

## Role of root traits and root phenotyping in drought tolerance

### ABSTRACT

The spatial distribution of all root parts in a particular growth environment is collectively referred to as root system architecture (RSA). Root length, cortical cell file number, number of roots and cell size help in determining water uptake ability among various root types as an adaptation strategy under dry conditions. Using a combination of root phenotyping techniques that include laboratory, greenhouse, and field evaluations, root characteristics can be identified and selected. Roots play a significant role in the plant's capacity to recover from drought stress. Root attributes are influenced by environmental factors, are difficult to measure in the field, and are regulated by polygenes. Different root properties, including root number, root length, root angle, and root surface area, have quantitative trait loci that have been found. Crop yield and the efficiency with which nutrients and water are used could both be improved genetically by improving the root system architecture in drought-prone areas. Different alleles may be introduced into elite cultivars to create desired root phenotypes through molecular breeding in order to ensure crop productivity in a harsh environment.

**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 offer critical features inclusive of uptake of water and vitamins for plant increase, act as storage organs and anchor the plant to the soil. Roots interact with pathogenic and useful organisms within the rhizosphere. 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 interacts with the outside surroundings (soil moisture, temperature, nutrients and pH) and the enclosing microbial groups that effect the manner wherein a plant detects and responds to its environment. Different root traits permit plants to respond, adapt and thrive in specific 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 produce cultivars that can tolerate abiotic stress situations inclusive of drought or flooding”. Implementing “root breeding” requires the identification of underground root traits that permit a plant to utilize water and nutrients efficiently [1].

“Understanding the root phenes that bring about better yields and improved stress tolerance could offer tangible objectives for breeders to choose plants with the precise root phenotypes to use as parents and develop breeding lines for crop improvement. The achievement of breeding applications to enhance RSA depends on the particular trait, the heritability of the trait, the potential to appropriately and correctly phenotype roots of more than one genotype, farming system used (row plants vs. swards or pastures), soil properties and the goal 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 prime aim for plant breeders to enhance abiotic stress tolerance in plants. The sort of root distribution required for specific plants relies upon the environment, as abiotic stresses faced by roots have a substantial impact in crop yield. Strong root improvement is critical for survival of seedlings in soils which go through rapid surface drying, while sufficient moisture remains available in deeper soil layers. Therefore, knowledge of plant responses to abiotic stresses is useful to develop stress resistant crop varieties. Several root characters inclusive of morphological plasticity, root tip diameter, gravitropism and rhizosheaths permit the plants to evolve and respond to numerous environmental elements and are beneficial for enhancing water use efficiency in crops. Therefore, it is important to understand the RSA regulating mechanisms for crop improvement” [3].

## **2. KEY ELEMENTS OF ROOT SYSTEM ARCHITECTURE IN RELEVANCE TO CROP PRODUCTIVITY:**

The key role of roots in plant growth has reignited interest in understanding the molecular mechanisms that regulates RSA in crops. The capacity of the soil to function as a living ecosystem that supports plants, animals, and ultimately humans is referred to as soil health and it is influenced by the root system. Strategies such as crop rotation are advantageous because they interfere with the life cycles of pests and diseases, affect nutrient availability and enhance soil health. In addition to preventing soil erosion and water leaching, plant roots are essential for soil phytoremediation. The latter makes use of a plant's ability to extract and store heavy metals or other harmful substances in above-ground organs that can be easily picked and disposed of. Roots are able to connect with the above-ground plant sections, detect and respond to biotic and abiotic challenges through signalling pathways. Root morphology and physiology impact the growth and development of above ground plant organs through altered root to shoot transport of mineral nutrients or various organic signalling molecules such as hormones, proteins, and RNAs. Plant roots uses morphological plasticity to adapt and respond to soil moisture levels as low water availability constitutes an important abiotic stress resulting in considerable crop losses. Finding root characteristics that boost soil's ability to forage for water and keep productivity high during times of reduced water supply is one field of research that has practical applications [1]. Root traits that are of practical value for crop and forage production systems are described below.

### **2.1. ROOTING DEPTH**

“One of the most frequently evaluated features is rooting depth because crops with deeper roots have better access to stored water and nutrients like nitrogen (N), a soluble nutrient that tends to leach into

the deeper layers of soil. Although the physical and chemical characteristics of the soil have a significant impact on rooting depth, crop breeding strategies can take advantage of other additional factors that impact rooting depth" [1]. "The anatomical characteristics where a significant association between a particular type of root structure and plant performance under stress has been shown [4] are most noteworthy". Increasing the duration till flowering is an efficient way to enhance root depth. A higher rate of root system elongation, commonly known as "root vigour,"and/or a narrower angle of descent may cause an increase in root system depth. While descent rate particularly refers to the rate at which maximum root depth increases, root vigour refers to the overall rate of root system elongation. The processes that control cell division and expansion at the root apex contribute to faster root growth. "Genotypes with these features may be selected in screens that directly measure the root elongation rate. A modification of shoot growth would presumably give more resources for root growth since root vigour also depends on photoassimilation and water allocation to root tips for growth" [5].

Under water stress, maize genotypes with fewer cortical cells file number and larger cortical cells have deeper roots due to lower metabolic costs associated with soil exploration. Similar to the cortical cell file number, the larger root cortical aerenchyma reduces the metabolic expenditures associated with sustaining root biomass and allows for improved root exploration. Gravitropism is another factor that defines rooting depth and is relevant to crop breeding. It is a physiological reaction that causes plant organ growth to be directed either toward or away from the gravity vector. In order to improve the plant's ability to access water deeper in the subsurface layers and maintain yield under limited rainfall conditions, selection for faster growing and deeper roots may be beneficial. The root system of the plant as well as the tissues above ground respond differently to scenarios of deep subsoil water availability compared to periods of frequent rainfall. An important characteristic that might influence a plant's ability to absorb nutrients from the soil is the size of its root system, which should be compared to the size of the plant's above-ground components [1].

## **2.2. ROOT HAIRS**

"Root hairs are thin protrusions that originate from the epidermal cells and are crucial for the intake of nutrients and water. Environmental factors like drought, heavy metals, and interactions with pathogenic and soil microbes have an impact on the development of root hairs in plants" [6]. A significant portion of the total root surface area is made up of root hairs, which help the plant absorb water to a degree of over 50% [1]. By anchoring and extending the zone of soil root influence, root hairs help roots develop through soil and contribute to water uptake. This process is further aided by a reduction in gradients in matric potential along the root-soil interface. By enhancing the root-soil contact and gaining access to smaller pores than the main root axis can, root hair multiplication enables roots to utilise Phosphorus (P) supplies that would otherwise be inaccessible to them. The physical features of root hairs, such as their length and density, are important for nutrient uptake. Increased root hair lengths have shown to improve nutrient depletion zones through improved nutrient uptake, particularly for nutrients with low diffusivities. Because of this, the amounts of phosphorus (P) absorbed by root hairs can be significant, sometimes almost equivalent to those absorbed by the rest of the plant's root system [7].

## **2.3. ROOT BRANCHING**

“One important factor affecting the overall RSA is the formation of root branches or lateral roots. The root surface area, total root length, and root biomass are all increased by lateral roots. The soil's ability to hold nutrients and water affects the optimal lateral root density” [1].

## **2.4. ROOT SURFACE AREA**

The ability to withstand drought stress is improved with increased root surface area, which is the entire area of the root system in contact with the soil. In situations where the subsurface layers have limited water, greater root masses and root length densities increase yield performances by speeding up water absorption. The decrease in number of nodal roots during water scarcity reduces the metabolic expenses associated with soil exploration, allowing greater axial root length, greater rooting depth, and ultimately better water uptake from the soil [8].

## **2.5. ANATOMY**

The pathways through which water and nutrients enter and are transferred are determined by cell size, number, and density. By altering root cortical tissues to air spaces, root cortical aerenchymae, cortical cell size, and the cortical cell file number helps in limiting the nutrient and carbon costs of soil exploration. 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 and lowering the metabolic cost of accessing water in deep soil domains.

## **3. IMPORTANCE OF ROOTS TRAITS IN DROUGHT TOLERANCE**

“Roots are a natural yet frequently underexploited target for crop improvement under drought stress as the organ most important for the uptake of water. The spatiotemporal capture of water within the soil profile is significantly influenced by the architecture of root system. Several phenes contribute to overall root system architecture and have important roles in resource capture, particularly in resource-limiting environments. Root phenotypes can significantly 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. Roots with shallow angles can more easily obtain nutrients like P that are concentrated in the shallow soil layers. The performance of plants is also influenced by the length and density of lateral roots” [11].

A plant's root systems are critical for acquiring water and nutrients as well as serving as an anchor to sustain the structure of the plant. Understanding root structure and function in relation to its developmental processes on an architectural and anatomical scale is crucial for comprehending the potential of root phenotypes. Under limited nutrient and drought conditions, variation in root architecture has been found to positively influence soil exploration and acquisition of water and nutrients. 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 these breeding goals can be accomplished by identifying root traits and phenes that facilitate the exploration and effective utilization of water and nutrients, progress in this area has been hampered by the challenge of phenotyping underground traits with higher throughput. Regardless, crop breeding programs have improved yields by focusing on a variety of traits such as increasing shoot biomass, altering the ratio of harvested grain to shoot biomass, improving disease resistance and expanding the length of the growing season. Early flowering and a shorter time between germination and harvest have been linked to yield improvements through breeding, which may have happened as a result of inadvertently selecting more effective root systems. Understanding the variability and contributions of particular root traits, or phenes, within a given species would allow the identification of those traits capable of improving the efficiency of the root system more efficiently and leading to higher plant productivity” [14].

“The two methods for evaluating RSA are field-based and lab-based phenotyping. Each offers benefits and has disadvantages for root breeding. The breeding field trials and the field-based technique can be used jointly to evaluate both above and below-ground traits simultaneously in the field. This would make it possible to link changes in RSA to above-ground 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. Root length, root branching, and root diameter are examples of local root metrics that can be defined by 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 only record a static evaluation of root structure metrics since the roots must be removed from the medium before root characterization. “Ex situ platforms have the potential to be implemented across a wide range of developmental stages and enable relatively quick assessment of static root metrics. The characterization of local root metrics is provided by ex situ field platforms developed for both maize and wheat, with some inclusion of image-based analysis. These platforms maintain flexibility in the timing of the development of analysis, but they significantly increases the time required for digging, washing, and subsequent analysis. Solid media like soil or sand or liquid media like hydroponic, aeroponic, or germination paper are examples of controlled environment platforms that are complimentary to these field platforms. 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 have increased the potential to describe the architecture of the global root system and to assess dynamic root metrics. Transparent growth media, such as gellan gum or phytigel, are each distinct platforms that recreate the global root system architecture using 2D imaging technology or 3D root scanning. Dynamic root measurements are delivered in each of these systems by repeated imaging up to 18 days post germination. An in situ automated controlled environment soil-based platform was developed for the characterisation of multiple crops, in contrast to the comparatively labour-intensive transparent media platforms. Although this rhizotron does not offer some of the global metrics that are available in the transparent media platform, it excels in its capacity to supply data throughout later developmental phases and significantly reduces the labour costs associated with digging and washing roots. Due to limited throughput and high instrumentation costs, novel in situ platforms incorporating X-ray tomography, nuclear magnetic resonance imaging, or electrical capacitance have not been implemented in broad screening trials. 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. The collection of root cellular morphology data, such as tissue or cellular anatomy, is not included in the current root system architecture platforms. Although sample preparation required for microscopy makes measuring cellular morphology rather time-consuming, significant variation in root anatomy metrics has been observed in the root cortical aerenchyma, root vasculature, and root hairs. It has been proposed that variation in root cortical aerenchyma is a potentially significant trait since it may reduce the carbon cost of sustaining 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 the concept of cheaper roots is appealing, it will be important to ensure that root strength and the capacity to grow through areas of compacted soils or high bulk density is maintained [2].

Generally, “root phenotyping techniques in the laboratory are more beneficial for basic research activities for knowledge of RSA and the genetics of root anatomy. Validation experiments are useful to determine the performance of plants with particular root phenes that permits assessment 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 and determine desirable root phenes under different cultivation and crop management practices” [1]. “Root length, diameter, dry weight and total absorbing surface area were positively correlated with grain yield in rice” [16]. “The desirable root ideotype refers to the plasticity of the roots that are able to respond to and readily adapt to different growing conditions. Strategies to determine root plasticity would require assessment of RSA and overall performance in various stress situations such as low soil moisture, excessive temperatures and/or nutrients. The best RSA can also differ for different crops at different locations due to phenotype–

environment interactions in addition to the particular agricultural practices (i.e., frequency of harvest or animal grazing). Although RSA has essential implications for the utilization of soil water and nutrients, direct choice for root architectural traits in breeding applications has formerly been restricted due to the absence of appropriate phenotyping techniques 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 scope for machine-learning approaches to interpret the dynamic nature of root system architecture. Canopy temperature is 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. More effective phenotyping strategies that enable evaluation of large plant populations in the field are urgently required to facilitate the incorporation of root traits directly into plant breeding programs” [17].

## **5. PHENOTYPING**

Image-based phenotyping of plant roots is based on the non-destructive optical analyses of plant traits. The main objective is to characterize the plant's anatomical, biochemical and physiological properties. Root traits are more related to drought tolerance in comparison to above ground plant parts and are key factors to maintain crop yield under drought. Root phenotyping is as important as shoot phenotyping, because root architecture and function determine the ability of plants to uptake moisture and nutrients. Therefore, root phenotyping is important for crop breeding, although under field conditions roots phenotyping is difficult. 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 have greater ability to absorb water, especially in herbaceous plants. “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 in turn 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” [3].

## **6. TECHNIQUES USED FOR ROOT PHENOTYPING**

Roots are an essential plant organ and its phenotyping is as important as shoot phenotyping, as the overall performance of the plant 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 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 such as growing plants in paper rolls, gels, in air regularly sprayed with nutrient solution or in aerated aqueous solutions. Plants are also grown in hydroponics and involve measurement of 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 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. 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 usually at or before harvest and is characterized and measured manually in two-dimensions at a single time point. Trenching provides an accurate measure of the extent of the root system in its natural setting. Trenching requires more time and effort to excavate and measure.

**2. SOIL CORE METHOD:** The soil core method and standard excavation method are considered as the best techniques to explore density, depth and angle of root. Vertical root length densities or weights are measured using soil core method. Soil core method is the quickest method that can be used to assess the maximum depth of roots in soil samples. Soil cores of about 2 m 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 is present in the center of the surface. The roots are then gently washed and the main root branches are analyzed for different root traits. Different techniques such as simple counting to imaging along with custom image analysis software are used to determine the basic root traits such as root dimensions, structure and root branching.

**4. MINI-RHIZOTRON:** Mini-rhizotron systems consisting of Plexiglas tubes containing small camera or scanner is inserted in the soil to assess the surroundings of the root soil. Limited genotypes may be monitored through mini-rhizotrons. 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 **biomass** which inspects the applied current response. One electrode is inserted at the base of the stem and the other is inserted in the rooting medium for high throughput analysis **of root biomass**. However, recent studies have shown that root capacitance may be associated with root circumference or its cross-sectional area. Soil-root interactions and root phenotypes based on electrical properties can be measured by electrical capacitance technique but it does not provide details about root function, architecture and root anatomy" [3].

**2. MAGNETIC RESONANCE IMAGING (MRI):** "Magnetic resonance imaging (MRI) utilizes 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) helps to determine 3-D root architecture in undisturbed soil cores in detail. It is an

excellent tool for root phenotyping in comparison to other destructive methods. Roots are excavated from the soil and then washed, imaged and analyzed with commercially available softwares" [3].

## 7. IDENTIFICATION OF QTL/GENES

"Reproducible and accurate phenotyping is essential to identify quantitative differences in RSA of plant materials and to 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. A number of genes involved in RSA are known either from gene mutants or from QTL studies. However, the details of how these QTL affect the root phenotype and their role in different soil types and environments, are comparatively limited" [1].

"Root traits are considered to be complex, which is controlled by polygenes and are difficult to quantify under field conditions and are highly prone to environmental effects. Identification of drought tolerance-related QTLs using marker-assisted selection is a promising approach. Various researchers have studied the linkage of QTL with traits that are responsible for increasing root system's foraging capacity in crop plants. Root traits are difficult to phenotype and QTL mapping is an alternative technique used in breeding programs. Different alleles may be incorporated into elite cultivars to ensure crop productivity under a stressful environment by producing desired root phenotypes" [3]. "*DEEPER ROOTING 1 (DRO1)* QTL 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, where 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	QRI.7H, QRI.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 choose for and modify particular root traits are restricted in spite of the significance of the root systems for utilization of water and nutrients. Genetic gains in forage and grain production are essential objectives for plant breeding applications and can be increased with the knowledge of root traits. One drawback is the need for non-destructive root phenotyping to accurately reflect and capture the RSA. These techniques need to permit non-stop tracking of root improvement and its reaction to specific developing situations in addition to high-throughput structures to correctly examine different genotypes as a part of the breeding program.

**Author's contributions** This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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