Review Article

Root phenotyping and root traits for drought tolerance.

ABSTRACT

The spatial distribution of all root parts in a particular growth environment is collectively referred to as root system architecture (RSA). Roots have special features such as root length, number of roots, cortical cell file number and cell size, which help in determining water uptake ability among various root types as an adaptation strategy under dry conditions. Identification and selection for root traits can be obtained using a combination of root phenotyping strategies that encompass laboratory, greenhouse and field evaluations. Prior knowledge of the RSA of different genotypes or breeding lines can be used to compare the productivity of a particular genotype in relation to root size when the plant is exposed to water deficits. Roots form indispensable biological plant structures that largely contribute to the plant's ability to recover from drought stress. Root traits are controlled by polygenes and are difficult to quantify under field conditions and are prone to environmental effects. QTLs for different root traits such as root number, root length, root angle and root surface area have been identified. Genetic improvement of root system architecture under drought conditions could enhance productivity, nutrient and water use efficiency of crops. To ensure crop productivity under a stressful environment, different alleles may be incorporated into elite cultivars to produce desired root phenotypes through molecular breeding.

KEYWORDS: *root system architecture, root number, root length, root angle, root surface area*

ABBREVIATION

RSA - root system architecture, N - nitrogen, P- phosphorus, RTD - root tissue density, SSA - specific surface area SRL - specific root length, ABA - abscisic acid, QTL - quantitative trait loci, WUE- water use efficiency, DRO1 - *DEEPER ROOTING 1*

1. INTRODUCTION

Roots provide essential functions such as uptake of water and nutrients for plant growth, act as storage organs and anchor the plants to the soil. Roots are site of interactions with pathogenic and beneficial organisms in the rhizosphere. The plasticity of root growth and development in response to changing moisture and nutrient status of the soil provides opportunities for exploring natural variation to identify beneficial root traits to enhance plant productivity in agricultural systems. The spatial distribution of all root parts in a particular growth environment is collectively referred to as root system architecture (RSA). RSA is dynamic and is affected by the external environment (soil moisture, temperature, nutrients and pH) and the enclosing microbial communities that impact the way in which a plant detects and responds to its surroundings. Different root characteristics enable plants to respond, adapt and thrive in different environments. An increasing global population requires agricultural production systems and cultivars that can continue to be productive in erratic weather patterns and are capable of more efficient resource capture from the soil. Breeding programs have traditionally focused on the above ground plant parts (forage, seed or grain production) for the generation of food, feed and fiber. Breeders aim to develop improved cultivars that can tolerate a variety of abiotic stress conditions such as drought or flooding. These approaches include selection of individuals with improved plant growth characteristics such as grain or biomass yield, seed production, leaf surface area, number of tillers and disease resistance. Strategies to implement “root breeding” require the identification of the underground root traits that enable a plant to efficiently utilize water and nutrients in different environment [1].

Understanding the root phenes that result in higher yields and increased stress tolerance would provide tangible targets for breeders to select individuals with the ideal root phenotypes to use as parents and develop breeding lines to advance through the crop improvement process. The success of breeding programs aimed at modifying RSA is dependent on the specific trait under selection in different crops, the heritability of the trait, the ability to accurately and efficiently phenotype roots of multiple genotypes, the specific farming system used (row crops *vs.* swards or pastures), soil properties and the target set of breeding environments [2]. 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. 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. 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. Several root characters such as morphological plasticity, root tip diameter, gravitropism and rhizosheaths allow the plants to adapt and respond to various environmental factors and are useful for improving water use efficiency in crop species. Therefore, it is important to understand the RSA regulating mechanisms for crop improvement. Different types of roots have special features such as primary root length, length and number of lateral roots, crown root number and cortical cell file number and cell size, which help in determining water absorption/uptake ability among various root types as an adaptation strategy under dry conditions [3].

2. KEY ELEMENTS OF ROOT SYSTEM ARCHITECTURE RELEVANT FOR CROP PRODUCTIVITY:

The key roles of roots as part of plant development have sparked renewed interest in understanding the molecular mechanisms that control RSA in crops. The root system also contributes to soil health, which refers to the continued capacity of the soil to function as a living ecosystem that sustains plants, animals and ultimately humans. Strategies such as crop rotation are beneficial because they disrupt pest and disease cycles, affect nutrient availability and improve soil health. Plant roots also keep the soil in place, reduce water leaching and soil erosion and are key for soil phytoremediation. The latter takes advantage of the ability of some plants to extract heavy metals or other toxic compounds with their roots from contaminated soils and to accumulate and store these in aboveground organs that can be easily harvested and disposed of. Roots sense and respond to abiotic and biotic stresses and are able to communicate with the aboveground plant parts via signaling pathways. Root morphology and physiology impact the growth and development of aboveground plant organs through altered root to shoot transport of mineral nutrients or diverse organic signaling molecules including hormones, proteins and RNAs. Low water availability (drought) represents an important abiotic stress resulting in significant crop losses and therefore plant roots utilize morphological plasticity to adapt to and respond to soil moisture levels. Research areas of practical value include identifying roots traits that increase the capacity of soil foraging for water and maintain productivity during limited water availability [1]. Root traits that are of practical value for crop and forage production systems are described below.

2.1. ROOTING DEPTH

Rooting depth is one of the most frequently evaluated traits because crops with deeper roots have better access to stored water and nutrients such as N, a soluble nutrient that tends to leach into the deeper layers of the soil. Although rooting depth is strongly influenced by the soil's physical and chemical properties, additional factors that impact rooting depth can be exploited for crop breeding programs [1]. Most notable are the anatomical traits in which a strong correlation between a specific type of root anatomy and plant performance under stress has been demonstrated [4]. One effective approach to increase root depth is to increase the time to reach flowering. An increase in root system depth may result from a faster rate of root system elongation, also referred to as 'root vigour' and/or a narrower angle of descent. Root vigour refers to the overall rate of root system elongation, whereas descent rate refers specifically to the rate at which maximum root depth increases. Faster root growth depends on processes within the root apex that determine cell division and expansion. Genotypes with these features may be selected in screens that directly measure the root elongation rate. Root vigour also depends on photoassimilate and water allocation to root tips for growth, suggesting that manipulation of shoot growth would provide extra resources to root growth [5].

Maize genotypes with reduced cortical cell file number and large cortical cell size have deeper roots under water stress conditions because of the reduced metabolic costs associated with soil exploration. Similar to the cortical cell file number, the larger root cortical aerenchyma enable enhanced root exploration by decreasing root metabolic costs that come with maintaining root biomass. Another factor that specifies rooting depth and has relevance to crop breeding is gravitropism. Gravitropism is a physiological response manifested as a redirection of plant organ growth toward or away from the gravity vector. Selection for faster growing and deeper roots could enhance the plant's access to water deeper in the subsoil layers, enabling plants to maintain yield under limited rainfall conditions. Scenarios of water availability deep in the subsoil *vs.* periodic rainfall periods have different effects on crop growth and development and are reflected in the aboveground plant tissues as well as in the roots. The size of a plant's root system is a key trait that can affect the uptake of resources from the soil and should be considered in relation to the size of the aboveground plant parts [1].

2.2. ROOT HAIRS

Root hairs are slender projections originating from the epidermal cells and play an important role in water and nutrient uptake. Development of root hairs in plants is affected by environmental factors such as drought, heavy metal and interactions with pathogenic and soil microorganisms [6]. Root hairs account for a large percentage of the total root surface area and contribute to almost 50% of water absorption by the plant [1]. Root hairs assist with root growth through soil by means of anchoring and contribute to water uptake by extending the zone of soil root influence which is further facilitated by reducing the gradients in matric potential near the root soil interface. Root hair proliferation allows roots to exploit otherwise non-accessible stocks of Phosphorus (P) likely by increasing the root-soil contact and having access to finer pores than the main root axis can enter. Physical characteristics of root hairs such as length and root hair densities are significant in nutrient acquisition. Increased root hair lengths have shown to enhance zones of nutrient depletion via enhanced nutrient uptake especially for root uptake for nutrients with low diffusivities. As a consequence, the quantities of P taken up by root hairs can be significant, reaching quantities almost equivalent to those taken up by the rest of the plant root system [7].

2.3. ROOT BRANCHING

The formation of root branches or lateral roots is a significant determinant of overall RSA. Lateral roots add to the total root biomass, total root length and root surface area. The ideal lateral root density is influenced by the nutrient and water availability of the soil [1].

2.4. ROOT SURFACE AREA:

Root surface area which represents the total area of the root system that is in contact with the soil improves drought stress tolerance with an increase in area. Greater root masses and root length densities improve yield performances by enhancing the water uptake rate when the subsoil layers have limited water. The reduction in the number of nodal root during water-deficit stress reduces the metabolic costs of soil exploration, permitting greater axial root elongation, greater rooting depth and hence greater water acquisition from soil [8].

2.5. ANATOMY:

Cell size, number and density determine the pathways through which water and nutrients enter and are transported. Root cortical aerenchymae, cortical cell size and the cortical cell file number help limit the nutrient and carbon costs of soil exploration by altering root cortical tissues to air spaces. The diameters and distribution of the xylem vessels, especially metaxylem affect drought stress tolerance in cereals [9] and soyabean [10] by improving root hydraulic conductivity which reduce the metabolic cost of accessing water in deep soil domains.

3. IMPORTANCE OF ROOTS FOR DROUGHT TOLERANCE:

Drought is the most substantial threat to crop yields worldwide and is expected to offset yield increases attained from technological advances. Roots are a natural and often underexploited target for crop improvement under drought stress as the organ most responsible for the uptake of water. Root system architecture largely determines the spatiotemporal capture of water within the soil profile. Several phenes contribute to overall root system architecture and have important roles in resource capture, particularly in resource-limiting environments. Root phenotypes can substantially improve the capture of both mobile resources such as water or nitrogen (N) and immobile nutrients such as phosphorus (P). Root-growth angle is a key phene for accessing nutrients in the soil, especially in poor soils. Roots with steep angles are ideal for accessing mobile nutrients that quickly move through the soil profile and become more concentrated at greater depth, such as water and N. Shallow-angled roots are better able to access nutrients more concentrated in the shallow soil layers, such as P. The length and density of lateral roots is also important for plant performance [11].

Root systems play important roles in water and nutrient acquisition of a plant as well as anchorage to support the plant structure. Continuous shoot improvements of the last decades neglected the phenotypic variation of root architectural, morphological, and anatomical traits despite the widely available genetic resources. The key to understand the potential of root phenotypes lies in the understanding of root structure and function relative to its developmental processes on architectural and anatomical scale. Variation in root architecture has been shown to positively influence soil exploration and acquisition of water and nutrients in nutrient limited and drought conditions. For instance, rice promotes deep rooting which enhances growth and yield under drought [12] similar to well-known observations in maize under drought and low nitrogen conditions [13].

4. STRATEGIES FOR ROOT PHENOTYPING AND THEIR UTILIZATION IN BREEDING PROGRAMS

Developing plants with the capacity to grow and remain productive in marginal soils with reduced water and fertilizer inputs is a major target of crop and forage breeding programs worldwide. Although the identification of root traits and phenes that facilitate the exploration and effective utilization of water and nutrients can be used to achieve these breeding objectives, the challenge of phenotyping underground traits with higher throughput has hindered progress in this area. Regardless, crop breeding programs have increased yields by selecting for a combination of traits such as increasing shoot biomass, shifting the ratio between harvested grain *vs.* shoot biomass, improving disease resistance and expanding the length of the growing season. Yield increases through breeding have been associated with earlier flowering and decreased number of days between germination and harvest that could have resulted from inadvertently selecting for more efficient root systems. Understanding the variability and contribution of specific root traits, or phenes, in a given species will enable the identification of those traits capable of enhancing the efficiency of the root system more effectively and will result in increased plant productivity [14].

There are two approaches to assessing RSA: field-based or lab-based phenotyping. Each offers benefits and has disadvantages for root breeding. The field-based methodology can be utilized alongside breeding field trials so that both aboveground and belowground traits can be assessed together under field conditions. This would allow changes in RSA to be correlated with aboveground selection. In-field phenotyping often prevents the direct viewing of the entire root system and is generally a one-time measurement of the root system, with the exception of mini-rhizotrons [15].

Root phenotyping platforms can be defined by the combination of growing environment and rooting media and broadly grouped into ex situ or in situ methodologies. Methodologies can also be described as static (single time point) and dynamic (rate changes over time) metrics across both local (individual roots) and global (root system architecture) regions of the roots. Local root metrics include root length, root branching and root diameter which can be described in static or dynamic observations. Root surface area, root density and root volume are examples of global root metrics.

4.1 EX SITU PLATFORMS: Ex situ platforms require the roots to be extracted from the medium prior to root characterization and therefore capture only a static assessment of root architecture metrics. Ex situ platforms enable relatively rapid assessment of static root metrics and have the potential to be implemented across a wide range of developmental stages. Ex situ field platforms developed for both maize and wheat provide the characterization of local root metrics, with some inclusion of image-based analyses, maintain flexibility in developmental timing of analyses, but significantly increase the time required for digging, washing and subsequent analysis. Controlled environment platforms that are complementary to these field platforms include solid media such as soil, sand or Turface, or liquid media such as hydroponic, aeroponic, or germination paper. Controlled environment platforms are often limited to vegetative stages, but provide detailed characterization of root metrics through image analyses and are easier for the application of stress treatments. The use of appropriate platforms for root phenotyping can provide valuable information for the characterization of phenotypic variation in germplasm and transgenic experiments [2].

4.2 IN SITU PLATFORMS: In situ platforms enable direct imaging of roots within the growth medium and have created opportunities to characterize dynamic metrics. Recently developed in situ platforms expand the ability to assess dynamic root metrics and have increased the potential to describe global root system architecture. Transparent growth media, such as gellan gum or phytagel are each unique platforms that utilize 2D imaging technology or 3D root scanning to recreate global root system architecture. In each of these platforms, repeated imaging up to 18 days post germination delivers dynamic root metrics. By contrast with the relatively labour-intensive transparent media platforms, an in situ automated controlled environment soil-based platform was developed for characterization of multiple crops. Although this rhizotron does not provide some of the global metrics that are available in the transparent media platform, it is superior in its ability to provide data during later developmental stages, and greatly decreases labour costs required for digging and washing roots. Novel in situ platforms utilizing X-ray tomography, nuclear magnetic resonance imaging or electrical capacitance are in development, but have not been implemented in broad screening experiments due to low throughput and expense of instrumentation. The technical precision and comprehensive phenotypic analyses of the in-situ platforms exceed the ex-situ platforms, but are often more expensive and have a greatly reduced throughput. Current root system architecture platforms do not include the collection of root cellular morphology data such as tissue or cellular anatomy. Although the measurement of cellular morphology is relatively time intensive due to the sample preparation required for microscopy, significant variation in root anatomy metrics has been observed in root cortical aerenchyma, root vasculature and root hairs. Variation in root cortical aerenchyma has been suggested as a potentially important trait because it may lower the carbon cost of maintaining active roots. Therefore, future advances in the ability to collect root system architecture information, including root anatomical descriptions, has the potential to contribute to the development of new root traits that will increase crop productivity. Although cheaper roots are an attractive concept, it will be important to ensure that root strength and the ability to grow through regions of compacted soils or high bulk density is maintained [2].

Generally root phenotyping strategies in the laboratory are most useful for basic research activities as part of the discovery phase to identify genetic variation for RSA and understanding the genetics of root anatomy. The evaluation phase includes understanding the RSA and how the root anatomy and physiology changes in response to different abiotic and biotic stress in controlled single-variable experiments. Validation experiments are useful to determine the performance of plants with particular root phenes that enable evaluation of biomass/yield production and identification of associations between traits and molecular markers. The utilization stage includes utilizing molecular markers for selecting the desirable individuals to use as parents for crossing and population development, to track desirable root phenes during the breeding pipeline and further understand the plasticity of roots in multiple soil types under varying cultivation and crop management practices. Additionally, studies have shown that the root morphology also influences the persistence and productivity of this perennial crop species [1]. In rice, root length, diameter, dry weight and total absorbing surface area were positively correlated with grain yield [16]. It is possible that the desirable root ideotype is not a specific root phene representing a static or defined root phenotype, but rather the trait of interest refers to the plasticity of the roots that are able to respond to and readily adapt to a myriad of growing conditions (deep roots to capture water from the subsoil combined with root branching to increase the uptake of nutrients (*i.e.*, P) closer to the soil surface using a "topsoil foraging" root architecture). Strategies to measure and capture root plasticity will require evaluation of RSA and plant performance in a range of stress conditions commonly found simultaneously in the field (low soil moisture, high temperatures and/or limiting nutrients). The ideal RSA may differ for different crops at different locations due to phenotype–environment interactions as well as the specific agricultural management practices implemented in the field (*i.e.*, frequency of harvest or animal grazing). Although RSA has important functional implications for the acquisition of soil water and nutrients, direct selection for root architectural traits in breeding programs has previously been limited by the absence of suitable phenotyping methods with physiological relevance. [1].

Powerful 3D image-based systems offer new opportunities to visualise roots in situ at high resolution, for example via MRI, to monitor root development and water transport. Accumulating image data from crop roots provides potential for machine-learning approaches to interpret and predict the complex and dynamic nature of root system architecture, however translating such knowledge into predictive breeding necessitates data from large breeding populations, which exceeds the scope of most present systems. From a breeding perspective, easily assayed proxies associated with root architecture present more cost effective current alternatives. One example is canopy temperature, which can be indicative of access to water via the root system and is measurable at high throughput in large populations by aerial phenotyping with a thermal camera. Therefore, more effective phenotyping strategies that enable evaluation of large plant populations in the field are urgently needed to facilitate the direct incorporation of root traits into plant breeding programs [17].

5. PHENOTYPING:

Image-based phenotyping of plant roots is based on the non-destructive (where possible) optical analyses of plant traits, and its main objective is to characterize the plant's anatomical, biochemical and physiological properties. Root traits are more related to drought tolerance compared with above ground plant parts and are key factors to maintain crop yield under drought. Root phenotyping is as important as shoot phenotyping, because plant's ability to uptake moisture and nutrients mainly depends on root architecture and function. Therefore, root phenotyping is important for crop breeding, although under field conditions, screening roots by phenotyping is a very difficult task. Root traits affect the amount of water and nutrient absorption and are important for maintaining crop yield under water stress conditions. Plants with higher main root diameter have more growth potential as it has direct relation with water absorption, and have more ability to explore compact soil. Fine roots are most permeable and thought to have greater ability to absorb water, especially in herbaceous plants. This role becomes even more important in water and nutrient-deficient soils due to an increase in climate variability under current cropping systems. Root architecture also has a significant impact on nitrogen use efficiency. 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. 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. 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 [3].

6. TECHNIQUES USED FOR ROOT PHENOTYPING

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. For root phenotyping, different techniques are used under laboratory as well as field conditions. Root phenotyping was first developed in the laboratory and then demonstrated in field to check its applicability. 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. 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, gels, in air regularly sprayed with nutrient solution or in aerated aqueous solutions. Plants are also grown in hydroponics by using transparent Plexiglas nail board sandwiches with mechanical resistance. These sandwiches were filled with glass beads of 1.5mmin size for the circulation of nutrient solution. 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 by using WinRhizo software. RSA can be analyzed through Smart Root software for the measurement of growth kinematics and branching angles of individual roots of a root system. These systems require some manual input for such analyses. Image processing becomes a more challenging task when soil is used as a growth medium [3].

6.1 FIELD PHENOTYPING:

1. TRENCH PROFILES: Root system is excavated layer by layer horizontally. The root system is characterized and measured manually in two-dimensions at a single time point, usually at or before harvest. Trenching provides an accurate measure of the extent of the root system in its natural setting from which other metrics could be inferred. Trenching requires a great deal of time and effort to excavate and measure.

2. CORE: 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.

Soil cores are taken for the measurement of vertical root length densities or weights by using excavation techniques. The core break method 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.

3. SHOVELOMICS: Shovelomics is a technique widely used for root system analysis for field studies. 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 to imaging along with custom image analysis software.

4. MINI-RHIZOTRON: 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. Through mini-rhizotrons, limited genotypes may be monitored. Different indirect methods are also used for analyzing RSA, such as root pulling resistance or analysis of abscisic acid (ABA) content in the leaf.

5. BURIED HERBICIDE TECHNIQUE: Buried herbicide technique is a method of assessing root depth of seedlings using a layer of herbicide (TRIK or diuron) buried 25 or 30 cm deep in soil-filled boxes [3].

6.2 LAB/GREEN HOUSE PHENOTYPING:

1. ELECTRICAL CAPACITANCE: An electrical capacitance measurement technique is also used for the measurement of total root mass which inspects the applied current response. One electrode is inserted at the stem base and the other is inserted in the rooting medium. This technique has been used in the field for high throughput analysis of root mass. However, recent studies have shown that root capacitance may be more associated with root circumference or its cross-sectional area at the soil or solution surface. 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 [3].

2. MAGNETIC RESONANCE IMAGING (MRI): Magnetic resonance imaging (MRI) relies on a strong magnetic field and radiofrequency fields to align protons (H+) in tissues. Manipulation of parameters allows water contained in plant tissues and in the soil matrix to be distinguished [3].

3. X-RAY COMPUTED TOMOGRAPHY (CT): 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. 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 [3].

7. IDENTIFICATION OF QTL/GENES:

Reproducible and accurate phenotyping is also critical to identify quantitative differences in RSA of plant materials and identify the underlying genetic mechanisms (quantitative trait loci (QTL) and genes associated with root phenes) to implement genomics-assisted breeding strategies. The use of molecular breeding strategies depends on the phenotypic data used to determine the estimated breeding value of a particular allele in a given genetic background and set of environments. Several traits influencing root depth appear to be under the control of multiple genes, suggesting the potential for improvement/modification through selection and breeding. The presence of natural variation in root morphological traits in a target crop species also indicates that selection for specific root traits could be achieved. A number of genes involved in RSA are known either from gene mutants with quantifiable changes in primary root length, root branching, root hair formation or from QTL studies. However, the mechanistic details of how these QTL affect the root phenotype, the effect of the QTL in a different genetic background and their role in different soil types and environments, are comparatively limited [1].

Different image-based softwares are used for the analysis of root traits, which have some advantages and limitations. 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. 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 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. To ensure crop productivity under a stressful environment, different alleles may be incorporated into elite cultivars to produce desired root phenotypes through molecular breeding [3]. *DEEPER ROOTING 1* (*DRO1*) quantitative trait loci results in steeper root angles and more robust seedling gravitropic responses and leads to rice plants that are more tolerant to drought. The enhanced drought tolerance of *DRO1* plants is due to their deeper root system. The *DRO1* gene encodes a plasma membrane-localized protein that is regulated by the plant hormone auxin, although the exact molecular function of DRO1 is still unknown. *DRO1* is negatively regulated by auxin and is involved in cell elongation in the root tip that causes asymmetric root growth and downward bending of the root in response to gravity. Higher expression of *DRO1* increases the root growth angle, whereby roots grow in a more downward direction [18].

Table 1: QTL/Genes of root traits associated with drought tolerance

Sl. No.	Crop	QTL/Genes	Trait	Reference
1	Rice	QUICK ROOTING 1 (QRO1), QRO2	Root length	[19].
		DEEPER ROOTING 1 (DRO1), (DRO2)	Root growth angle	[12].
2	Wheat	QMrl.sau-7B, QTrl.sau-4B, QAd.sau-7A, and QSa.sau-4B.	Root length	[20].
3	Barley	QRl.7H, QRl.5H	Root length	[21].

		QRv.S42IL.1H, QRv.S42IL.2H, QRv.S42IL.5H, QRv.S42IL.6H, QRv.S42IL.7H	Root volume	[22].
4	Maize	crown root angle (CRA2)	Root angle	[23].
		crown root length (CRL1)	Root length	[23].
5	Sorghum	qRA2_5, qRA1_5	Nodal root angle	[24].
		qRDW1_5	Root dry weight	[24].
6	Soybean	FR_Gm01, FR_Gm03, FR_Gm04, FR_Gm08, FR_Gm20,	Root surface area	[25].

8. CONCLUSION:

Breeding efforts that select for and modify specific root traits are limited despite the importance of the root systems for enhancing the acquisition of water and nutrients. Genetic gains in forage and grain production are important targets for plant breeding programs and these gains could be enhanced by understanding the root traits that contribute to improved plant performance. The challenge is to develop systems for non-destructive root phenotyping to accurately reflect and capture the RSA. These methods should allow continuous monitoring of root development and its response to different growing conditions as well as relatively high-throughput systems to efficiently evaluate a large number of genotypes as part of the breeding program.

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