used may be soil or sand filled pots; Large number of varieties can be evaluated in a shorter period of time; Easy to handle the experiments compared with the field; Less time is required for root washing as compared to the field.</td>
<td>Some roots may be destroyed during washing; RSA may be affected by the growth container; As plants are grown in controlled conditions which prevents their exposure to environmental conditions, therefore, the physiological relevance of roots need further evaluation.</td>
<td>Root Reader 2D; WinRhizo.</td>
<td>[127,129]</td>
</tr>
<tr>
<td>Field</td>
<td>Give true picture or presentation of root structure; It gives clear physiological and practical picture as plants grow by facing all the environmental factors.</td>
<td>Roots form an extensive network which is difficult to excavate all the roots; Labor intensive and time consuming; Root excavation is very tedious and intensive work; Washing of roots is also time consuming; Destructive method as roots may be destroyed during excavation and washing. Problems may occur due to variability in the field or soil conditions.</td>
<td>Shovelomics; DIRT; WinRhizo; X-ray computed tomography.</td>
<td>[44,48,112,127,130]</td>
</tr>
</tbody>
</table>

Note: RSA—root system architecture.
Table 3. Techniques/softwares used for image analysis for root phenotyping under controlled/field conditions.

<table>
<thead>
<tr>
<th>Software/Imaging Technique</th>
<th>Growth Environment/Application</th>
<th>Image Type</th>
<th>Medium</th>
<th>Crop</th>
<th>Parameters to be Observed</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZ-Rhizo</td>
<td>Laboratory</td>
<td>2-D</td>
<td>Agar plates containing MS medium</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Main root (length, vector length, angle, number); Laterals (position, length, vector length, angle and number); Higher order laterals (order, position, length, vector length, angle and lateral root number).</td>
<td>Non-invasive image procurement with a newly developed program for root exposure, measures multiple root system parameters. Under different nutritional and environmental conditions phenotypic explanation of each plant species is analysed.</td>
<td>Due to the complexity of the root system roots growing too close to each other may not be correctly analysed; analyse images in 2-D.</td>
<td>[91,131]</td>
</tr>
<tr>
<td>RootNav</td>
<td>laboratory</td>
<td>2-D</td>
<td>natural sandy loam soil</td>
<td>Wheat, rice, <em>Arabidopsis</em>, <em>canola</em></td>
<td>Total length; Tip angle; Emergence angle; Start distance; Convex hull area.</td>
<td>An innovative, semi-automated method used for root architectural information. Faster and easier to use than manual methods Length results are reliable with manual measures, and RootNav has been found to be also.</td>
<td>It has only been written in C# using the NET framework libraries. The tool runs under Windows XP, Vista 7 and 8. Database access is achieved only using the MySQL Connector library.</td>
<td>[95]</td>
</tr>
<tr>
<td>Root Reader 3D</td>
<td>Laboratory (transparent gellan gum system) Hydroponics and sand culture</td>
<td>3-D</td>
<td>hydroponic and sand culture gellen gum</td>
<td>Rice</td>
<td>Primary and total root length; Number of crown and lateral roots; Root initiation angle; Exploitation volume; Exploitation index; Surface area; Convex hull volume; Gravitropic response and narrowness index.</td>
<td>It provides a flexible foundation for more extensive root trait analysis, uses a silhouette-based back-projection algorithm combined with cross-sectional volume; analyse images in 3-D.</td>
<td>Many root traits and quantitative techniques, including advanced dynamic and topological analyses, have not yet been incorporated into the software. It also requires calibration after every reading.</td>
<td>[126,132, 133]</td>
</tr>
<tr>
<td>X-ray computed tomography</td>
<td>Laboratory/ Field</td>
<td>2-D; 3-D</td>
<td>Gelzan or aluminum soda cans, silica beads, or potting soil</td>
<td>Barley, Wheat, <em>canola</em></td>
<td>Individual root angle; Total root angle; Individual root length; Total root length.</td>
<td>Examine the root growth behavior in soil without disruption, capture 3-D root structure and totally non-destructive.</td>
<td>Resolution is limited to 1000–2000 × and does not clearly analyse the cross-section diameter; as it requires high resolution, calibration of gray levels to attenuate coefficients is complicated.</td>
<td>[130]</td>
</tr>
</tbody>
</table>
Table 3. Cont.

<table>
<thead>
<tr>
<th>Software</th>
<th>Environment</th>
<th>Dimension</th>
<th>Soil/Plant Type</th>
<th>Root Morphology</th>
<th>Root Analysis</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmartRoot</td>
<td>laboratory</td>
<td>2-D</td>
<td>Hoagland solution Lupin, Maize</td>
<td>Growth rates; lateral root density; inter-lateral distances; branching angles.</td>
<td>Provides vectoral representation of roots and gives discrimination between root branching and root overlapping. Initially, this issue attracted attention because overlaps create biases in the estimation of the total root length.</td>
<td>Unable to estimate variables such as total root length, at which most root analysis software are usually very good, analyse images in 2-D.</td>
</tr>
<tr>
<td>Shovelomics</td>
<td>Field</td>
<td>2-D</td>
<td>sandy loam soil maize</td>
<td>Numbers; angles and branching pattern of crown and brace roots; angles; branching.</td>
<td>Quantify root system architectural traits of mature root systems in relatively high throughputs; Nodal root angles or the number of axial and lateral roots are determined from excavated and washed root systems by rating or counting.</td>
<td>Developed only to automatize the quantification of root architectural traits of maize root crowns.</td>
</tr>
<tr>
<td>DIRT</td>
<td>Field</td>
<td>2-D</td>
<td>gellan gum gel cowpea</td>
<td>Root top and root tip angle; maximum and median width of root system.</td>
<td>Measures traits of monocot and dicot roots from digital images, automates the extraction of root traits by making a high-throughput grid.</td>
<td>Measurements are inspired by the Shovelomics standard for root excavation.</td>
</tr>
<tr>
<td>WinRhizo</td>
<td>Field</td>
<td>2-D</td>
<td>mesocosms consisting of PVC Maize</td>
<td>Axil root length; axil diameter; lateral root order; number and length.</td>
<td>It can do morphology (length, area, volume), topology, and architecture, made of a computer program and image acquisition components that can be combined to meet different needs.</td>
<td>Its limitations are that color is used to draw the root; Skelton and root distribution used in this software are the same, so it causes confusion.</td>
</tr>
</tbody>
</table>
Canopy temperature (CT) is one of the major physiological traits related to plant water status and several metabolic processes such as photosynthesis, respiration and transpiration. Canopy temperature depression (CTD) is also a good technique used for field phenotyping of crop plants under stress environments, especially droughts. Canopy temperature and CTD are considered as good indicators of yield [135,136] and transpiration rate [137] under drought conditions. CTD is the difference between air temperature and CT which is correlated with different physiological processes such as stomatal conductance [138], leaf water potential [139], and grain yield [135,140,141] under stress conditions. Measuring CTD through CT and air temperature is a nondestructive method used to check the plant water status under drought conditions [142,143]. Plant water balance is a direct measure of drought response in crop plants [143]. This is associated with the plant transpiration process which is the main cause of leaf temperature variation and is directly associated with leaf temperature and stomatal conductance [138,144]. Therefore, CTD has been used in breeding programs to screen out stress tolerant or susceptible genotypes especially under drought conditions. The phenomenon behind this is drought avoidance which occurs through cooler canopy temperature [81]. Cooler canopy temperature is involved in up to 60% yield variation [81]. The main processes linked with drought adaptation are increased root dry weight, transpiration rate and decreased CT during grain formation [81]. It is observed that transpiration through stomata results in cooler leaves if water is available for transpiration. When water is limited for transpiration, closing of stomata occurs which rises canopy temperature. This change in stomatal conductance as well as leaf transpiration in response to water stress can be detected through thermal imaging [145] and has the potential for selecting large numbers of plants for CO₂ availability [146] and water uptake capacity [147]. The gaseous exchange of water occurs through stomata due to which leaf surfaces are cooled by evaporation and leaf temperature is reduced. While, contrary to this, stomatal closure and reduced transpiration increases leaf or CT [148]. Cooler CT is linked with higher grain yield because of more stomatal opening, exchange of gases and maximum photosynthetic rates [138,149]. Cooler CT is also linked with deeper roots, [68] and higher grain yield [99]. Wheat varieties with cooler canopy temperature have been shown to produce more yield compared with genotypes having warmer canopies [140,150] and warmer CT is associated with reduced stomatal conductance [151].

Under field conditions, reduction in root growth occurs due to a reduction in below ground carbon partitioning at high temperature which may reduce root number, length and diameter at the reproductive stage [152]. However, root development may be promoted under moderate drought through sending an increased amount of carbon assimilates to roots. Thus, it results in increased primary root development and suppression of lateral roots under moderate drought [153]. CT is also associated with root development and it was observed that wheat genotypes with cooler canopy temperatures resulted in an increase of 40% root dry weight at deeper depth (upto 120 cm) [81]. Under drought stress, cooler genotypes exhibited a deeper root system by extracting 35% more water from the deeper soil layer (upto 90 cm) [154]. It shows that cool canopy temperature has been strongly associated with increased plant access to water due to deeper roots [81]. CT can be used as a phenotypic selection tool for breeder’s trials [148]. Therefore, canopy temperature can also be used for root phenotyping in crop plants under water stress. Besides its merits, this technique has some limitations; it does not measure plant temperatures correctly, since it depends strongly on microclimate of the plant stand. Therefore, it is carefully balanced with reference temperatures of transpiring canopies. Secondly, higher variability occurs due to rapid changes in environmental conditions in the field, e.g., on cloudy days [155,156]. Variability also occurs due to differences in canopy densities of different genotypes [157]. Differences in plant density in different varieties might be due to differences in the germination rate. This might be due to variation in soil properties and different sowing densities.

4. Use of Breeding and Molecular Approaches to Exploit Root Traits for Drought Tolerance

Drought tolerance through conventional breeding has been achieved in different cereals like wheat [158], rice [159] and maize [160]. However, the breeding approaches for improving crop
productivity under drought stress situations are laborious and time consuming as it requires careful management under field conditions [161].

Plant responses to stress conditions occur through various changes at developmental and physiological stages, brought about by altering the expression of stress inducible genes [162]. Root traits are considered to be complex, which is controlled by polygenes having a quantitative effect and are difficult to quantify under field conditions and highly prone to environmental effects. Genetic loci controlling such traits are called quantitative trait loci (QTL). Identification of drought tolerance-related QTLs is one of the promising approaches, using marker-assisted selection [163]. Lots of efforts have been made to identify genes and QTL controlling root traits. Various researchers have studied the linkage of QTL with those traits responsible for increasing root systems foraging capacity in crop plants. These characters showed a molecular mapping of root traits in wheat [164,165], which exhibits the presence of multiple QTLs for different root traits; for instance, root number, length, seminal root number and angle, total root biomass, root system depth, lateral root number and length, and root surface area. It is observed that the productivity, and nutrient and water use efficiency (WUE) of crops could be enhanced by genetic improvement of root system architecture under drought conditions.

The root traits are difficult to phenotype and its QTL mapping is an alternative technique used in breeding programs [166]. Rapid screenings at the seedling stage on the basis of root traits helped in identifying the contrasting lines for mapping QTLs in soybean [20]. To ensure crop productivity under a stressful environment, different alleles may be incorporated into elite cultivars to produce desired root phenotypes through molecular breeding. Different QTLs have been identified for major cereals and legumes such as wheat, rice, maize, barley, pearl millet, sorghum, chickpea and soybean for drought tolerance [167,168]. Identification of genes or molecular markers linked with root architecture and root growth is useful to improve root traits through breeding and molecular selection and are also used to identify QTLs for agronomic traits for drought tolerance [169]. Wild species may have one or more positive alleles at major gene loci that affect agronomic traits [170]. Gene mining from wild species in different plants like tomato [171], soybean [172,173], and rice [174] has proved to be successful for drought tolerance.

Different QTLs have been identified for both biotic and abiotic stress parameters along with physiological, agronomic and seed composition traits in soybean [175]. Several researchers have identified QTLs for RSA in maize and rice and evaluated their effects under different moisture levels on yield [176,177]. For instance, in rice, for greater root length, marker-assisted backcross was used to introgress alleles following four major QTLs affecting root traits [178–180]. Likewise, a major QTL for leaf ABA concentration was also found effective for root architecture as well as grain yield in maize [120,177,181]. Similarly, four major QTLs controlling grain yield and stay-green (Stg1–Stg4) character have also been identified in sorghum [182].

5. Conclusions

Among various environmental stresses, drought is one of the serious stresses which has a significant but negative impact on crop yield. To manage drought, different tools are used to enhance crop yield under drought scenarios. Phenotyping root is one of the drought management tools as roots are more prone to drought conditions and play a significant role in the plant’s life by extracting soil resources from deeper soil layers to carry on several metabolic functions in the plant’s body and its phenotyping helps to understand different root traits. Similarly, different root phenotyping methodologies and platforms are usually used along with some imaging and image analysis softwares to understand root traits and their role during drought conditions in plants. Different 2-D and 3-D techniques are used for root phenotyping and among all these, each has its advantages and drawbacks. X-ray based computed tomography is a non-invasive technique for 3-D visualization of root traits. Besides, this canopy temperature depression (CTD) has also been considered a good technique for phenotyping of crops facing drought under field conditions. On the basis of this review, it is observed that different root traits such as fine root diameter, specific root length, root area, root angle and root
length density are useful for improving plant’s productivity under drought conditions. It is, therefore, suggested that these all root traits may be included in breeding programs to improve crop production under drought conditions.

**Author Contributions:** Wrote the manuscript and prepared tables, A.W.; gave the idea about manuscript and revised it critically and contributed for intellectual contents, X.Z.; helped for collection of manuscript literature, Q.F. and Z.Y.

**Funding:** This work was supported by the National Key R&D Program of China (2016YFD0300401) and Chinese Academy of Sciences as a part of President’s International Fellowship Initiative (PIFI) Program for post-doctoral studies.

**Acknowledgments:** The authors are grateful to the Chinese Academy of Sciences for providing funds to complete this study.

**Conflicts of Interest:** The authors declare that there is no conflict of interest for this study. The study was conducted in the absence of any commercial or financial relationship that could be constructed as a potential conflict of interest.

**References**


44. Zhu, J.; Ingram, P.A.; Benfey, P.N.; Elich, T. From lab to field, new approaches to phenotyping root system architecture. *Curr. Opin. Plant Biol.* 2011, 14, 310–317. [CrossRef] [PubMed]


48. Pierret, A.; Moran, C.J.; Dousson, C. Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. *New Phytol.* 2005, 166, 967–980. [CrossRef] [PubMed]


65. Vadez, V.; Rao, J.S.; Bhatnagar-Mathur, P.; Sharma, K.K. DREB1A promotes root development in deep soil layers and increases water extraction under water stress in groundnut. *Plant Biol.* 2013, 15, 45–52. [CrossRef] [PubMed]


Benjamin, J.G.; Nielsen, D.C. A method to separate plant roots from soil and analyze root surface area. *Plant Soil* 2004, 267, 225–234. [CrossRef]


Armengaud, P. EZ-Rhizo software the gateway to root architecture analysis. *Plant Signal. Behav.* 2009, 4, 139–141. [CrossRef] [PubMed]


© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).