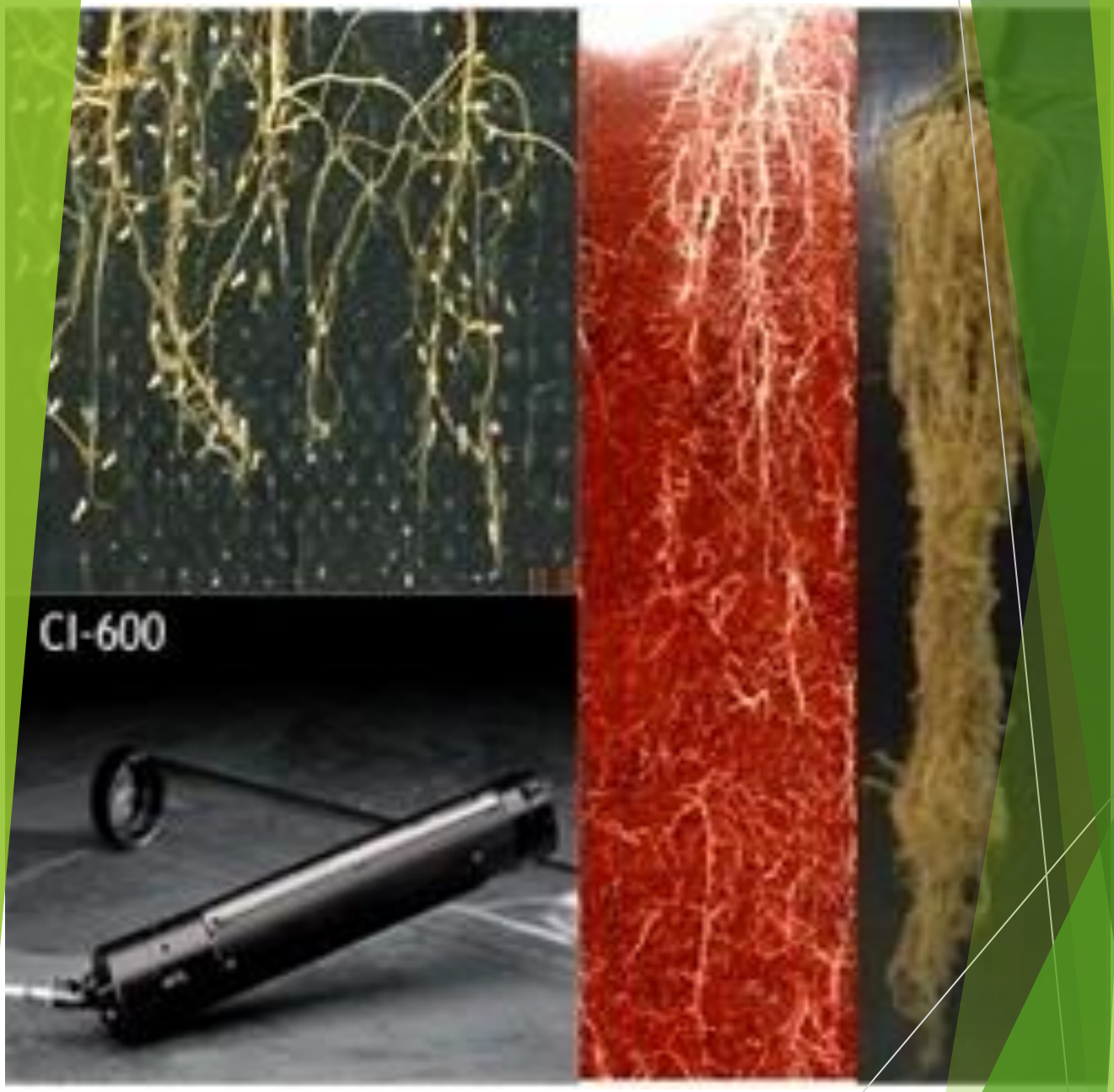
Recent Advances in Root Architectural Sampling and Monitoring Tools for Root Analysis in Rainfed Agriculture



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RECENT ADVANCES IN ROOT ARCHITECTURAL SAMPLING AND MONITORING TOOLS FOR ROOT ANALYSIS IN RAINFED AGRICULTURE

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This e-book is a compilation of resource text obtained from various subject experts of ICAR-CRIDA and ICAR-IIOR, Hyderabad. This e-book is designed to educate students, research scholars, and academicians related to agriculture and has been compiled on the occasion of 10-days training program on “Recent advances in root architectural sampling and monitoring tools for root analysis in rainfed agriculture” from 24th January to 02nd February, 2024 sponsored by ICAR, New Delhi.

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PREFACE

This e-book is an outcome of 10-days sponsored short course training program on “Recent advances in root architectural sampling and monitoring tools for root analysis in rainfed agriculture”. This book is intended for SAUs/Researchers/ICAR Institutes, and policy makers engaged in agriculture sector. This compilation would be helpful in selecting characteristics for breeding programs, potential of a crop to withstand climatic vagaries, tools and techniques for assessing impact of different practices on roots of agricultural and horticultural crops.

This e-book is a compilation of resource text covering all aspects of root architecture and root system in the soil. It is important because most of the resources that plants use from the soil are heterogeneously distributed and are subjected to local depletion. In order to extract/excavate root system with minimum losses and damage, we need to have appropriate methodologies for precision data. In this compilation, efforts are on to convey various root methodologies and monitoring tools for root study.

There is urgent need to compile recent tools and techniques and their hands on practices for better management and identifying indicators of stress in crops and horticulture The content of this e-book has been designed in such a way, so that it can provide updated information towards important tools and techniques in studying root architecture and gaining confidence in dealing with such estimates. This compilation is designed to meet the expectations of researchers of various disciplines like Agronomy, Horticulture, Soil Science, Microbiology, Genetics & Plant Breeding, Plant Physiology and Soil and Water Conservation Engineering, Environmental Sciences, Forestry etc for improved and precision data.

The valuable suggestions for future improvements are always welcome.

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CONTENTS

Chapter-1

SOIL CARBON ADDITIONS THROUGH ROOTS

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The carbon fixed in plants by photosynthesis and added to soil as above and belowground litter is the primary source of C in ecosystems (Warembourg and Paul, 1977). Although most carbon enters ecosystems via leaves, and carbon accumulation is most obvious when it occurs in aboveground biomass, more than half of the assimilated carbon is eventually transported below ground via root growth and turnover and exudation of organic substances from roots. In many arable systems, since aboveground plant residues are grazed or removed, root-derived C provides a significant C input and is thus a major contributor to soil organic carbon (Heal *et al*., 1997), especially in the subtropical and tropical systems.

Root contribution to soil organic carbon (SOC)

Belowground residues and root turnover represent direct C inputs into the soil system, and as such have the potential to make major contributions to SOC stocks. The tight coupling between root distribution and SOC distribution with depth is often cited as evidence for the importance of root inputs in maintaining SOC stocks (Jackson *et al.,* 1996; Jobbagy and Jackson 2000). In addition to the spatial location within the mineral soil, roots generally decay slower than aboveground residue (Rasse *et al.,* 2005; Silver and Miya 2001) which has been attributed to both litter quality and environmental factors (Crow *et al.,* 2009; Kogel-Knabner 2002).

Since, shoots are the only primary carbon source in ecosystems apart from roots, comparisons between the contribution of shoots and roots to soil organic carbon are inevitable. Many studies suggest that the relative contribution of plant roots to soil organic C stocks is larger than that of plant shoots. Long-term residue management studies suggest that above ground material has a limited impact on SOM levels as compared to root systems. Root biomass has considerable value for SOC storage because of the amount of C contained in these residues and

the fact that they are less easily mineralized, thus more likely to become chemically or physically stabilized in deeper soil layers (Bolinder *et al.* 1999). For roots to be predominant contributors to the soil organic carbon pool, the belowground C additions have to be large, and/or belowground C has to be relatively more resistant to mineralization than aboveground C.

Carbon allocation belowground

Carbon taken up by plants through photosynthesis is termed gross primary production (GPP). CO_2 uptake during photosynthesis is only temporary – respiration returns about half of the captured carbon to the atmosphere almost immediately. The remaining C is incorporated as structural material in shoots aboveground or allocated belowground. The fraction of GPP allocated belowground is significant. Studies indicate that roughly 40% of net fixed C is allocated belowground (Jones *et al*., 2009). Carbon allocated belowground is lost as root respiration, incorporated in structural material as root biomass or released into the rhizosphere soil as rhizodeposition.

Rhizodeposited C

Rhizodeposition was first defined by Whipps and Lynch (1985) as all material lost from plant roots, including water-soluble exudates, secretions of insoluble materials, lysates, dead fine roots, and gases, such as CO_2 and ethylene. The term rhizodeposition includes a wide range of processes by which C enters the soil including: (1) root cap and border cell loss, (2) death and lysis of root cells (cortex, root hairs etc), (3) flow of C to root-associated symbionts living in the soil (e.g. mycorrhizas), (4) gaseous losses, (5) leakage of solutes from living cells (root exudates), and (6) insoluble polymer secretion from living cells (mucilage) (Jones *et al*., 2009).

The amount of C inputs from the rhizodeposited C component is difficult to quantify under field conditions. Values of rhizodeposition, measured by ^{14}C labeling technique, may range between 30-90% of the carbon transferred to belowground components of various plant-soil systems (Whipps 1990). Common assumptions relating to rhizodeposited C are that it is equivalent to about 65 to 100% of the measurable root biomass (Bolinder *et al.*, 1999; Bolinder *et al*., 2007; Rasse *et al.*, 2005; Plénet *et al*., 1993).

Root biomass C

Root biomass C refers to the carbon present in live and dead roots at the time of harvest. In annual plants, the allocation of dry matter to roots changes during their life cycle and with growing conditions. Typically, relatively more assimilates are channeled to roots during early growth, but as development proceeds, the growing reproductive structures come to dominate and the amount of assimilate translocated to roots decreases. This change in allocation has been observed in many crops and is particularly pronounced in cereal crops as the stem elongates and the ear develops. Several studies have shown that the proportion of carbon translocated to roots decreases with time as the ear grows and this is reflected in reduced root mass (Gregory, 2006).

Since the physical quantification of root biomass is difficult, C inputs from the root biomass at harvest are usually calculated using estimates of shoot to root (S: R) ratios (or root: shoot ratios) at peak standing crop (Bolinder *et al.* 1999). Estimates of S:R ratios for common rainfed crops were found to range from 1.84 to 7.29 with a mean of 4.98 (Srinivas *et al*., 2017) (Table 1).

Table 1. Shoot: Root ratios of some rainfed crops at late flowering stage

Crop	Variety	S: R Ratio
Sorghum	SPV 462	3.20
	CSH 16	2.95
Greengram	ML 267	6.06
	LGG 460	6.38
Sunflower	Morden	4.85
	KBSH 44	5.64
Maize	Varun	2.49
	DHM 117	1.84
Castor	Kranthi	5.81
	PCH 111	5.23
Pigeonpea	PRG 158	5.09
	ICPH 2740	4.68
Cowpea	C 152	5.77
	APFC 10-1	5.25
Horsegram	CRHG 4	7.29
	CRIDA 18R	7.08
Mean		**4.98**

Source: Srinivas *et al*. (2017)

Estimates from shoot: root ratios as well as physical measurements of root biomass at harvest indicate that considerable amounts of C remain in root biomass at harvest. From a review of 45 studies, Amos and Walters (2006) estimated that in a range of climates and soil types, corn roots could contribute between 1.5 and 4.4 Mg C ha^{-1} $year^{-1}$. Root biomass represents up to 50% of residues incorporated into the soil from corn and soybean crops (Buyanovsky and Wagner, 1986).

Gan *et al.* (2009) quantified the carbon in different plant parts of wheat, oilseeds and pulses and found that while straw represented the largest stock of C, belowground C was considerable (Table 2).

Table 2. Carbon in plant parts of wheat, oilseeds and pulses at maturity, under rainfed and irrigated conditions in Saskatchewan, Canada

Crop/condition	C (kg ha^{-1})			
	Grain	Straw	Roots (0-100 cm)	Rhizodeposits (65% of roots)
Rainfed				
Canola	343	1371	534	347
Mustard	324	1026	306	199
Flax	362	1212	314	204
Chickpea	495	743	330	214
Drypea	290	678	224	145
Lentil	439	853	380	247
Wheat	639	1491	449	292
Irrigated				
Canola	516	1548	535	348
Mustard	495	1273	365	237
Flax	418	1323	220	143
Chickpea	768	901	295	192
Drypea	424	861	203	132
Lentil	619	968	300	195
Wheat	1004	2133	606	394

Source: Gan *et al.* (2009)

It is clear that in general shoots have more biomass than roots, yet roots contribute more to SOC than shoots, indicating that there must be other mechanisms by which root derived C is preferentially preserved in soil over shoot derived C. Biochemical recalcitrance of root material

(biochemical quality), physico-chemical protection through the interaction with minerals, physical protection from microbial decomposers through aggregation and reduced decomposition of roots present in lower soil depths are mechanisms that explain the preferential preservation of root C in soil as SOC (Rasse *et al*., 2005).

Biochemical recalcitrance (biochemical quality)

The importance of biochemical composition or "quality" in determining the rate of decomposition and mineralization of nutrients from plant materials has long been recognized (Swift *et al.*, 1979). The chemical composition or quality of residues exerts a significant control over their decomposition. Plants generally contain the same classes of compounds, but the proportions of each, which depend upon the species and maturity, influence the degree and rate of decomposition. Residues typically consist of three fractions which differ in decomposition rate; 1. easily decomposable sugars and amino acids, 2. slowly decomposable compounds comprising cellulose and hemicellulose, and 3. recalcitrant materials such as lignin (Van Veen *et al*., 1984).

Biodegradability of plant litter material is often characterized through biochemical fractionation, such as the method of Goering and Van Soest (1970). This method leads to the quantification of a series of organic molecule fractions displaying decreasing biodegradability. Within a given species, the lignin content of roots obtained by the method of Goering and Van Soest (1970) is on average more than double that of shoots (Table 3).

Table 3. Lignin, N and lignin/N ratios of root and shoot tissues of some rainfed crops at late flowering stage

Crop	Variety	Plant part	Lignin %	N %	Lignin/N
Sorghum	SPV 462	Root	8.54	0.90	9.49
		Shoot	4.91	1.32	3.72
	CSH 16	Root	9.16	1.02	9.02
		Shoot	5.76	1.37	4.22
Greengram	ML 267	Root	15.16	2.92	5.20
		Shoot	8.38	3.50	2.40
	LGG 460	Root	13.82	2.85	4.86
		Shoot	9.40	3.60	2.61
Sunflower	Morden	Root	13.28	1.32	10.10

		Shoot	9.90	2.24	4.43
	KBSH 44	Root	12.47	1.19	10.52
		Shoot	9.57	2.21	4.34
Maize	Varun	Root	7.79	1.01	7.75
		Shoot	4.62	1.45	3.20
	DHM 117	Root	8.70	1.06	8.21
		Shoot	4.26	1.55	2.75
Castor	Kranthi	Root	12.41	1.540	8.06
		Shoot	6.30	2.54	2.48
	PCH 111	Root	10.83	1.65	6.56
		Shoot	5.23	2.68	1.96
Pigeonpea	PRG 158	Root	19.44	2.40	8.12
		Shoot	15.97	3.54	4.51
	ICPH 2740	Root	18.86	2.42	7.81
		Shoot	16.07	3.66	4.39
Cowpea	C 152	Root	15.79	2.60	6.07
		Shoot	8.79	3.60	2.44
	APFC 10-1	Root	17.11	2.51	6.82
		Shoot	7.91	3.34	2.37
Horsegram	CRHG 4	Root	17.99	2.40	7.50
		Shoot	7.94	3.23	2.46
	CRIDA 18R	Root	18.80	2.49	7.55
		Shoot	9.04	3.21	2.82

Source: Srinivas *et al*. (2017)

Due to the higher content of lignin in roots, root residues decompose more slowly than aboveground biomass and therefore have greater influence on long term soil organic matter dynamics. The lignin to N ratio, which integrates the effects of the two most important characteristics governing plant residue decomposition, has been proposed as a better indicator of chemical recalcitrance than lignin content alone and used extensively to distinguish plant residues that are difficult to degrade, i.e. high lignin/N ratio, from those that are more easily biodegraded, i.e. low lignin/N ratio. In an evaluation of the decay rates of fine roots of four plantation tree species Raich *et al.*, (2009) found a highly significant negative correlation between fine root decay and fine root lignin: N, which supports the use of lignin: N as a decay-controlling factor within terrestrial ecosystem models. Srinivas *et al*. (2017) reported that across several crop plants, the lignin/N ratio of root tissues was 2-4 times that of shoot tissues (Table 3).

Numerous studies conducted under different conditions confirm the slower mineralization of root C. Srinivas *et al.* (2017) reported that averaged across crops, 50.22% of shoot C was

mineralized, while only 37.35% of root C was mineralized (Table 4) at the end of 120 days of laboratory incubation. This suggests that the proportional contribution of root C to the sequestration of C in soil, through long-term buildup of soil organic matter, is greater than that of other plant parts.

Table 4. Percent carbon mineralized at the end of 120 days of incubation from roots and shoots of rainfed crops

Crop	Variety	Part	% C mineralized
Sorghum	SPV 462	Root	48.90
		Shoot	54.02
	CSH 16	Root	45.46
		Shoot	56.69
Greengram	ML 267	Root	31.01
		Shoot	41.35
	LGG 460	Root	29.74
		Shoot	44.7
Sunflower	Morden	Root	34.11
		Shoot	45.20
	KBSH 44	Root	38.11
		Shoot	46.70
Maize	Varun	Root	34.96
		Shoot	53.49
	DHM 117	Root	34.15
		Shoot	54.14
Castor	Kranthi	Root	39.91
		Shoot	51.42
	PCH 111	Root	42.29
		Shoot	49.57
Pigeonpea	PRG 158	Root	37.14
		Shoot	46.46
	ICPH 2740	Root	36.95
		Shoot	48.86
Cowpea	C 152	Root	38.59
		Shoot	55.68
	APFC 10-1	Root	37.72
		Shoot	54.31
Horsegram	CRHG 4	Root	32.03
		Shoot	48.86
	CRIDA 18R	Root	36.51
		Shoot	51.99

Mean	Root	37.35
	Shoot	50.22

Source: Srinivas *et al.* (2017)

Physical protection from decomposition through aggregation

The organic material released by roots plays a major role in the interaction between root, microorganisms and the mineral soil. Roots improve aggregation directly by enmeshing soil particles and indirectly by stimulating microbial biomass which in turn synthesizes polymers that act as binding agents (Jastrow *et al.*, 1998; Tisdall and Oades, 1979). The existence of stable macroaggregates in soil is very important for the stabilization of SOM, because the formation of stable microaggregates is fostered within macroaggregates. Stable aggregates protect SOC from biodegradation by reducing the access of decomposers to these encapsulated substrates (Elliott, 1986; Oades, 1988).

Physico-chemical protection through interaction with minerals

Roots interact with mineral soil in a manifold manner. Plant roots produce many organic acids; lactate, acetate, oxalate, malate and citrate being the primary anion components. These molecules are generally considered as labile compounds that are mineralized within a few hours following release by roots. It is often ignored that due to their negative charge, these substances may become rapidly and readily sorbed to the mineral phase through cation bonding (Jones, 1998). For citrate, it was demonstrated that interaction with clay minerals and Fe oxides inhibits degradation (Jones and Edwards, 1998). These soil minerals possess most of the available surface area in mineral soils. Available surface largely governs the stabilization of organic compounds. Sorption of root-derived organic acids to the mineral phase may be more effective in subsoils with low contents of organic matter because mineral surfaces are not yet saturated with organic matter.

Reduced decomposition in deeper soil layers

Depending on the plant species, roots can transfer C to considerable depth in the soil profile. There is considerable variation between both plant types and individual plant strains (cultivars) as to the maximum depth to which they produce roots, but 2 m for angiosperms (and

much more for trees) is not at all uncommon (Kell, 2011). Plant root depths vary greatly in the same soil for different plants, for different cultivars of the same plant and even between different mutants of the same parent (Kell, 2001). Most presently cultivated agricultural crops have root depths that do not extend much beyond 1 m, but a few crop plants can produce roots exceeding 2 m (Kutschera *et al.*, 2009). Using data from experimental root measurements and modeling, Metselaar *et al.* (2009) estimated the rooting depths of globally important agricultural crops (Table 5) and found that averaged across all crops the depth within which 95% of roots were present (D95) was 90 cm, while depth within which 50% of roots were present (D50) was 19 cm. Deeper root systems have the potential to sequester SOC (Smith, 2004) deeper in the soil profile, where SOC turnover times to atmospheric CO_2 is slower due to unfavourable conditions for microbial activity with respect to moisture, temperature and nutrient availability.

Table 5. Average depth to 50% roots (D50) and depth to 95% roots (D95) of major agricultural crops

Crop	D50 (cm)	Weighted D50 (cm)	D95 (cm)	Weighted D95 (cm)	Number of observations
All crops	28	19	172	90	603
Barley	19	16	97	63	10
Rye	27	24	216	154	6
Rapeseed	16	14	99	73	30
Potato	33	30	125	83	50
Sugarbeet	47	45	154	129	11
Rice	11	10	53	25	91
Cotton	41	33	291	162	98
Maize	42	30	252	64	52
Pulses	25	23	155	41	49
Sunflower	24	14	181	32	28
Soybean	23	16	166	89	41
Wheat	19	13	128	42	80
Others	38	24	259	45	57

Source: Metselaar *et al*. (2009)

Strategies for enhancing C sequestration by roots

The scope for sequestering atmospheric C into plant roots is substantial. Any strategy that increases the quantity of C allocated belowground, enhances the recalcitrance of belowground inputs, or retards the decomposition of belowground C, will result in greater C sequestration in soil. In agroecosystems, such strategies include crop improvement through breeding or

biotechnology, choice of cultivars, crops and cropping systems (intensive cropping, intercropping, mixed cropping, rotational cropping, alley cropping with tree components, etc.), and soil and crop management practices. Since potential for C sequestration in deeper soil layers is large, crop cultivars that express deeper and denser rooting characteristics will present greater opportunities for C sequestration. There is considerable scope for increasing the depth of roots by appropriate breeding strategies (Kell, 2011). Switching from annual crops to perennials or converting annual crops to perennials through breeding can also help sequester carbon as perennials have deeper and more extensive root systems that allow them to survive climatic extremes such as droughts and floods.

Subsoil C sequestration can be achieved by higher inputs of stable organic matter to deeper soil horizons. This can be achieved directly by selecting plants/cultivars with deeper and thicker root systems that are high in chemically recalcitrant compounds like suberin. Furthermore, recalcitrant compounds could be a target for plant breeding/biotechnology to promote C sequestration (Lorenz and Lal, 2005). Breeding crops that could cover present cropland areas but that had roots a metre deeper in the soil could double the amount of carbon captured from the environment (Kell, 2011). This could be a significant weapon in the fight against climate change.

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Chapter-2

MINIRHIZOTRON STUDY OF ROOT SYSTEM – *IN SITU* ROOT SCANNER

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Due to the inaccessibility of root systems, special techniques are required to investigate plant roots, their morphology, growth patterns, standing stock, distribution and turnover, and to construct belowground carbon budget. Traditionally, destructive techniques such as soil coring, in-growth cores, whole root system excavation, and trenching have been used to investigate root processes, while more recently, minirhizotrons are being used. Minirhizotrons provide a nondestructive, in situ method for viewing roots and are one of the best tools available for directly studying roots. They permit the simultaneous measurement of fine root production and disappearance, which cannot be accomplished using coring, in-growth cores or excavation approaches.

Minirhizotron techniques have improved significantly since they were first proposed (Bates, 1937) and are widely utilized to study the dynamics and functions of fine roots in agricultural and natural plant communities. Their greatest strength is their ability to monitor (from birth to death) specific root segments at frequent time intervals without significantly impacting fine root processes. They can be used to characterize fine root production, phenology, growth, mortality and lifespan, and are useful in developing ecosystem carbon budgets (Majdi, 1996).

As defined by Brown and Upchurch (1987) ‘‘a minirhizotron system is a multicomponent assembly that visually or photographically records normally inaccessible plant roots growing within the soil’’. A variety of methods have been used to view roots in minirhizotrons including: root periscopes, endoscopes, boroscopes, fiber optics, fiber optics with cameras, illuminated mirrors, and miniature color video cameras. Today, a minirhizotron system typically consists of a minirhizotron tube that has been inserted into the soil, a color micro-video camera, a camera control unit to focus the camera and adjust the light levels, a VCR for recording root images on video tape, and a monitor for viewing images as they are collected. In recent times scanners are

being increasingly used in place of cameras and scanned images can be stored and viewed on portable computers.

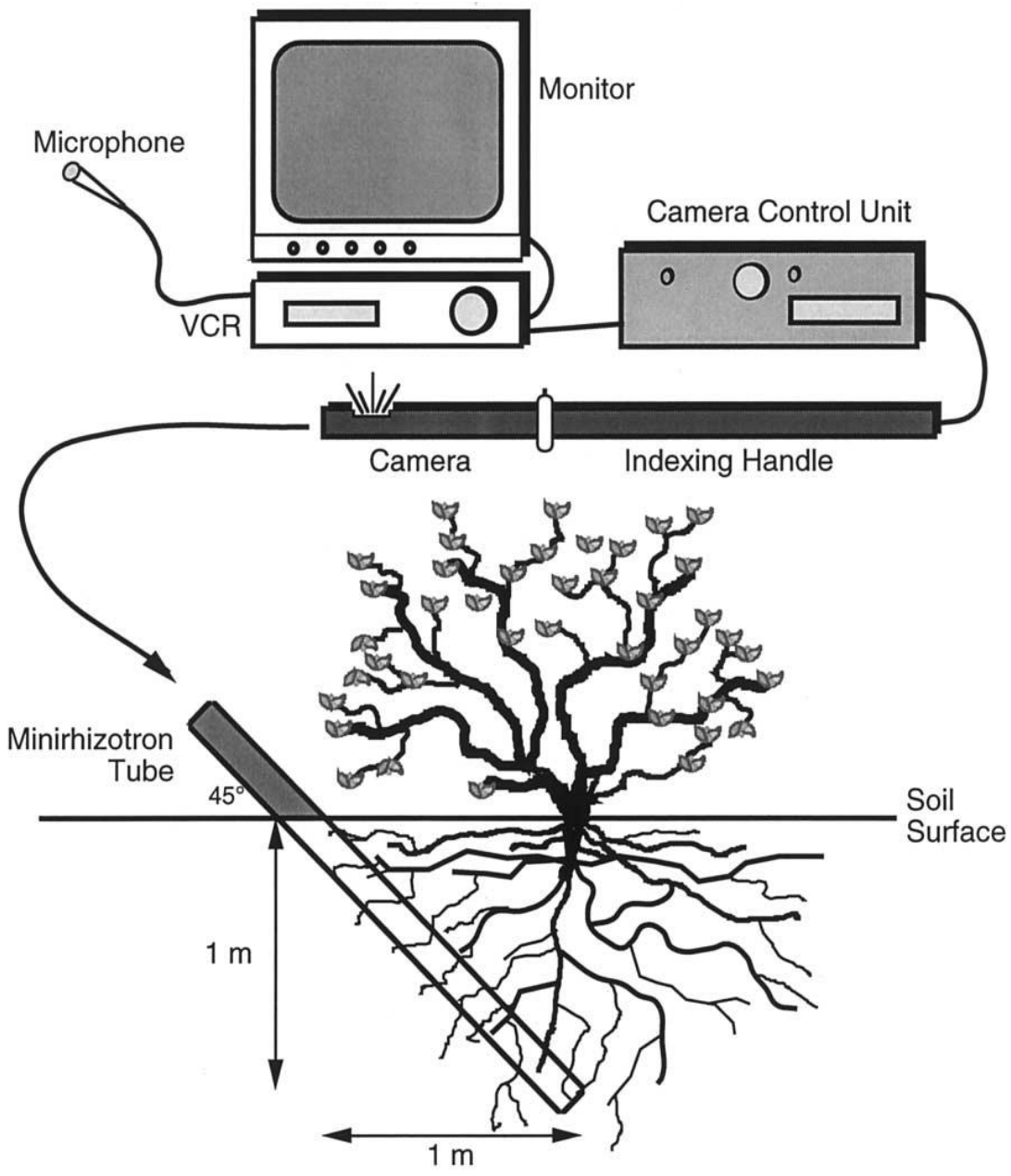

Fig. 1. Schematic diagram of a video camera based minirhizotron system

Minirhizotron tubes have been made of Plexiglas, cellulose acetate butyrate (CAB), polycarbonate (lexan), acrylic, and glass. Material used does not appear to affect root growth; the

choice of material will depend upon cost and availability. Polycarbonate tubes are thought to be more scratch resistant than other plastics.

Minirhizotron tubes should be sealed or covered to exclude moisture. Machined polycarbonate and PVC plugs and rubber stoppers have been used to seal the belowground end of minirhizotron tubes. Typically, the aboveground portions of minirhizotrons are insulated and covered with a light-tight cap, painted or covered with black electrical tape to reduce thermal fluctuations in tubes and exclude light that can affect rooting along the minirhizotron tube.

Two primary objectives guide installation of minirhizotron tubes. First, the installation procedure should minimize disturbance to the soil surrounding the tube. Second, the procedure should optimize the contact between the soil and the tube surface. Ideally, minirhizotron tubes should be installed in such a way, that they become an integral part of the soil matrix and do not affect the root growth. Roots encountering a minirhizotron tube should grow just as if they encountered a rock or other large object in soil.

Minirhizotron tubes have been installed in the soil at different angles. Review of 25 recent minirhizotron studies in long-lived species showed that a range of minirhizotron angles have been used. The most common angles were either 30 or 45° off vertical, each accounting for 24 and 28% of the studies, respectively. Four studies used horizontal tubes (90°) and three used vertical tubes (0°).

The following root parameters can be determined from minirhizotron images. Root number, root length, root length density (RLD) on area basis, RLD on volume basis, root biomass density (RBD) on volumetric basis. The greatest strength of Minirhizotrons is that they allow the researcher to track appearance, growth, and disappearance of individual roots over time, permitting estimation of root production and turnover (mortality), and fate of individual roots over extended periods of time.

Most studies report that the minirhizotrons underestimate root length in the upper soil layers compared to root length density, and overestimates root length in the deeper soil layers when compared to the soil core method. Underestimation has been attributed to poor soil/tube wall contact and inhibition of root growth caused by light entering the topsoil. In deep soil layers, root growth tends to be overestimated because of preferential root growth along the tube.

While there are several methods for studying roots, no single method can serve all the requirements of the researcher. The following table provides a comparison of methods based on several criteria.

Table 1. Evaluation of methods to measure roots

Method	Accuracy	Work	Analysis	Dynamic	Cost	Throughput
Monoliths	+++	+++	+++	---	---	---
Soil cores	++	+++	+++	--	---	---
Trench walls	++	+++	++	-	---	---
Mesh bags	-	+	++	+	--	--
2D rhizotrons	+	+	+	++	+	+
Minirhizotrons	-	+	+	++	+	+
Optical scanners	+	-	+	++	++	+
Electrical capacitance	--	---	-	+	-	++
Ground penetrating radars	?	---	--	+++	++	++
Computed tomography methods	+++	--	++	++	+++	+

Accuracy is the accuracy of the method to measure root length, Work describes the amount of work involved in sample collection, Analysis describes the amount of work required to extract numerical data, Dynamic indicates the suitability of the method to assess roots over time, Cost compares methods in terms of financial investment and throughput considers the suitability of the method for high throughput studies.

Operating procedure for root scanner based minirhizotron system

1. Install minirhizotron tubes in the soil at desired angle by using suitable augers that produce holes of the same diameter as the outer diameter of the minirhizotron tubes. The installation is best done several months ahead of sowing of field crops to allow the soil around the tube to stabilize, so that it is identical to the bulk soil.
2. Load the software required to run the root scanner in the portable computer.
3. Fully charge the portable computer.
4. Connect the root scanner to the computer using the provided USB cord.

5. Open the root scanner software and calibrate the scanner.
6. Set the scan specifications such as image file type, resolution, brightness and contrast and select a folder to store images.
7. Insert the scanner into the tube to the desired depth using the stops/marks provided on the connector.
8. Run the scan. The image is captured, processed based on the file type chosen and stored in the designated location.
9. The scanned images can be analyzed for different parameters using any root image or general image analysis software.

Precautions

1. The bottom ends of the minirhizotron tubes should be sealed effectively to prevent water from entering the tube. Water in the tube can ruin the scanner optics and electronics.
2. Water tends to condense on the inner surface of the tube and can introduce noise into the images. Condensed moisture should be removed by gently cleaning the inner surface of the tube with soft cloth.
3. The section of the tube projecting above the soil surface should be taped or covered to prevent light from entering into the tube.
4. The top end of the tube should be closed to prevent water from entering into the tube.

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Chapter-3

INSTRUMENTATION RELATED TO ROOT STUDIES: CHNS ANALYSER

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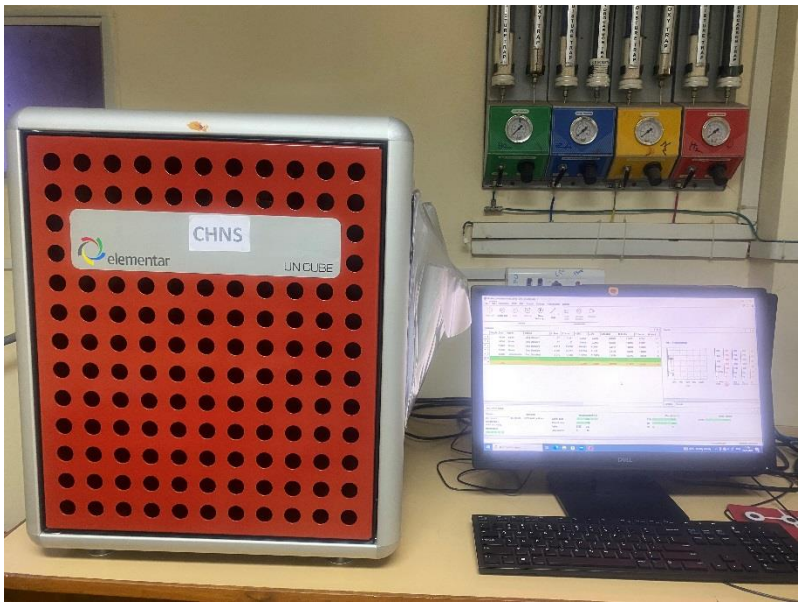
Nitrogen and carbon determination by combustion analysis is very common for soils, plants, leaves, roots, sediments, filtered material and animal tissues. Nitrogen and carbon provide important information for agricultural and environmental research. The importance of soil and plant testing has increased in the last years, as many of the traditional methods are no longer suitable for routine analysis, for their time-consuming sample preparation and for the required use of hazardous reagents. For this reason, the need for an efficient analytical technique has become critical. As the demand for improved sample throughput, reduction of operational costs and minimization of human errors has increased dramatically, a simple and automated technique which allows fast analysis with an excellent reproducibility is the key for efficient nitrogen and carbon determination. CHNS elemental analysers provide a means for the rapid determination of carbon, hydrogen, nitrogen and sulphur in organic matrices and other types of materials. They are capable of handling a wide variety of sample types, including solids, liquids, volatile and viscous samples, in the fields of agriculture, environment, food and energy. The analysers are often constructed in modular form such that they can be set up in a number of different configurations to determine CHN, CHNS, CNS, CN or N depending on the application. CHNS analysis requires high temperature combustion in an oxygen-rich environment and is based on the classical Pregl-Dumas method. This combustion can be carried out under both static conditions i.e. introduction of a set volume of oxygen or dynamic conditions i.e. a constant flow of oxygen for a set period of time. Often, catalysts are also added to the combustion tube to aid conversion. In the combustion process, where temperatures can reach up to 1800^{o}C, carbon is converted to carbon dioxide; hydrogen to water; nitrogen to nitrogen gas/oxides of nitrogen and sulphur to sulphur dioxide. If other elements such as chlorine are present, they will also be converted to combustion products, such as hydrogen chloride. A variety of absorbents are used to remove these additional combustion products as well as some of the principal elements, sulphur for example, if no determination of these additional

elements is required. The combustion products are swept out of the combustion chamber by inert carrier gas such as helium and passed over heated (about 600^{o} C) high purity copper. This copper can be situated at the base of the combustion chamber or in a separate furnace. The function of this copper is to remove any oxygen not consumed in the initial combustion and to convert any oxides of nitrogen to nitrogen gas. The gases are then passed through the absorbent traps in order to leave only carbon dioxide, water, nitrogen and sulphur dioxide. Detection of the gases can be carried out in a variety of ways including (i) a GC separation followed by quantification using thermal conductivity detection (ii) a partial separation by GC ('frontal chromatography') followed by thermal conductivity detection (CHN but not S) (iii) a series of separate infra-red and thermal conductivity cells for detection of individual compounds. Quantification of the elements requires calibration for each element by using high purity 'micro-analytical standard' compounds such as acetanilide and benzoic acid.

Combustion elemental analysers are manufactured in a variety of configurations to suit specific applications, and the choice depends on the elements of interest, the sample type and size, and the concentration of the analyte. All instruments require two gas supplies: (i) an inert carrier gas (helium recommended); and (ii) high purity oxygen (minimum 99.9995%). The strict specification for oxygen is associated with the need to reduce the nitrogen 'blank' contribution to an inconsequential level. Additionally, GC-type gas filters are also usually fitted to prevent trace organic species and water entering the combustion system. The choice of sample introduction systems will depend on the application and the sample type. For solids or viscous liquids, samples are weighed out into tin capsules; for liquids, samples can be sealed in individual aluminium vials or introduced via a liquid auto-sampler. Both capsules and vials are pre-cleaned and dried to avoid trace contamination from oils and water acquired during their manufacture. Instruments are marketed with either simple 'one

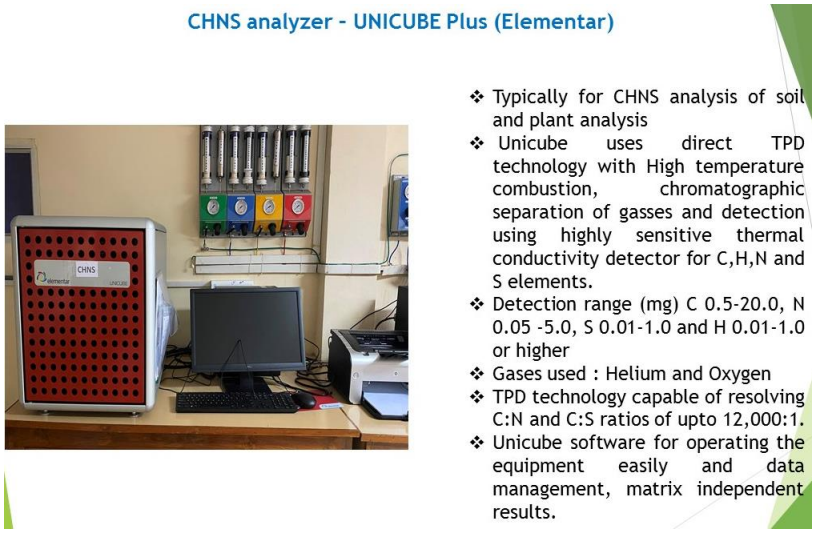


shot' introduction interfaces or a carousel type autosampler. In some instances, a microbalance is directly interfaced with the analyser to allow the automatic recording of the weight of each test portion. The combustion section of the analyser is designed to achieve both complete combustion of the sample and conversion of oxides of nitrogen to nitrogen gas (N_2). Although different approaches have been chosen by different manufacturers, the use of high purity copper is universal for the reduction stage. In some instruments, the combustion and reduction stages are housed in separate furnaces. In others, the reactions are combined in a single two-tier furnace. Catalysts are usually added to the combustion section to aid complete combustion and absorbents to remove potential contaminants. Both the catalysts/absorbents and copper metal are packed into readily exchangeable tubes made of ceramic material or high quality silica. Instruments are classified as either 'static' or 'dynamic' in terms of their combustion characteristics. In the 'static' type, a pre-set volume of oxygen is added to the combustion tube before the sample is introduced. In the 'dynamic' type, the oxygen is added to the tube at the same time as the sample introduction and continues to flow for a set time. In the majority of applications, either method is applicable. For slow burning materials such as coals and cokes, where multiple additions of oxygen are required for complete combustion, the 'static' system is preferred. The detection system within the analyser can take several forms depending on the combustion mode and sample size. With small test portions, the combustion gases can be separated on a GC column and quantified using a thermal conductivity detector. If larger test portions are required, an instrument employing 'frontal' chromatography can be chosen. The latter approach employs a GC column with thermal conductivity detection but provides a step-wise profile for integration. Yet other detection approaches require no separation step but use separate infra-red and thermal conductivity cells to respond to individual elements. Control of the instrument is established through a computer module, which is used to set up the program of work, report instrument diagnostics, and manage the calibration procedures.

The choice of instrument depends on a number of factors associated with the sample type. In instances where obtaining a homogeneous sample can prove difficult, food analysis for example, a greater weight of test material is required to provide a representative test portion. The larger the test portions and the higher the content of organic matter, the more oxygen will be needed to carry out the combustion successfully. This in turn means that larger capacity reduction tubes are needed to remove the excess oxygen and provide capacity for a reasonable number of combustions before

replacement. For such applications, a macro-analyser would be required for gram-sized samples. For less heterogeneous samples, a micro-type analyser designed for milligram quantities would be appropriate. Another important consideration is the amount of ash that is formed during the combustion and its removal. The ash will comprise the remains of tin and aluminium containers and the inorganic residues from the test portion. Instruments are manufactured with both vertical and horizontal furnace configurations. With vertical systems, ceramic crucibles are placed in the combustion tubes to accommodate the ash. This allows extended auto-sampler operation but can lead to back pressure problems if the ash is not removed on a regular basis. In horizontal systems, the ash is removed after each combustion although this arrangement is difficult to automate.

CHNS elemental analysers have been used in analytical laboratories for over several decades. The method is used extensively across a wide range of applications, including pharmaceuticals, chemicals, oil industry, agriculture, energy and food. In plant analysis, the determination of nitrogen (as a surrogate for protein) is very important for evaluating nutritional quality and is increasingly being undertaken by combustion analysis.



Chapter-4

INFLUENCE OF SOIL AND WATER CONSERVATION MEASURES ON ROOT NETWORK

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Soil loss and its quality degradation due to soil erosion remains a major factor of low crop productivity in tropical and subtropical countries. Soil and water conservation (SWC) ts should therefore be implemented toward soil erosion control for protection/ conservation of nutrients, soil organic matter and soil physical properties. The main factors causing soil erosion can be classified into three groups: (1) energy factors, e.g., rainfall erosivity; (2) protection factors, e.g., plant cover and land management; and (3) resistance factors, e.g., soil erodibility. Soil erosion is a major threat to the soil resource, soil fertility, productivity, and, lastly, to food and fiber production (Boardman et al. 2009). According to Ighodaro et al. (2013), soil erosion is the biggest environmental problem the world faces second only to population growth. Worldwide, erosion on cropland averages to about 30 t/ha/year, and each year about 10 million ha of cropland is rendered unproductive and abandoned due to soil erosion (Nyawade et al. 2018a, b). Pimentel (2006) reports that soil erosion is highest in Asia, Africa, and South America where soil losses range from 30 to 40 t/ha and that it is severe in small farms located in marginal areas where soil quality is poor and the topography often steep (Fig 1). Soil erosion is severe in small farms located in marginal areas where soil quality is poor and the topography is often steep. Although the problem is as old as settled agriculture, its extent and impact on human welfare and global environment are more now than ever before. However, human alterations of land use and cover have caused erosion rates to increase for many areas of the world, resulting in considerable land and environmental degradation (Valentin et al. 2008; Martínez-Casasnovas et al. 2009; Farhan et al. 2014; Tesfahunegn et al. 2014). While temporary solutions such as increased fertilizer have offset some of the effects of erosion on soil productivity, they are not complete substitutes for topsoil (Gachene et al. 1997) and represent the greatest input cost for compensating yield losses caused by erosion (Pimentel 2006).Studies conducted on a humic Nitisol at Kabete, Kenya, indicated that soil erosion by water can lead

to substantial loss in maize growth and grain yield (Gachene et al. 1998) .Thus, in order to maintain long-term productivity and preserve soil and environmental quality, it is important to implement SWC practices that prevent and minimize soil erosion, rather than manage the effects of erosion after it has occurred (Yannelli et al. 2013). Runoff and soil loss prediction has been widely used as a tool to guide in SWC planning (Murillo et al. 2011; Renschler and Harbor 2009; Gobin et al. 2004).

Fig 1. **A view of the stream passing through slopy lands affected by soil erosion**

Table 1: Effect of soil erosion on maize growth and yield

Soil loss (t/ha)	Leaf area index	Crop height (cm)	Grain yield (kg / ha)
0.77	4.85	247.3	828.6
40.54	4.67	235.0	601.6
171.31	5.52	226.7	517.8
247.27	2.89	163.4	300.0

Source: Gachene et al. (1998)

Agronomic Measures of Soil and Water Conservation (SWC)

Agronomic SWC approaches have gained considerable application particularly in sub-Saharan Africa due to their low cost of adoption compared to the structural SWC measures. These techniques are categorized into five groups: crop management, soil management, contour farming, organic matter and fertilizer management, and agroforestry.

Crop Management Practices

The use of cropping systems and mulching to conserve soil and water in arable lands is increasingly gaining interest and is often evaluated in runoff plots (Fig 2). Good crop management reduces soil erosion by water and wind to tolerable levels and can improve soil fertility. To achieve these practices, select appropriate crops for the soil and slope, plant early, and use suitable cropping

systems and rotations to keep the soil covered. Besides their role in crop diversification, intercropping systems are important in soil erosion control and soil fertility improvement. In a study conducted in central Kenya to quantify the losses of soil organic matter due to soil erosion under different potato-based cropping systems, Nyawade et al. (2018b) observed 34% reduction in soil organic matter losses when Dolichos lablab was intercropped with potato crop. This observation was attributed to the Dolichos's indeterminate growth pattern which enabled it to provide effective postharvest cover. Mulching is particularly applicable where crop residues have limited competitive use rather than for soil erosion. The mulch protects soil surface against raindrop impact, decrease runoff velocity by impacting roughness, and thus improves water infiltration capacity. Mulching also enhances burrowing activity of earthworms which improves percolation of water through the soil profile, thus improving soil moisture storage in the root zone. Integrated use of contour hedge- rows and mulching remarkably reduced runoff from 100 mm to 20 mm and soil loss from 100 to 2 Mg ha^{-1} in a sloping semi-arid land in Eastern Kenya (Kinama et al. 2007).

Fig 2. Runoff plots installed at right angle to the contours and parallel to the slope

Soil Management Practices

Designing sustainable soil management practices remain a major challenge in East and Central African region. Losses of SOC, N, and P due to soil erosion and poor cropping systems are estimated to be at rates of 270 million tons per year in Africa. Given the inherent low fertility of many soils in tropical and subtropical regions, the use of farmyard manure and inorganic fertilizer is particularly important due to their ability to improve both soil water content and soil properties. For instance, extensive work on highly weathered soils in Kenya (Otieno et al. 2018), Tanzania (Okalebo et al. 2006), Uganda (Pincus et al. 2016), Democratic Republic of the Congo

(Munyahali et al. 2017), and Angola (Rodrigues et al. 2018) has demonstrated the potential of crop residues to restore soil fertility. Improvement of soil productivity in degraded soils therefore not only requires the application of chemical fertilizers but also strategies that, for instance, integrate legume cover crops into cropping systems (Nyawade et al. 2018b; Nyawade 2015).

Conservation Tillage

Proper tillage systems which cause minimum disturbance to the soil and have the ability to increase soil water content are essential for effective SWC. Such systems include conservation tillage (zero and minimum tillage), contour tillage, contour ridging, and ripping. The concept of conservation tillage, though not new, is gaining popularity in East Africa for sustainable crop production, especially in dry areas. After several decades of SWC efforts in Africa, conservation tillage has been recognized as the missing link between biological methods of agro-forestry, farm inputs, and mechanical approaches such as terracing. The method aims at reducing labor in land preparation through tillage systems that promote soil fertility and soil water conservation. Conventionally, tillage is conducted to pre- pare a seed bed and also to control weeds. However, conventional tillage has been found to destroy the structure of the soil and cause com- paction. This has negative effects on soil aeration, root development, and water infiltration, among other factors. More important, but less noticeable, is the destruction of soil microbiology by disturbance and turning over of soil, which is then exposed to drastic atmospheric and climatic conditions. Conservation tillage, therefore, takes care of this by applying four main principles: (1) zero or minimum soil turning, (2) permanent soil cover (3), stubble mulch tillage, and (4) crop selection and rotations. An important aspect of conservation tillage practice involves ripping the land with tined implements or subsoiling the land immediately after crops are harvested, to break the plow pans. Suitable equipment includes animal-drawn subsoilers, rippers, “ridgers,” planters, and weeders.

Grass Strips and Vegetative Buffers

Grass strips are the least costly and least labor- demanding soil conservation structures. They combine characteristics of both biological and structural measures. Grass strips are a popular and easy way to terrace land, especially in areas with relatively good rainfall where grass is used also as fodder. The grass is planted in dense strips, about 0.5-1 m wide, along the contour, at intervals equivalent to calculated terrace spacing. These lines create barriers that minimize soil erosion and runoff through a filtering process. Silt builds up in front of the strip, and with time,

benches are formed. The spacing of the strips depends on the slope of the land. On gentle sloping land, the strips are made with a wide spacing (20-30 m), while on steep land, the spacing is about 10-15 m. The grass needs to be trimmed regularly to prevent spreading to the cropped area. The grass is cut and normally used as livestock fodder or as mulch. Many grass varieties are used, such as napier, guinea, and guatemala grass. The main drawback with grass strips is that they harbor rodents, and in dry areas, they may not survive the dry spells.

Organic Matter and Fertilizer Management

Adding manure and fertilizers to the soil supplies the plant nutrient for vigorous crop growth. The elevated nutrient levels boost crop growth, thus covering the ground quickly, protecting it from erosion, and allowing water to seep in.

Contour Farming and Contour Bunds

Contour farming entails preparing the land, planting, and weeding across the slope. It slows down runoff, thus giving the water time to infiltrate into the soil. Experiments show that contour farming alone can reduce soil erosion by as much as 50% on moderate slopes. For slopes steeper than 10%, a combination of measures should be recommended. Trash lines made by laying crop residues along contour slow down runoff and trap eroded sediment. However, these structures can be destroyed by termites. Grass barrier strips planted along the contour are effective soil conservation measures on soils that absorb water quickly and on slopes as steep as 30%. The effectiveness of contour farming in water and soil conservation depends not only on the design of the system but also on the soil, climate, slope aspect, and land use. The beneficial effect is least marked on compact or slowly permeable soils because these soils become saturated quickly compared to highly permeable soil. Contour bunds have shown great potential to reduce runoff and soil loss in Southern Africa (Thierfelder and Wall 2009). Contour bunds are soil conservation structures that involve construction of an earthen bund by excavating a channel and creating a small ridge on the downhill side. The difference from earthen bunds is in the fact that contour bunds are used for draining excess runoff from steep cultivated slopes, while earthen bunds are used for runoff harvesting usually on relatively less steep lands. Thus, contour bunds resemble narrow channel terraces which in Kenya are referred to as fanya chini terraces. Contour bunds are used for prevention of flooding and are popular in the highland areas of Ethiopia. Contour ridges have received application in semiarid areas to harvest water and in higher rainfall areas for growing potatoes.

Structural Measures of Soil and Water Conservation

Structural measures of SWC in croplands often aim at changing the slope profile and are primarily constructed to control runoff and soil erosion. Their construction usually involves earth movements and requires substantial initial inputs of labor or money. These structures are often located along the contour and in marginal rainfall areas where rainfall needs to be conserved on-site. The appropriate type of physical structure depends on climate and need to retain or discard the runoff, farm sizes, soil characteristics (texture, drainage, and depth), availability of an outlet or waterway, labor availability and cost, and the adequacy of existing agronomic of vegetative conservation measures.

Diversion Ditches

Diversion ditches are surface drainage structures constructed across the field to intercept and divert surface runoff from the slope above and drain it to a safe outlet. They are used to protect cultivated lands, collect water for ponds or other storage schemes, and control development of gully heads. They are referred to as cutoff drains when constructed at the boundary between cropland and the adjacent non-arable land. Channel may either be grass lined or earth lined. Grass-lined channel is more stable and is suitable for broad shallow channels, while narrow and deep channels are often earth lined. Short ditches (250 m long) are suitable in areas with highly erodible soils. On stable soils like red clay loams, the length of the channel should not be more than 500 m.

Infiltration Ditches

These are used to harvest water from roads or other sources of runoff. They consist of a ditch, 0.7-1.5 m deep, dug along the contour, upslope from a crop field. Water is diverted from the roadside into the ditch which is closed at the other end.

Water Retention Pits

These are series of pits that trap runoff and allow it to seep into the soil. The size of the pits depends on the projected amount of runoff. A typical size is 2 m^2 and 1 m deep. Vegetation such as bananas and tree crops should be planted around the pits and kept from children and livestock.

Waterways

These structures divert runoff safely from hillslopes to valley bottoms where it joins a stream or river. It may be natural or artificially made and well stabilized with grass, stone, masonry, or concrete. Reinforced concrete waterways are more efficient but have not been widely adopted by smallholder farmers due to their high cost. Creeping grasses such as signal grass (*Brachiaria*

humidicola) and bahia grass (*Paspalum notatum*) are suitable for lining waterways as they cover the ground closely and require little maintenance. Generally, grass species that are weedy and those that can block the waterways and divert runoff should be avoided.

Terraces

Terracing by excavating ditches, construction of earth and some stone bunds, and vegetative barriers are normally defined as SWC structures and are primarily promoted to reduce soil erosion. On sloping lands, terracing is necessary for reducing overland flow rates, thereby contributing to water and nutrient conservation. Although terracing steep lands especially in the Eastern African region has been an indigenous technology among some communities, new methods have been evolving over the years as the need to be innovative with ever-decreasing space for cultivation grows with the population, especially in the densely populated and erosion-prone highlands. Therefore, simply put, terraces are earth embankments built across a slope to intercept runoff water, reduce soil erosion, and increase *in situ* water infiltration. Level terraces can reduce runoff by 92% and soil loss by 87-95%, while graded terraces can reduce runoff by 77-92% and soil loss by 98%. Terraces can be designed to channel excess water into waterways or stable outlet. In East and Central Africa, three main types of terraces are used on croplands: bench terraces, fanya juu terraces, and channel terraces. Fanya juu terrace is made by digging a trench and throwing the soil upslope to form an embankment along a contour, while in fanya-chini, the soil is thrown downslope. Terraces are stabilized with the non-weedy grass species. In humid and sub-humid areas, farmers prefer fodder grasses like napier grass for stabilization (Fig 3).

Fig 3 Well-developed level bench terraces with bananas planted along the terrace ditch

Impact of SWC measures on root network

Any SWC measure adopted in croplands would result in soil moisture conservation due to infiltrated water into soil profile as these measures are provided across the slope and reduce the

velocity of flowing water on the surface. The agronomic measures are temporary particularly designed for the regions where annual average rainfall is <800 mm and slopes are <6%. Above these values in the regions, the mechanical measures like Contour bunding, graded bunding, terracing are adopted basically for soil moisture and soil conservation by cutting the velocity of flow.

Mechanical forces active on steep slopes tend to overturn plants, which respond by developing a specific asymmetrical architecture in the root system. This asymmetric architecture is the consequence of preferential lateral root emergence and elongation in the up-slope and down-slope directions. Root systems show a normal symmetrical architecture when the same species is grown on plane soil. The asymmetrical root architecture on steep slopes seems to increase the plant's stability by modifying the distribution of mechanical forces into the soil. This hypothesis is supported by the observation that lateral roots developing in the up-slope or down-slope directions present considerable anatomical modifications in shape and tissue-organization compared with lateral roots from plants growing on plane soil.

In response to anchorage reinforcement to avoid uprooting, plants growing on a slope develop an asymmetric root system architecture called ''bilateral-fanshape''. This architecture seems to derive from a preferential elongation of lateral roots in the two main directions (up-slope and down-slope) and represents the most efficient means of distributing self-loading forces. These up- and down-slope roots are structural roots and present a considerable shape eccentricity at their base, the mechanical importance of which is still under investigation. This bilateral-fan shape architecture might partly result from a change in the mechanical function of existing laterals and partly from new late lateral root emergence from parental roots even if secondary growth is well developed. The great interest for this second hypothesis arises from the fact that lateral roots emerging from secondary growth would enable a plant growing on a slope to continuously adjust its anchorage function of self-loading forces variations. A question that needs to be answered is whether or not it is possible to speak of an adaptation to slope conditions. This would be at the basis of the search for an ecotype better adapted to slope conditions.

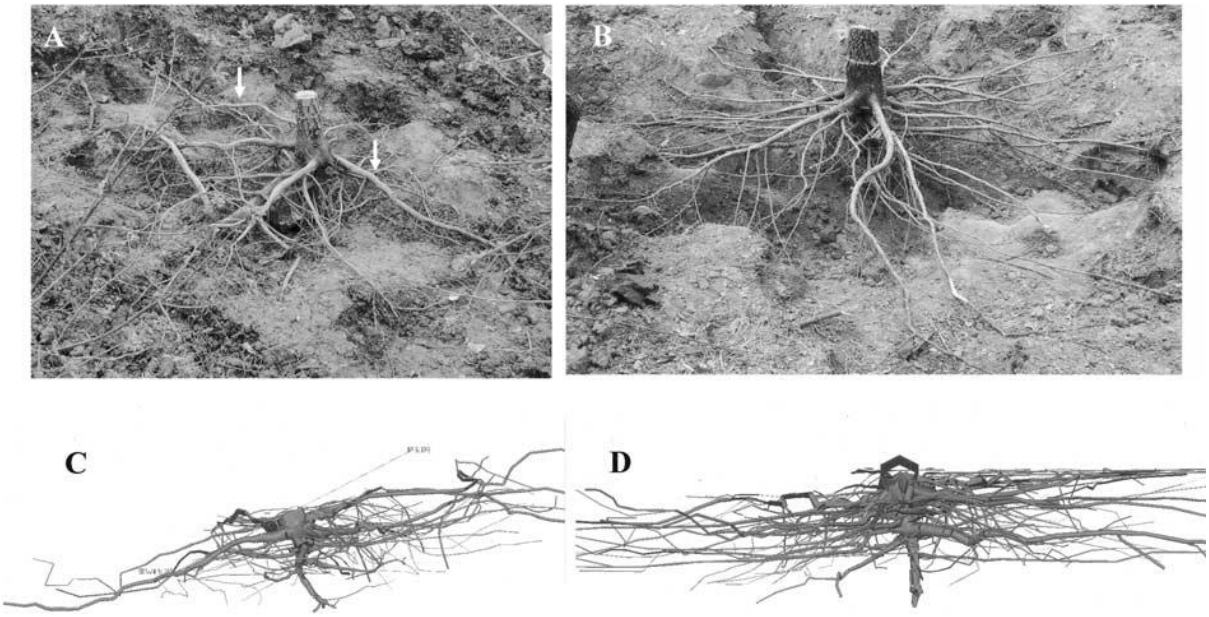
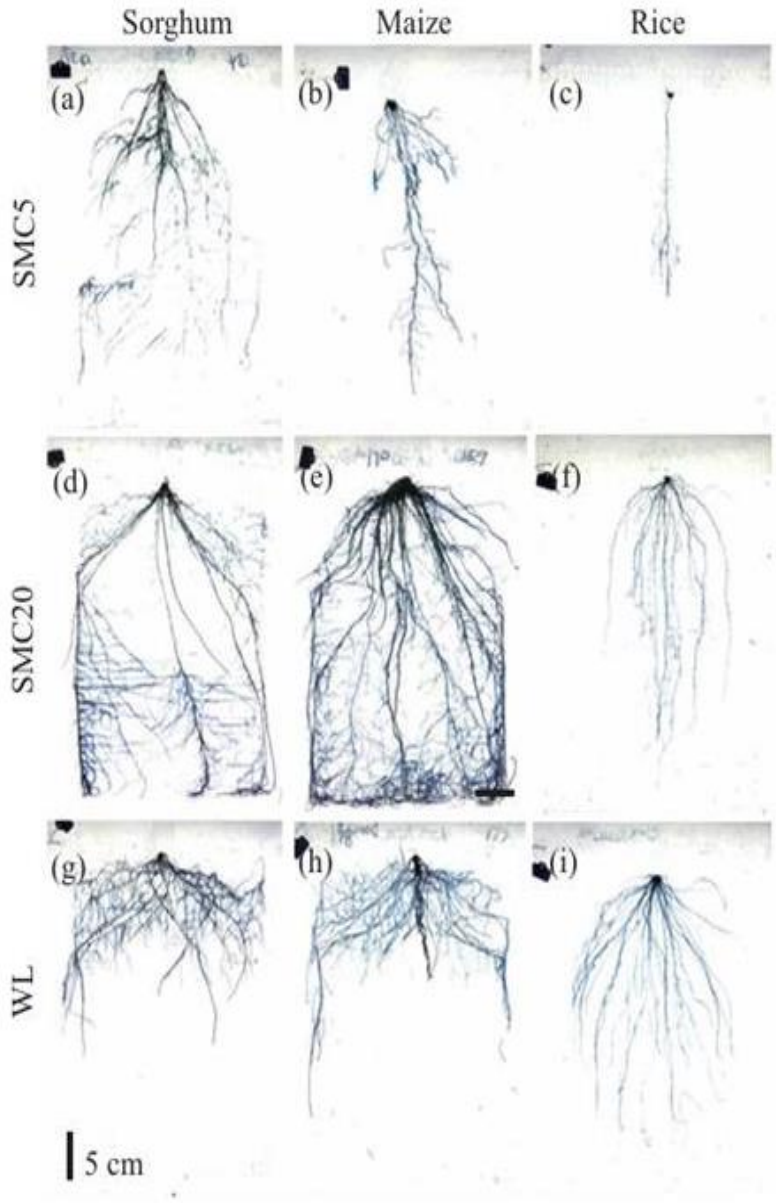


Fig 4. Root system of Quercus cerris growing on a slope (A) or on a plane (B). The two root systems were hand excavated and then taken into the laboratory and placed on a stand in their original orientation. The growth direction of each lateral root has been reconstructed in digital form with the Polemus 3D digitizer. (C) The computerized image of the root system shown in (A). (D) The computerized image of the root systems shown in (B).

Root characteristics under different soil moisture regimes

Water stress such as drought and waterlogging are considered to be a major limiting factor in crop production. Roots play important roles in crop adaptation to water stress. This study aimed to characterize the vertical root distribution patterns and analyze the root-shoot relationships of different cereal species with different water requirements in response to different soil moisture conditions.



., Root system profiles of sorghum (a, d, and g), maize (b, e, and h), and rice (c, f, and i) grown under 5% w/w of soil moisture content (SMC5; a-c), 20% w/w of soil moisture content (SMC20; d-f) and waterlogged soil (WL; g-i) at 35 days after sowing. Root systems were sampled using the root box pin-board method (Kano-Nakata et al. 2012). Plant with average growth size was selected for photo documentation.

Sorghum, maize, and rice were grown under 5% w/w soil moisture content (SMC5), 20% w/w soil moisture content (SMC20) and in waterlogged soil (WL) for 35 days using root box pin-board method. For sorghum and maize, the optimal soil water condition was SMC20 which produced the greatest shoot and root growth, while rice had greatest shoot and root growth under WL. Sorghum significantly increased root to shoot ratio in both water stress conditions, suggesting that sorghum prioritizes carbon partitioning of assimilates towards the roots. Although whole root

dry weight and total root length were reduced by water stress, vertical distribution of root traits varied with soil water conditions and promoted root response was observed in specific soil layer. A highly positive relationship between root and shoot traits was observed in rice, suggesting that root and shoot trait responses are coupled with changing soil water conditions. Further studies are needed to con- firm root architectural changes focusing on different root component traits as well as other root traits related to root architectural structure.

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Chapter-5

INSTRUMENTATION FOR SOIL MOISTURE MEASUREMENT INCLUDING CALIBRATION AND VALIDATION

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Importance of precise soil moisture information is well understood in various fields like agriculture, hydrology, meteorology, environmental studies etc. Soil moisture is very dynamic, both temporally and spatially, therefore its continuous monitoring is necessary. There are various methods available to retrieve soil moisture status. All these methods have their own advantages and disadvantages and should be used with caution depending upon the requirements and demand of the project. An attempt has been made to describe different soil moisture estimation methods right from conventional methods like gravimetric soil moisture techniques to most advanced tools like Synthetic Aperture Radar Polarimetric (PolSAR) techniques. All these methods have not only been assessed individually but have also been compared and evaluated for their relative advantages and limitations. Soil moisture is estimated both by direct and indirect methods. Direct methods involves the determination of moisture in the soil while indirect methods estimate amount of water through the properties of water in the soil. In direst methods moisture is estimated thermo- gravimetrically either through oven – drying or by volumetric method.

Oven drying method

Soil sample is collected in a moisture can and wet weight of the sample is recorded. The soil sample is dried in hot air oven at 105 ^{o}C until constant weight is obtained and dry weight of the sample is recorded.

$$\text{Moisture content (on weight basis)} = \frac{\text{Wet weight - Dry weight X 100}}{\text{Dry weight}}$$

Volumetric method

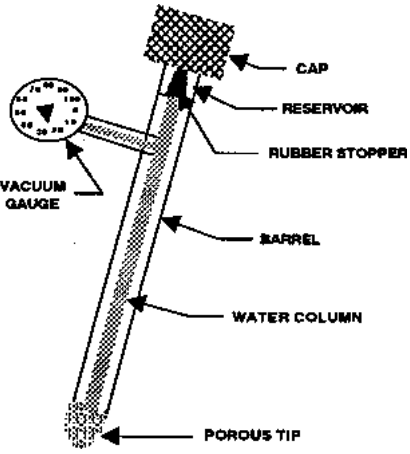
Soil sample is taken with a core sample or with a tube auger whose volume is known. The amount of water present in the soil sample is estimated by drying in the oven. The

volumetric moisture content can also be estimated from the moisture content estimated on dry weight basis.

The most common instrument used for estimating soil moisture by indirect methods is; tensiometer, gypsum block, neutron probe, pressure plate and pressure membrane apparatus.

Tensiometer

Tensiometer is a sealed, airtight, water-filled tube (barrel) with a porous tipon one end and a vacuum gauge on the other, as shown in Figure 1. A tensiometer measures soil water suction (negative pressure), which is usually expressed as tension. This suction is equivalent to the force or energy that aplant must exert to extract water from the soil. The instrument must be installed properly so that the porous tip is in good contact with the soil, ensuring that the soil-water suction is in equilibrium with the water suction in the tip. The suction force in the porous tip is transmitted through the water column inside the tubeand displayed as a tension reading on the vacuum gauge. Soil-water tension is commonly expressed in units of bars or centibars. One bar is equal to 100 centibars (cb). The suction at the tip is transmitted to the vacuum gauge because of the cohesive forces between adjacent water molecules. As the suction approaches approximately 0.8 bar (80 cb), the cohesive forces are exceeded by the suction and thewater molecules separate. When this occurs, air can enter the tube through the porus tip and the tensiometer no longer functions correctly. This condition is referred to as breaking tension. Tensiometers work in the range from 0 to 0.8 bar. The suction scale on to the vaccum gauge of most commercial tensiometers reads from 0to 100 cb.



Tensiometers are quite affordable for scheduling irrigation. The cost ranges from $25 to $50 each, depending on length of the barrel, which ranges from 6 to 72 inches. The only other equipment required is a small hand-held vacuum pump used for calibration and periodic servicing. Tensiometers are easy to use but may give faulty readings if they are not serviced regularly. Tensiometers are best suited for use in soils that release most of their plant-available water (PAW) at soil-water suctions between 0 and 80 cb. Soil

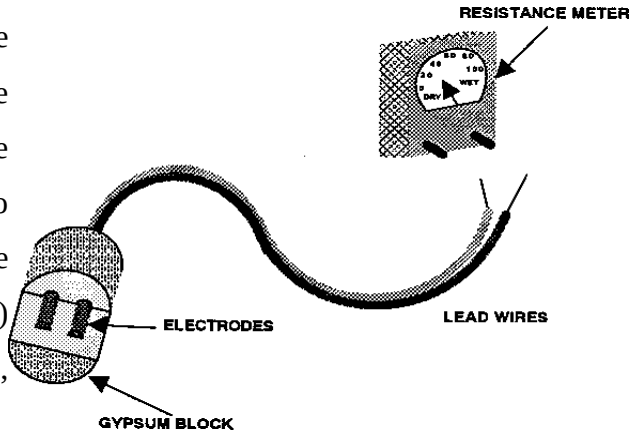
textures in this category are those that consist of sand, loamy sand, sandy loam, and the coarser-textured range of loam and sandy clay loam. Many clayey and silty soils still retain over 50 percent of their plant-available water at suctions greater than 80 cb, which is outside the working range of a tensiometer. Tensiometers are not recommended for clayey and silty soils unless irrigation is to be scheduled before 50 percent depletion of the plant-available water, which is the normal practice for some vegetable crops such as tomatoes.

Gypsum block or Electrical resistance blocks

Electrical resistance blocks consist of two electrodes enclosed in a blockof porous material, as shown in Figure 2. The block is often made of gypsum, although fiberglass or nylon is sometimes used. Electrical resistance blocks are often referred to as *gypsum blocks* and sometimes just *moisture blocks*. The electrodes are connected to insulated lead wires that extend upward to the soil surface.

Resistance blocks work on the principle that water conducts electricity. When properly installed, the water suction of the porous block is in equilibrium with the soil-water suction of the surrounding soil. As the soil moisture changes, the water content of the porous block also changes. The electrical resistance between the two electrodes increases as the water content of the porous block decreases. The block's resistance can be related to the water content of the soil by a calibration curve.

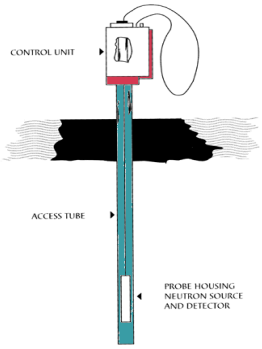
To make a soil-water reading, the lead wires are connected to a resistance meter containing a voltage source. The meter normally reads from 0 to 100 or 0 to 200. High readings on the scale (corresponding to low electrical resistance) indicate high levels of soil-water, whereas, low meter readings indicate low levels.



Because of the pore size of the material used in most electrical resistance blocks, particularly those made of gypsum, the water content and thus the electrical resistance of the block does not change dramatically at suctions less than 0.5 bar (50 cb). Therefore, resistance blocks are best suited for use in fine- textured soils such as silts and clays that retain at least 50 percent of their plant- available water at suctions greater than 0.5 bar. Electrical resistance

blocks are not reliable for determining when to irrigate sandy soils where over 50 per cent of the plant-available water is usually depleted at suctions less than 0.5 bar.

Neutron moisture meter



Soil moisture can be estimated quickly and continuously with neutron moisture meter without disturbing the soil. Another advantage is that soil moisturecan be estimated from large volume of soil. This meter scans the soil about 15cm diameters around the neutron probe in wet soil and 50 cm in dry soil. It consists of a probe and a scalar or rate meter. This contains a fast neutronsource which may be a mixture of radium and beryllium or americium and beryllium. Access tubes are aluminum tubes of 50-100 cm length and are placed in the field when the moisture has to be estimated. Neutron probe is lowered in to access tube to a desired depth. Fast neutrons are released from the probe which scatters in to soil. When the neutrons encounter nuclei of hydrogen atoms of water, their speed is reduced. The scalar or the ratemeter counts of slow neutrons which are directly proportional to water molecule. Moisture content of the soil can be known from the calibration curve with count of slow neutrons.

Pressure membrane and pressure plate Apparatus

Pressure membrane and pressure plate apparatus is generally used to estimate field capacity, permanent wilting point and moisture content at different pressure. The apparatus consists of an air tight metallic chamber in which porousceramic pressure plate is placed. The pressure plate and soil samples and saturated and are placed in the metallic chamber. The required pressure of 0.33 or 15 bar is applied through a compressor. The water from the outlet till equilibrium against applied pressure is achieved. After that, the soil samples are taken out and oven- dried for determining the moisture content.

Phene Cell

The Phene cell works on the principle that a soil conducts heat in relation to its water content. By measuring the heat conducted from a heat source and calibrating the conductance versus water content for a specific soil, the Phenecell can be used reliably to determine soil-water content. Because the Phene cell is placed at the desired soil depth, a separate cell is needed for each depth at each location to be monitored.For irrigating small acreages, the total

cost ofusing the Phene cell is less than that of the neutron probe. For large acreages, the neutron probe may be more cost effective.

Time Domain Reflectometer

The time domain reflectometer (TDR) is a new device developed to measure soil-water content. Two parallel rods or stiff wires are inserted into the soil to the depth at which the average water content is desired. The rods are connected to an instrument that sends an electromagnetic pulse (or wave) of energy along the rods. The rate at which the wave of energy is conducted intothe soil and reflected back to the soil surface is directly related to the average water content of the soil. One instrument can be used for hundreds of pairs of rods. This device, just becoming commercially available, is easy to use andreliable.

Selecting the Right Device

When cost, ease of use, and reliability are considered, tensiometers and electrical resistance blocks are usually the most practical devices for measuring soil-water in North Carolina. For best results, tensiometers and electrical resistance blocks must be properly installed, maintained, and calibrated for the primary soil types in each field. Installation Focedures for tensiometers and resistance blocks are described in the next section. The gravimetric method can be used to calibrate tensiometers and electrical resistance blocks on the farm.

Preparing and Installing Measuring Devices

Tensiometers

Before a tensiometer is installed, the porous tip should be soaked in waterovernight. The tube should then be filled with boiled (air-free) water, and the gauge and tip should be tested using a small, hand-held vacuum pump (availablefrom tensiometer manufacturers). The vacuum pump should also be equipped with a vacuum gauge. It is used to create a vacuum in the tensiometer.

After the porous tip of the tensiometer is saturated, attach the vacuum pump to the top of the tensiometer with the cap removed. Use the pump to evacuate air from the tensiometer barrel. The vacuum gauge reading on the pump and on the tensiometer should be the same. Furthermore, this reading should remain constant for severalseconds, indicating that air is not leaking through the porous tip.

If tension cannot be maintained, the tip or barrel has probably been damaged or cracked. The most common cause of failure is a crack in the porous tip resulting from rough handling. A cracked tip allows air to enter the barrel so that tension forces in the soil are not correctly transmitted to the gauge. Tips, seals and gauges can be replaced by the tensiometer manufacturer.

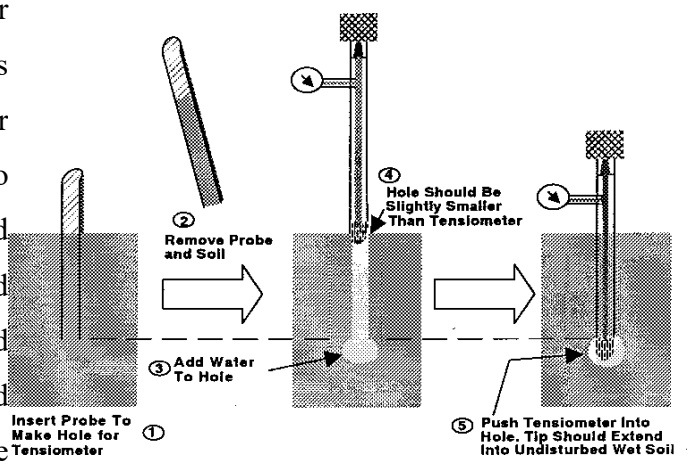
After the vacuum pump test has been completed, the rubber seal in the cap should be tested. Fully assemble the tensiometer and place it on a table or surface so that the porous tip is exposed to the air. Water will begin evaporating from the tip. Within a few minutes, the tension reading on the gauge should begin to increase. If it does not, the rubber stopper in the cap is not providing a good seal and should be replaced. Otherwise, the tensiometer is ready for installation. It should be transported to the field with the tip submersed in a container of water or wrapped in a moist cloth so that tensionis not broken before installation.

A probe slightly smaller than the diameter of the porous tip (for example, a steel rod, broom handle, or tube) is used to make a hole in the soil for the tensiometer. The depth of the hole should be about 1/4 to 1 inch less than the actual depth for the porous tip (Figure 3). Pour 1/4cup of water into thehole to moisten the soil at the bottom. Insert the tensiometer and gently push it down to the desired depth, usually one- half the effective root zone depth. To ensure good contact between the soil and the porous tip, push the tip into the undisturbed soil just below the depth created by the probe. After the probe has been installed, the soil and porous tip usually reach equilibrium within 24 hours, and the instrument is then ready to use. The tensiometer should be installed to one-half the effective root depth. The porous tip must be in good contact with the adjacent soil.

Field experiences with tensiometers have been mixed. When properly installed and maintained, tensiometers are reliable. Unsatisfactory results are usually caused by inadequate maintenance. Sandy soils, which are best suited for tensiometers, have low levels of plant-available water. In coarse, sandy soils the water content may decrease from field capacity to less than 20 per cent of the plant-available water within three days. At this depletion rate, tension can exceed 80 cb within three days, breaking the water column (tension). The soil may then appear dry and the crop may show visible signs of stress. Because tension was broken and the tensiometer is no longer functioning correctly, however, the gauge shows a low tension (high soil moisture). Thus, the irrigatorconcludes that the tensiometer is

unreliable. Tensiometers should be read every day (sometimes twice a day in very sandy soils) until you obtain a feel for how fast the soildries after rainfall or irrigation.

Whenever tension is broken, the tensiometer must be serviced. This includes refilling the instrument with boiled water and checking it with the vacuum pump. Adding a little food coloring to the boiled water makes it easier to see whether water is still present in the tensiometer. Air bubbles in the water column tend to collect at the top of the barrel and appear clear compared to the colored water. The water column should always befree of air bubbles, and water should always be stored in the reservoir. It may benecessary to add water to the reservoir during the season even if tension is not broken.



Electrical Resistance Blocks

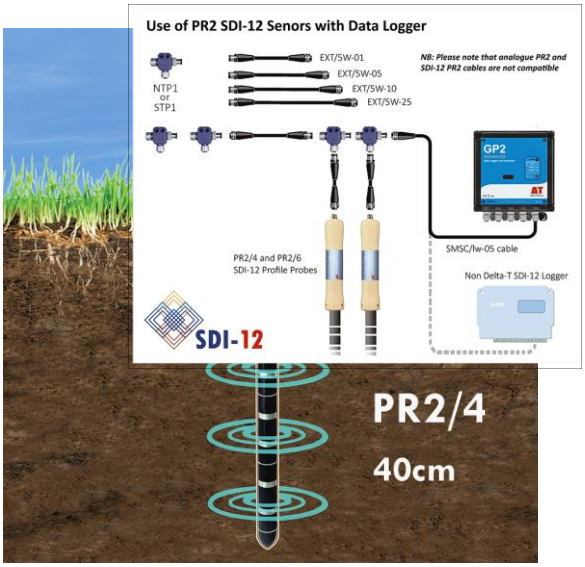
Like tensiometers, electrical resistance blocks should be soaked overnightbefore they are installed in the field. A soil probe should be used to make a hole to the desired installation depth. The hole should be slightly larger than the moisture block so the block slips in easily. After placing the resistance block inthe hole, backfill the hole with a thick soil slurry using soil from the installation depth. Since fine-textured soils do not dry as rapidly as sandy soils, resistance blocks do not need to be read as frequently as tensiometers. Normally, three to four readings per week are adequate.

The electrical resistance of soil-water is affected by substances dissolved in the water. The exchange of water between the soil and the block over the course of the irrigation season may gradually alter the electrical resistance of the block and eventually alter the calibration. This is not a serious problem in North Carolina soils unless highly saline water is used for irrigation. Since electrical resistance blocks are inexpensive, however, new calibrated blocks should be installed at the beginning of each growing season.

Positioning Soil-Water Measuring Devices

If tensiometers or electrical resistance blocks are used, at least one deviceshould be located in each of the major soil types in the irrigated field. For most soils irrigated in North Carolina, the effective root depth is about 12 inches. The soil-water measuring device should therefore be installed to a depth of 6 inches. In soils with a dramatic textural change within 12 inches of the soil surface, such as a loamy sand surface texture overlying a sandy clay loam, one device should be installed in the center of the effective root zone portion of each layer. Soil-water measuring devices should be installed in the plant row. Install them as soonas possible after planting so that roots will grow around them and water extraction will resemble natural field conditions. Flag each device so that it can be easily found in the growing crop. Mark the end of each row containing a device.

Time Domain Reflectometry



Time domain reflectometer is based on the principle of the dielectric constant of water which is much larger than that of other matter such as soil, solid and air. The dielectric constant of water is 80 as compared to other matter, solid (3–8) and air (1). It is an electromagnetic technique which is used in soil moisture measurement. This method applies a voltage signal to transmission line inserted into the soil. The voltage signal requires time to travel from source to the end of the transmission line and back again. The TDR instrument samples the return signal and converts the time measurement to length unit by using the relative propagation velocity (v). From the travel time (t) and the length (l) of the probe, which has been travelled along twice, the propagation velocity v=2l/t is calculated. The velocity of pulse changes due to in change soil moisture or dielectric properties. The advantage of this method is that it allows reliable measurement of volumetric water content to be made within a short time and it does not require soil-specific calibration . Over the full moisture range, it allows reliable measurement and gives accurate results within an error limit of ±1%. Moreover, this technique gives a measurement within 30 sec and performs long-term in situ measurement in an automated fashion over a given location. The disadvantage of this method is that it is

relatively expensive equipment due to complex electronics and potentially applicable under a highly saline condition or in highly conductive clay soils. Moreover, it requires soil-specific calibration for soils having large amounts of bound water (i.e., those with high organic matter content, volcanic soils, etc.). TDR is environment sensitive and provides limited applicability in highly saline soils.

Capacitance and Frequency Domain Reflectometry

Frequency Domain Reflectometry (FDR) is smaller than TDR. Frequency domain reflectometry provides an estimation of soil moisture with a change in the frequency of a signal as a result of dielectric properties. The electrical capacitance of a capacitor that uses the soil as a dielectric depends on the dielectric content. When this capacitor is connected to an oscillator, forming an electrical circuit, change in soil moisture can be measured by changes in the circuit operating frequency. The main advantage of this method is that it provides accurate measurement (±0.01 ft3ft3) after soil specific calibration, and it can read in high salinity level, where TDR fails. Moreover, the FDR provides better resolution than TDR. On the other hand, the disadvantage of this method is that the sensing sphere of influence is relatively small (about 4 cm) and for accurate measurements, it is extremely critical to have good contact between the sensor and soil. Moreover, this technique is highly sensitive to temperature, bulk density, clay content, and air gap than TDR and requires soil specific calibration.

Retrieval of spatial distribution of soil moisture using remote sensing methods

Remote sensing methods provide better results for near-surface soil moisture over a large area with spatial and temporal variability of soil moisture. The near-surface soil moisture seems insignificant but it is this thin layer that controls all the agricultural activity. In this paper, we reviewed all the remote sensing methods. Optical and thermal remote sensing methods are not applicable to estimate soil moisture under vegetation cover, but the microwave remote sensing methods are applicable to estimate soil moisture under the vegetation cover due penetration of weather capability. Thus, our review concludes that the point measurement methods provide point estimation, so point measurement methods cannot be extended to the large area with high

accuracy. The active microwave remote sensing methods are applicable to estimate the spatial distribution of soil moisture over large agricultural areas with high spatial resolution but it cannot be proved high temporal soil moisture over the large area. On the other hand, passive microwave remote sensing is capable of estimating soil moisture with better temporal resolution at longer scale as far as the area of observation is concerned.

Table 1: Overview of remote sensing methods used for soil moisture estimation over large area

Methods	Measured Parameter	Strengths	Limitations
Optical Remote Sensing	Reflectance	1. High spatial Resolution 2. Multiple Satellites Available 3. Large Area Soil Moisture Estimation 4. Simple to Use	1. Change of Color of Soil can decrease the accuracy 2. Poor Penetration Capability 3. Restricted Its Use for Soil Moisture Underneath Crop Cover
Thermal Remote Sensing	Thermal Inertia	1. Good Spatial Resolution, 2. Multiple Satellites Available. 3. Large Area Soil Moisture Estimation	1. Poor Temporal Resolution. 2. Weak Relationship to Soil Moisture when is High Amount of Vegetation Cover. 3. Not able
Active Microwave Remote Sensing	Dielectric Constant	1. Large Area Soil Moisture Estimation 2. Direct Sensitivity toward the Soil Moisture 3. High Spatial Resolution 4. Cost Effective	1. Surface Roughness and Vegetation Cover and Noise Parameters
Passive Microwave Remote Sensing	Brightness Tem	1. Regional and Global Scale Soil Moisture Map 2. Cost Effective	1. Very Coarse Spatial Resolution of the Order of 25 to 50 km

Calibration and validation of soil moisture sensors

1. Collect soil samples from a representative area and depth. It's important that you collect the type of soil you'll be measuring in your study.

2. Sieve out or manually remove any rocks, plant material or non-organic material from the samples.
3. Dry the soil samples – The most efficient way to do this is in an oven. The Australian Department of Sustainable Natural Resources recommends a temperature of 105°C to 110°C.
4. Soil samples can also be air dried on paper in a warm, dry room; however, this is likely to take days or even weeks.
5. Place the dried soil into plastic containers that are large enough for the sensing area of your soil moisture sensor to be completely buried without touching the sides of the container. We recommend at least 2-3 cm of soil between the sensor and the closest edge of the container.
6. You need 1 container for each calibration point you wish to use.
7. Create a range of moisture in each sample by adding water, where the first container is kept dry and the final container is fully saturated. You can use the soil moisture sensor to check that each container is increasingly moist. Be sure to measure from the driest to the wettest, or make sure the sensor is fully dried between each container.
8. It is important that the soil in each container is well mixed so that the moisture level is consistent.
9. Measure and record the sensor output in each container, then take a sample from each container.
10. Weigh these samples on an analytic balance and record the wet weight.

Place each sample into an oven to dry. Once dried fully, record the dry weight of each sample.

11. Plot the graph between observed values and estimated values of soil moisture and it should have linear relationship for the accuracy given by the manufacturer.

The validation process is to verify the instrument in different soils for soil moisture measurement of soil samples collected from the field. For probes of different lengths, we need to repeat the same process of collecting soil samples at different depths with varying soil moisture from different locations.

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Chapter-6

SEMI-PERMANENT STRUCTURES FOR ROOT STUDIES

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Roots play an important role in maintenance of water balance of plants. In addition to water and nutrient uptake, roots also play a key role in providing anchorage, regulation of plant growth and development through hormonal production and microbial interactions. Roots assume much more significance especially under water limited conditions. Among the several factors contributing to drought resistance, root traits are preferred to other avoidance traits like leaf rolling, epicuticular wax and stomatal closure as they tend to reduce growth and yield while deep roots maintain growth and reduce yield losses (Lakshmamma *et al* 2016). Generally, root length and root /shoot ratio increase and root weight and shoot length are affected under water limited conditions (Masalia *et al* 2018, Rauf *et al* 2009).

Root length and leaf cuticular wax are the traits associated with drought avoidance and has the potential to sustain sunflower yield under drought (Praveen *et al* 2020). Though the importance of root traits is established unequivocally, genetic improvement of root traits by conventional breeding methods has been rather slow due to difficulty in measuring the root dynamics, their interactions with the below ground environment and a lack of understanding of the rhizosphere. Comas *et al* 2013 demonstrated an increase in biomass and yield when root growth was better. Considering the importance of root traits in general and more so in dryland crops, it's essential to generate data on root traits.

Methodologies for the study of root system have ranged from tedious manual excavations in the field, through pot studies in sand, hydroponic culture to video monitoring of root growth *in-situ*. The methods used to study roots tend to be labour intensive or require costly equipment and facilities and therefore, seldom exploited.

Semi-permanent raised structures

Root studies were taken up by growing plants in semi-permanent raised structures on the ground. This method offers the advantage of conducting the experiment similar to field conditions.

The height of the structure varies with the crop and depends on the depth to which the roots of that crop can penetrate. Castor genotypes were screened in 30m x 2.4m x 1.5m (L x B x H) structure and sunflower in 30m x 1.5m x 1.2m. Each structure can accommodate 33 castor lines and 50 sunflower lines with two replications. The structure has a central permanent wall with side collapsible walls that are constructed with hollow cement bricks. The structure was secured by erecting wooden poles on either side which are held together tightly with a wire. Drain pipes were provided at different heights to drain excess water. The structure was filled with red soil and watered regularly to allow compaction.

Raised structure before sowing

Sunflower 30 DAS

Preparation for harvesting

Washing of soil

Washing of roots with jet of water

After washing

When the bulk density of the structure reaches as that of field, sowings were done. The crops were grown by following recommended spacing and fertilizer with irrigation as and when necessary. The plants were allowed to grow till they attain maximum root growth i.e. 60 days for sunflower and 100 days for castor. Various shoot observations like SCMR, SLA, LAI, TDM, plant height, leaf number, stem girth and root traits like length, volume, weight were recorded at harvest. On the day of harvest, side walls were carefully removed and the adhering soil washed off with a jet of water. Based on an index developed with root and shoot characters (Sarada *et al* 2009), genotypes possessing the best characters were identified.

Control Vs Stress root growth

Breeders need a genotype that performs well both and control and stress conditions. When the root traits were studied under both conditions at 2 locations, all the root traits showed strong significant correlation between control and stress conditions and therefore, screening for root traits in control condition holds good even for stress situation.

Character	**Bengaluru**			**Hyderabad**		
	Control	**Stress**	**r - value**	**Control**	**Stress**	**r -value**
Root length (cm)	40.4	36.9	0.85*	56.6	21.8	0.85*
Root volume (cc)	56.8	35.8	0.81*	28.3	15.0	0.81*
Root weight (g/plant)	8.3	4.9	0.80*	5.3	2.4	0.81*

Plant height (cm)	93	68	0.61	68	45	0.60
Leaf number	14	12	0.79*	24	19	0.87*
Stem girth (mm)	10.3	12.0	0.71*	12.9	9.8	0.67
TDM (g/plant)	47.6	28.8	0.64	43.0	16.8	0.89*
WUE (g/litre)	0.89	0.74	0.60	1.5	1.6	0.89*

Modelling as an alternate approach

As the root system development and shoot growth are highly related, shoot morphological criteria were used to identify deep root systems.

Traits	Correlation coefficient
Root Wt Vs TDM	0.95
Root Vol. Vs TDM	0.93
Root length Vs TDM	0.84
Root/Shoot Vs TDM	0.21
SCMR Vs TDM	0.10
Petiole Wt Vs Root Wt	0.72
Petiole Wt Vs TDM	0.82
LW Vs TDM	0.97
Stem girth Vs TDM	0.95

Canonical correlation analysis done to study the inter-relationship between root and shoot characters revealed shoot weight, LAI followed by plant height and number of leaves are the important variables contributing towards the root characters in Sunflower. Among the root characters, root dry weight and root volume are the important characters which has more influence on shoot characters.

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Chapter-7

NEW INSIGHTS INTO RHIZO-MICROBIOME AND PLANT GROWTH

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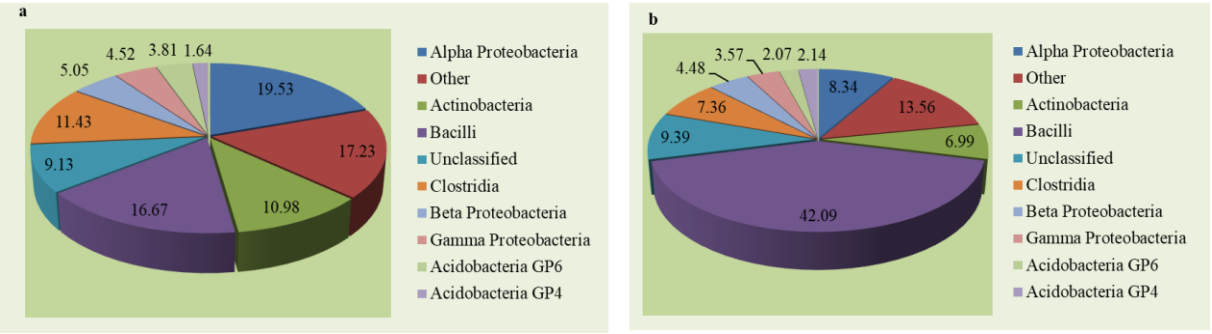
Plants and microorganisms are co-evolved over the years. Plant microbiome includes the microorganisms which are present on the above ground plant parts, inside the tissues as well as below ground roots. The term "Rhizosphere" is derived from the two German words, "Rhizo" meaning root and "Sphere" meaning area or zone. The rhizosphere is the narrow region of soil that is directly influenced by root secretions (Hinsinger, 1998). The microorganisms which are associated with the rhizospheric region are known as root microbiome or rhizo-microbiome. The rhizo-microbiome is also been referred as secondary genome of plants (Lareen et al. 2016; Molefe et al. 2023). The rhizo-microbiome plays very important roles such as; mobilization or acquisition of nutrients, plant growth promotion, and disease management (Edwards et al. 2015).

The composition of rhizo-microbiome is determined by root exudates and soil physico-chemical properties. Plants secrete various compounds such as sugars (glucose, sucrose, maltose, fructose galactose), amino acids (aspartic acid α-alanine, β-alanine, asparagine, arginine), enzymes (amylase, protease, phosphatase, invertase), organic acids (citric malic oxalic, and lactic acids) etc. (Molefe et al 2023). These root exudates serve as carbon and nitrogen sources for the growth and multiplication of microorganisms. They also impact the composition of rhizo-microbiome by acting as attractants or stimulants, signalling molecules, and repellants (Olanrewaju et al. 2019).

Through culture dependent techniques such as plating and other traditional methods it was possible to study only a small fraction of the microorganisms present in rhizosphere/bilk soil. With the development of high-throughput sequencing technologies such as next generation sequencing, now it is possible to study composition of microorganisms through metagenomic approaches. Srivastava et al (2020) studied the composition of wheat rhizospheric microorganisms through metagenomic approach by sequencing 16S rRNA gene amplicons. The composition of rhizo-microbiome was dominated by phylum proteobacteria (68%), firmicutes (13%), bacteroidetes (3%), actinobacteria (3%) and acidobacteria (3%). Soybean rhizo-microbiome was dominated by Actinobacteria, Acidobacteria, Chloroflexi, Cyanobacteria, Tenericutes, Chlamydiae, Chlorobi,

Deferribacteres, and Verrucomicrobia phyla. At class level, Bacilli, Mollicutes, Gammaproteobacteria, Clostridia, Epsilonproteobacteria, Thermomicrobia and Chlamydiae were dominant in the soybean rhizosphere (Mendes et al 2014). Plants influence the composition of the rhizo-microbiome by secreting certain chemical compounds. Under nitrogen and phosphate deficient conditions plants secrete strigolactones and these compounds attract arbuscular mycorrhizal fungi to improve the uptake of nutrients such as phosphorus (Carvalhais et al. 2019; De Cuyper and Goormachtig 2017).

Soil moisture stress also affects the composition of rhizo-microbiome. Effect of soil moisture stress on the composition of rhizo-microbiome of maize, sorghum and groundnut was assessed by sequencing V3-V4 region of the 16S rRNA gene of metagenome extracted from well-watered and moisture stressed rhizospheric soil of respective plants. In the maize rhizosphere, the classes of Actinobacteria, Alpha Proteobacteria and Bacilli were 10.98 %, 19.53 % and 16.67 % in drought stressed plants, whereas in well watered plants it was 6.99 %, 8.34 % and 42.09 %, respectively (Fig.1 a and b). Similarly, in the sorghum rhizosphere, classes of Clostridia, Bacilli, and Alpha Proteobacteria were 19.97 %, 62.10 %, and 5.79 % in drought stressed sorghum plants, whereas in well watered sorghum plants it was 37.33 %, 54.07 %, and 2.21 %, respectively (Fig.1 c and d). In case of groundnut, the classes of Clostridia, Bacilli, Gamma Proteobacteria were 57.37 %, 38.90 % and 1.48 % in drought stressed plants, whereas in well watered plants it was 25.16 %, 54.50 %, and 7.05 %, respectively (Fig.1 e and f) (ICAR-CRIDA, 2019).



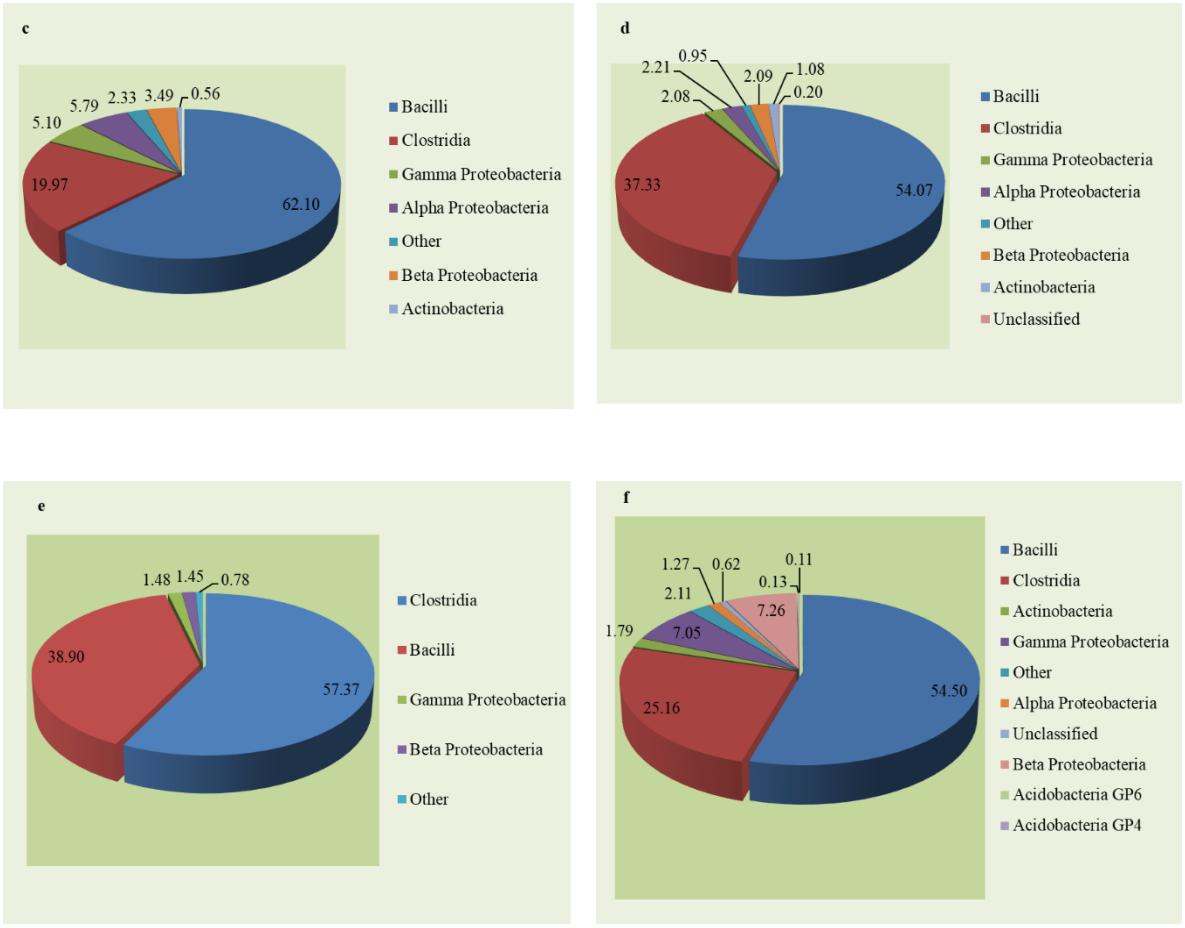


Fig 1. Composition of rhizo-microbiome in (a) moisture stressed maize (b) well-watered maize plants (c) moisture stressed sorghum (d) well-watered sorghum plants (e) moisture stressed groundnut (f) well-watered groundnut plant. (Source: ICAR-CRIDA, 2019)

Influence of rhizo-microbiome on plant growth

Microorganisms residing in the rhizosphere perform several functions which influence the plant growth and development. They are major players in nutrient cycles and help plant to acquire nutrients. Synthesize phytohormones such as auxins, cytokinins, gibberlins and abscisic acids which aid the plants at various stages of development. Arbuscular mycorrhizal fungi help plants by providing phosphorus and other nutrients as well as protect the plants from biotic and abiotic stresses (Fasusi et al. 2023).

Rhizophagy cycle is an evolving ecological process where plants take up bacteria or fungi from the soil. This process makes use of root exudates to help bacteria to enter root cells and reproduce. Further, root cells produce reactive oxygen species (ROS) at the site where survival

reproduction of endophytes happened. The ROS through oxidation process break down the cell contents of endophytes. The nutrients released by degradation of endophytes are used by root cells of plant (Chiaranunt and White 2023; Adeleke et al. 2022a; Verma et al. 2022).

Manipulation of rhizo-microbiome

Rhizo-microbiome can be manipulated to get desired benefits with the introduction of useful microorganisms. In the beginning, single cultures such as *Pseudomonas*, *Bacillus*, *Trichoderma* and others were used plant growth and disease management. Later on, consortia of two or more compatible microorganisms with diverse or synergistic functions are used to get desired effects. Rhizo-microbiome transplantation though not a new concept is gaining attention to overcome the adaptability issues of bioinoculants. Since this concept also make of uncultivable microorganisms which constitute up to 98-99% of total microorganisms (Park et al. 2023). Rhizo-microbiome transplantation technology has been used improve drought tolerance, disease management and improve physiological properties of plants (Zolla et al. 2013; Panke-Buisse et al. 2015; Jiang et al. 2022).

Rhizo-microbiome is an important component of plant growth and development as constituent microorganisms provide immense benefits to plants. Rhizo-microbiome plays key role in eco-friendly sustainable agricultural production.

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Chapter-8

ROOT ARCHITECTURAL SAMPLING METHODOLOGY IN THE FIELD

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During drought, root system of the plant is the interface between soil and drought. Hence, it is essential to understand the root dynamics and its concomitant effect on shoot parameters under varied drought situations which help us in monitoring drought impact and possible manipulation to some extent.

Field Root Architecture Method

In order to study the roots in the field conditions, a trench is generally dug along the signified rows of the selected plants. The trench measurement can be subjective to the stage and type of the plant to be studied. The field study here was on Horsegram. A trench of 100X155X45cm^3 (LWH) is to be dug on four sides of the chosen plants isolating a rectangle monolith of 60X35X45cm^3 (LWH) in the field. These trenches are dug by hand using crow bars and spades.

Trench profile of Horsegram plants

Pin Board: A PVC board of 61X 21 cm^2 size preset with 4 mm holes, on it 2.5 cm grid lines in alternate rows is prepared and painted black. After trenching, two boards are to be placed on either opposite sides of the monolith.

Spokes: 10mm gauge GI wire of length 65cm are pressed against the monolith by inserting the spokes gradually into one side of the pin board holes pushing to the other side, by passing it slowly through the soil and root. Before insertion of spokes the surface of monolith is to be watered to loosen the soil for easy drive of the spokes.

Washing: The monolith needs to be soaked for several hours and the root system is carefully washed free of soil. The entire plant and its root system is held by the spokes of the pin board. Once the

desired root architecture is pinned with spokes, to be pushed towards the pin board on one side and the other board is removed watchfully. The mounted root architecture on the pin board is then arranged on a plane surface or in a tray half filled with water. The spokes are removed slowly and earbuds are placed in the same position to uphold or retain the architecture of the root system. The digital images are generated using high end digital camera at a perpendicularly fixed boom stand.

Root storage: After sampling and photography, the roots are to be cut for sub sampling, store in water of glass bottles in a refrigerator at 4ºC until they are scanned using scanner and analyzed using "WinRHIZO".

Root Scanning: The cut roots are spread on transparent acrylic tray of the scanner in a thin film of water using plastic forceps without sharp edge. Root scans are stored in tiff format and data are generated in text file, later be converted into Excel files for further analysis. After scanning, the roots are dried at 40°C for four days for root dry weights.

The bottled roots are scanned in greyscale at 300 dpi (dots per inch) using a desktop scanner for acquiring images. Images are analyzed using WinRHIZO software.

Root Parameters

Total Root length (cm) and root surface area are the output from the scanned image analysis with WinRHIZO software, average root diameter (mm) is the average diameter of all the roots analyzed and total root lengths at various root thicknesses were generated. Root Length Density (RLD) is the root length per unit volume of the soil was calculated from the above-generated data. Root shoot ratio was calculated with the available root and shoot weights.

The major advantage of this type of method was examining the natural array of roots and easy to record the root data but the demerits are limited to 2D area and requires some skills, labour-intensive, huge loss of fine roots during digging and washing process, could not study the root growth in situ during the plant growing period.

Chapter-9

MINI-RHIZOTRON ROOT ARCHITECTURE SAMPLING METHODOLOGY: NET HOUSE GREENGRAM EXPERIMENT

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Plants vary in their capacities of root proliferation and responses to heterogeneous environments and specifically of species and varieties. With the dynamic status of soil moisture, shift in root distribution may be observed both horizontally and vertically. Crop production in drylands is ensconced with the problems of late onset of monsoon, and intermittent dryspells/ droughts during the crop growth period.

Understanding root distribution soil profile wise in order to estimate the capabilities of the plant to extract soil moisture and nutrients for its sustenance is incomplete without the standard methodologies for root architectural study. Till now the plant root studies mostly were in the form of excavating the total root biomass and estimating the root parameters of the total root system. However, the data did not give much clue about the distribution of roots at different soil depths in the soil profile which primarily affect the acquisition of soil moisture and nutrients. But after long periods of root research, root box-pinboard methods came into practice. Mostly these methodologies work with the narrow or thin root boxes which were helpful to get root architecture but to thwart this type of criticism on restricted space for the plant, in India we worked with the objective of field single plant spacing (FSPS), by providing boxes of size equivalent to the single plant spacing when grown in the field while methodology of extracting root architecture was one another objective being addressed. Pin board methodology has been in vogue since 60's. However, the improvements in this method took place in terms of materials used to fabricate root boxes, methodology to sample etc. Therefore, an attempt was made to indigenize the above-mentioned pin board methodology to suit the short duration legume like mungbean root architecture sampling requirements.

Moisture within the soil profile is heterogeneous and the root systems must forage for this limited resource. Therefore, root distribution varies within the soil moisture heterogeneity due to variation in topography, water infiltration, differential root competition etc. Root system in some

cultivars grows vertically with more root angle to the soil surface might have the genetic component (vegapareddy et al., 2010) to exploit more moisture from deeper layers of the soil with the top soil layers dried out. For the transient soil moisture availabilities and deficits, root system of the plant should be geared up to seek soil moisture for either maintenance of the root under dry soil conditions or for sustaining the roots.

Net House Mini-Rhizotron Experiment

Mungbean crop was grown in Red Sandy Loam soil profile filled root chambers of 30x15x45cm^3 dimension. Two seeds of two mungbean cultivars (ML267 and WGG37) were sown in each chamber and thinned to one plant after germination. As per the recommendation, fertilizer was supplied to each chamber. Each chamber uniformly received fertilizer and water content as per the treatment. The soil with 75% sand, 3% silt and 22% clay has neutral pH (7.2), normal EC (0.16ds/s^{-1}), low nitrogen content (171 kg ha^{-1}), medium available phosphorus (17.7 kg ha^{-1}) and high potassium (307 kg ha^{-1}).

Methodology of sampling for Root Architecture (CRIDA Indigenous Root Chamber-pin board method)

The methodology explained by Price et al., (2002) was Indigenized to suit the size of the root chamber in which the plants were grown.

Construction of Root chambers: As our test crop is mungbean, the field spacing of 30 x15cm^2 was taken into account and acrylic chambers of 30cm X 15cm x 15cm rectangle boxes were constructed and three boxes were arranged one above the other so as to have the total depth of 45 cm. The boxes were glued with a tape to avoid leakage of water from it. A drainage hole was made to the bottom most boxes. On one side (length side) of the box, acrylic sheet opens like a door which is fitted with the hasps and hinges.

Pin Board: A PVC board with spokes (motor bike spokes of 16.5 cm length, 3.5 mm diameter with a screw at the bottom part) fixed at grid lines distance of 2.5 cm in alternate rows and painted black.

Sampling Protocol: For sampling, the door of each chamber was opened, fitted with the black pin board by pushing the spokes into the soil until it touches the box pin board and turned it around lifting up the chamber leaving whole soil mass with root system on the pin board.

Root washing: Roots were washed by keeping the pin board in the wheel barrow. After washing, the mounted root system on the pin board was placed in the water tray, was photographed after it was allowed to gently align on its own.

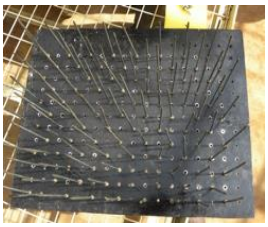
Photography: By placing the black pin board with root system in the water tray, digital images were generated using high end digital camera at a perpendicularly fixed object distance using boom stand. **Root Scanning and image analysis:** After sampling and photography, the roots were cut for sub sampling, stored in water of glass bottles in a refrigerator at 4ºC until they were scanned using flat bed scanner of STD 4800 and analyzed TRL (cm) using "WinRHIZO" (Regular 2009c Version). Root scans were stored in tiff format and data were generated in text file, later converted into Excel files for further analysis. After scanning, the roots were dried at 40°C for four days for root dry weights.

According to Price et al. (2003), the root boxes made of glass and of 1.5 cm in width and 70cm and 50cm in length and depth respectively, while in our case it is an acrylic box of 30x15x45 cm dimension which led to changed pinboard size and the pins. In our expt, we used the bike spokes of 16.5cm length in place of nails but could not paint them black. These spokes have got bolts to fix and remove at the bottom. On contrary to the nails, the spokes were of 3.5cm in diameter. Further to take the photographs without spokes obstructing the vision, earbuds were fixed in place of spokes.

Figure 1. Comparison of Root architecture of greengram varieties a) ML267 and b) WGG37 at 42DAS

Root chamber with mung bean plant

Different depths of soil in root chamber



Arrangement of spokes on pin board

Inserting pin board into the root chamber

Turning around and lifting up the chamber from soil profile

Whole soil profile on pin board

Washing the root system

Root scanner with WIN RHIZO software

Sample ready for digital imaging

CRIDA Indigenous Root Chamber-pin board method of Mungbean Root Architectural sampling

Generally, the first casualty under drought situation is the root system which is an interface between the plant and the soil. Consequently, the water saving mechanisms of the plant would come

into the scene along with the water capturing abilities of the root system. Till now the drought management measures were formulated and validated based on the study of only above ground biomass. However, the study of below ground biomass and yield may help us in finding the moisture sensitive stages of the crops accurately and to manage it effectively. As soil dries, plants typically reduce root growth and carbon allocation to those roots in dry portions of soil and preferentially grow roots in regions of high soil moisture.

Epilogue

Though every researcher appreciates the efficiency of 3D imaging, still 2D imaging techniques are easily accessible, technically simple and may be considered as a possible relevant option for observing positioning of roots. In this methodology, care was taken to consider field level single plant spacing for the chamber size. 2D Root architecture image and root measurement at different soil profile depths could be possible in this methodology. Root box-Pin board method may be helpful in studying the effect of soil and fertility factors on root architecture in addition to soil moisture. Therefore, this indigenized Root box-Pin board method is recommended for study of root architecture under controlled conditions.

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Chapter-10

DIGITAL IMAGE PROCESSING TOOLS AND TECHNIQUES

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Digital image is collection of pixels (also called picture elements or image elements) arranged in columns and rows like an array, or a matrix. An image is defined as a two-dimensional function, **F(x, y),** where x and y are spatial coordinates, and the amplitude of **F** at any pair of coordinates (x, y) is called the **intensity** of that image at that point. Digital Image is composed of a finite number of pixels, each of it has a particular value at a particular location. As we know, images are represented in rows and columns as follows:

$$f(x,y) = \begin{bmatrix} f(0,0) & f(0,1) & f(0,2) & \dots & f(0,N-1) \\ f(1,0) & f(1,1) & f(1,2) & \dots & f(1,N-1) \\ \vdots & \vdots & \vdots & & \vdots \\ f(M-1,0) & f(M-1,1) & f(M-1,2) & \dots & f(M-1,N-1) \end{bmatrix}$$

The right side of this equation is digital image by definition. Every element of this matrix is called pixel.

Types of an image: The binary image as its name suggests, contain only two-pixel elements i.e., 0 & 1, where 0 refers to black and 1 refers to white. This image is also known as Monochrome. The image which consists of only black and white colour is called Black and White image. The most famous image format is 8-bit. It has 256 different shades of colours in it and commonly known as Grayscale Image. In this format, 0 stands for Black, and 255 stands for white, and 127 stands for Gray. A 16-bit format is actually divided into three primary colours which are Red, Green and Blue. That famous RGB format. Number of colours depend on the number of bits per pixel (bpp). The following table shows some of the bits and their colour:

Bits per pixel	Number of colours
1 bpp	2 colors

8 bpp	256 colours
16 bpp	65536 colours
24 bpp	16777216 colours (16.7 million colours)
32 bpp	4294967296 colours (4294 million colours)

This table shows different bits per pixel and the amount of colour they contain.

Colour images are usually of the 24 bpp format, or 16 bpp. Pixel, that 0 value denotes black colour. But there is no fixed value that denotes white colour. In case of 1 bpp, 0 denotes black, and 1 denotes white. In case 8 bpp, 0 denotes black, and 255 denotes white. In case of 8bpp, the pixel value that denotes Gray colour is 127 or 128 (if you count from 1, not from 0).

Image storage requirements

The size of an image depends upon Number of rows, Number of columns, Number of bits per pixel. The formula for calculating the size is given below.

Size of an image = rows * cols * bpp

Assuming it has 1024 rows and it has 1024 columns. And since it is a gray scale image, it has 256 different shades of gray or it has bits per pixel. Then putting these values in the formula, we get 1024 * 1024 * 8 = 8388608 bits.

But since it's not a standard answer that we recognize, so will convert it into our format.

8 bits = 1 bytes; 1024 bytes = 1kb; 1024kb = 1 Mb

Converting it into bytes = 8388608 / 8 = 1048576 bytes.

Converting into kilo-bytes = 1048576 / 1024 = 1024kb.

Converting into Mega-bytes = 1024 / 1024 = 1 Mb.

Mega pixels of the image

An image of dimension: 2500 X 3192.

Its pixel resolution = 2500 * 3192 = 7982350 bytes.

Dividing it by 1 million = 7.9 = 8 mega pixel (approximately).

Aspect ratio

Aspect ratio is the ratio between width of an image and the height of an image. It is commonly explained as two numbers separated by a colon (8:9). This ratio differs in different images, and in different screens. The common aspect ratios are:

1.33:1, 1.37:1, 1.43:1, 1.50:1, 1.56:1, 1.66:1, 1.75:1, 1.78:1, 1.85:1, 2.00:1, etc.

Aspect ratio maintains a balance between the appearance of an image on the screen, means it maintains a ratio between horizontal and vertical pixels. It does not let the image to get distorted when aspect ratio is increased.

Spatial resolution: Spatial resolution states that the clarity of an image cannot be determined by the pixel resolution. The number of pixels in an image does not matter. Spatial resolution can be defined as the number of independent pixels values per inch. In short what spatial resolution refers to is that we cannot compare two different types of images to see that which one is clear or which one is not. Clarity of the image depends on its spatial resolution.

Histogram of an image: An image histogram, shows frequency of pixels intensity values. The x axis shows the Gray level intensities and the y axis shows the frequency of these intensities.

Digital Image Processing

Images are processed in order to extract some useful information. It is called digital image processing. Digital image processing is the use of algorithms and mathematical models to enhance the quality of image, extract meaningful information from image, and automate image-based tasks.

The basic steps involved in digital image processing are:

1. Image acquisition: This involves capturing an image using a digital camera or scanner, or importing an existing image into a computer.
2. Image enhancement: This involves improving the visual quality of an image, such as increasing contrast, reducing noise, and removing artifacts.

3. Image restoration: This involves removing degradation from an image, such as blurring, noise, and distortion.
4. Image segmentation: This involves dividing an image into regions or segments, each of which corresponds to a specific object or feature in the image.
5. Image representation and description: This involves representing an image in a way that can be analysed and manipulated by a computer, and describing the features of an image in a compact and meaningful way.
6. Image analysis: This involves using algorithms and mathematical models to extract information from an image, such as recognizing objects, detecting patterns, and quantifying features.
7. Image synthesis and compression: This involves generating new images or compressing existing images to reduce storage and transmission requirements.
8. Digital image processing is widely used in a variety of applications, including medical imaging, remote sensing, computer vision, and multimedia.

Classic Image Processing Algorithm

Morphological Image Processing: Leveraging the opening and closing operations, we can smooth the image using morphological image processing. From the binary images, the imperfections can be removed by simple thresholding. Dilation and erosion are the two fundamental operations that can be done using morphological image processing. Through dilation, the pixels can be added to the boundary of an object. While in erosion, we can remove the pixels from the boundary of an object in an image.

Gaussian Image Processing: This technique is also known as Gaussian smoothing. Leveraging it, the noise and details contained in an image can be reduced. The Gaussian image processing blurring technique is similar to looking at an image through a translucent screen. The Gaussian filters are low-pass filters that mostly weaken at high frequencies. The Gaussian filter gives more weight to the pixels located at the center than the other pixels located at different points. Therefore, Gaussian image filtering is used to enhance an image at different scales.

Fourier Transformation: Into the sine and cosine components, the image can be broken down into Fourier transformation. An image consists of three things, i.e. magnitude, phase, and spatial frequency. Related to contrast, the magnitude can be used. For increasing or decreasing the brightness of an image, the spatial frequency can be increased/decreased and for color information

of an image, the phase needs to be checked. After the Fourier transformation of an image is done, we can use it in image filtering, compression, and reconstruction.

Edge Detection: For detecting the discontinuity in the brightness of an image, an edge detection image processing technique is used. Leveraging the Sobel edge detection algorithm, we can make separate measurements of an image using a kernel. Edge detection image processing is highly beneficial as most of the information of an image is enclosed at the edges.

Wavelet Image Processing: For non-stationary signals, wavelet image processing is used. It measures the time and frequency of a wave. Through wavelet image processing, for low-frequency components, a good frequency resolution can be obtained. While for refining the edges of an image, wavelet image processing is chosen because it does not blur the image. Only the noise is reduced in the image. Overall, the quality of an image is not degraded while applying traditional filters.

Image Processing Tools

Open CV: OpenCV is one of the largest computer vision libraries. There are around 2 million customers downloading OpenCV every week. It is one of the easiest libraries to use. OpenCV supports both Python and C++ languages. With the key functionalities of human face detection, optical flow, and search for stereo machines, OpenCV is a cross-platform, supports Android version, and has a good in-built performance testing system. Supporting a continuous integration system, OpenCV is designed for developing open infrastructure.

Being an open-source library, it is well-optimized and designed for real-time computer-based applications. Leveraging it, most of the video and image processing jobs can be very easily done.

Scikit-image: Scikit-image is an open-source library, and it leverages machine learning's built-in functions. It contains a collection of algorithms for image processing. With a set of only a few functionality features, through Scikit we can perform a number of operations on images. With the usage of NumPy arrays, through Scikit, we can easily rotate, rescale and apply morphological operations on an image. To implement threshold, edge detection, and Gaussian smoothing-like operations, the Scikit library is the best one to choose from.

Some Scikit-image examples are the detection of features and objects in an image, segmentation of objects, filtering and restoration, geometrical transformations, manipulating colour channels, and many more.

PIL/Pillow: It is one of the most powerful libraries as it supports a wide range of operations on images. Apart from rotating, resizing, grayscaling, or cropping an image, through PIL, we can get the image details such as file format, pixel format, size of an image, etc. Adjacently, PIL can operate various other manipulating operations like uploading an image, displaying an image, or flipping an image.

NumPy: Through multi-dimensional arrays, the images are represented in the NumPy library. The types of arrays used are called NdArrays. Therefore, a numpy array of three dimensions can be used for depicting a colored image. To perform simple operations like flipping an image, extracting the content, or analyzing the image, NumPy library can be leveraged.

Mahotas: Most of the algorithms in Mahotas are in C++ programming language. Having minimum dependencies Mahotas is an independent module. Only for doing the numerical calculations, Mahotas library is dependent upon the C++ compiler. It does not require any NumPy module. Watersheds, morphological operations, thresholds, convolution, SLIC superpixels, spline interpolation, colorspace conversions, speeded-up robust features are some of the most popular and best algorithms for image processing in Mahotas library.

SciPy: For processing tasks and image manipulation, SciPy is a Python core scientific module. For processing and manipulation tasks of images, SciPy can be used. The current package of SciPy includes binary morphology, linear and non-linear filtering functions, object measurements, and B-spline interpolation.

Simple ITK: ITK is an open-source, cross-platform system that provides an extensive set of software tools for image analysis. ITK stands for Insight Segmentation and registration toolkit. Simple ITK is mostly available in C++, but it supports many programming languages like that of Python. Simple ITK is an image analysis toolkit that supports operations like image segmentation, registration, and general filtering operations.

PgMagick: For leveraging the GraphicsMagick library, PgMagick is a Python-based wrapper. Pymagick supports a number of tools and libraries through which reading, writing, and manipulation of images can be done. Over 88 formats that include TIFF, PDF, PNG, JPEG, JPEG-2000, DPX, GIF, PNM, etc. the PgMagick is supportable.

PyCairo: Cairo is a two-dimensional graphics library for drawing vectors graphically. For the graphics library, PyCairo is a set of Python libraries. The most interesting thing to note and work with vector graphics is that whenever you resize or transform them, the vectors do not lose any clarity.

SimpleCV: For building computer vision applications, SimpleCV library provides an open-source framework. For assessing SimpleCV, you do not have to learn complicated things like file format, color space, etc. You can easily access high powered computer vision libraries.

Through SimpleCV, beginners can also write simple machine vision tests. The video streams, images, cameras, and video files can be made interoperable in SimpleCV.

Basics of Image Processing in Python

Each image has its own story, and it contains a lot of information that can be used in distinct ways. For extracting meaningful information from the image, Python programming language is widely used.

Install Required Library: Leveraging the pipe, we can install the required library. First, there is a need to install the required library like OpenCV, Pillow, or another one.

Image Open and Show: By typing the image processing Python code for upload and display of an image, the file of an image can be opened and displayed. The image can also be rotated using the code shown below:

```
import required library
From PIL import image
Open image
im = Image.open ("cat.png")
image rotate & show
im.rotate (90).show()
```

Rotating an image: The image can be rotated as per the need. After rotating the image, the portion of the image having no pixel values is filled with transparent pixels.

```
import CV2
import imutils
image=CV2.imread (r"URL.png")
rot=imutils.rotate (image, angle=90)
CV2.imshow("Rotated", rot)
CV2.waitkey(0)
```

Resizing an image: The quality of an image either upgrades or downgrades when interpolation happens at the time of resizing. Therefore, resizing of an image should be done carefully.

```
import CV2
import matplotlib.pyplot as plt
#read image
Img = CV2.imread ("myimage.png")
print ('Image width is', img.shape [0])
print ('Image height is', img.shape [1])
CV2.resize (img, (400,400))
```

Shifting of an image: For shifting an image from one place to another. The image can be made upwards, downwards, left, right or centrally aligned.

```
Import CV2
Import numpy as np
Img = CV2.imread ('image.png')
M=np.float 32 ([0,1,2],[2,2,4]])
img=CV2.warpAffine(img,M, (w,h))
CV2.imshow('Image Translation',img)
CV2.waitkey(0)
CV2.destroyAllWindows()
```

Edge Detection: Edge detection is an image processing technique for identifying the boundaries of an object or region in an image.

Sobel Edge Detection: Leveraging the Sobel operator, the edges of an image are detected. The edges are marked due to a sudden change in intensity.

```
sobelx=CV2.Sobel (scr=img_blur,ddepth=CV2.CV_64F, dx=1, dy=0, ksize=5)
sobely=CV2.Sobel (scr=img_blur,ddepth=CV2.CV_64F, dx=1, dy=0, ksize=5)
sobelxy=CV2.Sobel (scr=img_blur,ddepth=CV2.CV_64F, dx=1, dy=1, ksize=5)
CV2.imshow ('Sobel X', sobelx)
CV2.waitkey (0)
CV2.imshow ('Sobel Y', sobely)
CV2.waitkey (0)
CV2.imshow ('Sobel X Y using Sobel () function', sobelxy)
CV2.waitkey (0)
```

Canny Edge Detection: It is one of the most popular edge detection methods. Consisting of four stages i.e. extracting the edges from the image, reducing the noise, suppression of false edges and hysteresis thresholding.

```
Edges = CV2.canny (image =img_blur, threshold1=50, threshold2=100)
CV2.imshow ('Canny Edge Detection', edges)
CV2.waitkey (0)
```

Convert and Save Image: The format of an image can be easily converted into another desired format. After that, the image can be saved by typing the code as shown below in the image:

```
im.save ("cat.png")
```

Resize Thumbnails: The size of the image can be changed using the thumbnail method of pillow library as shown in below image:

```
im.thumbnail ((400,400))
im.show ()
```

Converting to Grayscale Image: From the original colored image, grayscale image can be created using the code written in the image below:

```
cat_gray=image.open ('cat.png').convert ('L')
cat_gray.show()
```

Basic functions within ImageJ

Loading images

1. Select the File−>Open option. In the explorer window, navigate to the file location to open the image.

2. To load multiple image slices in a stack, select File−>Import−>Image Sequence. Navigate to one of the images in the sequence. A pop-up window will appear allowing you to enter a common string pattern. Any image file with this string pattern in the current working directory will be loaded as part of an image sequence.

Click Plugins−>LOCI−>Bio-Formats Importer. A pop-up window will appear al- lowing you to specify how the data is visualized and how much of the data is loaded. This option is specific to ImageJ with the Bio-Formats plug-in and is convenient for loading higher dimensional data (3-D, time series, multi-color) or images with uncommon file types.

Non-destructive contrast enhancement

Images may appear blank upon loading into ImageJ, even if they are supposed to have signal in them. This arises because computer displays are built to show only 256 gray levels. Because some images contain 4096 or 65,536 levels, the images are scaled in such a way that does not maximize the dynamic range of a particular image. Non-destructive contrast stretching does not change the image data itself, only the visualization.

Identify the following common and essential menu items:

Image−>Adjust−>Brightness/Contrast

Process−>Subtract Background

Process−>Filters.

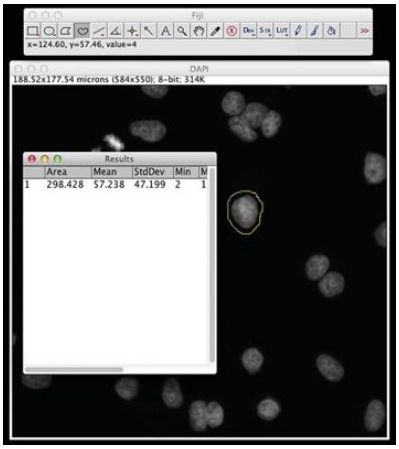

Figure The freehand selection tool (see Lasso). The freehand selection tool uses the cur- sor to create a user-defined region of interest. Shown are HeLa nuclei stained with 4′6-diamidino- 2-phenylindole (DAPI) to identify nuclei. Microscopy was performed at 20× magnification.

Adjust brightness and contrast (B&C) by clicking Image−>Adjust−>Brightness/ Contrast.

Using the Auto selection, ImageJ will automatically scale brightness and contrast based on an analysis of the image histogram.

For RGB images, use Image–>Adjust–>Color Balance.

Lasso

In the main ImageJ window, select the Freehand selections tool. Use the lasso tool to manually draw an elliptical region of interest around one of the cells. This can be used to circle a clump of cells, a single cell, or a subcellular region like the nucleus.

Select Analyze–>Measure to take measurements under the manually defined region of interest.

Background subtraction

The rolling ball method of background subtraction is commonly used to correct for uneven backgrounds in an image by subtracting signals at a predefined distance (radius) from the brightest pixels. The radius should be set to at least the size of the largest object that is not part of the background. Smaller radius size definitions will subtract foreground signal. The methods below describe how background subtraction is performed on fluorescence images using ImageJ. In this background subtraction approach, a background image is approximated as a smoothed version of the input image, followed by subtraction from the original image.

Load an image (either immunofluorescence, brightfield IHC, or unmixed IHC) into ImageJ. If the image has multiple colour channels, split the channels by running Image–>Colour–>Split Channels. Apply the subsequent steps to each channel.

Select Process–>Subtract Background. Make sure Light Background is unchecked for fluorescence images or checked for brightfield images. Enable the preview and adjust the rolling ball radius field. Note that the rolling ball radius should be bigger than the average nucleus' radius. It may be useful to toggle the Create Background (do not subtract) option to see what is being subtracted from the image. Finish this step by unchecking Create Background and then the OK button.

Chapter-11

REVIEW OF ROOT METHODS

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Plant root sampling is a crucial aspect of studying plant biology, ecology, and soil science. Different methods are used to sample plant roots, depending on the research objectives, plant types, and soil conditions.

Plant root sampling methods

Destructive

Soil Core Sampling: Core methods involve removing cylindrical core samples from the soil profile and washing the soil from the roots. Cores are usually extracted at various depths in the profile, at various predetermined distances from the plant bases, and in some predetermined time sequence. Use a soil corer to extract cylindrical soil cores containing roots.

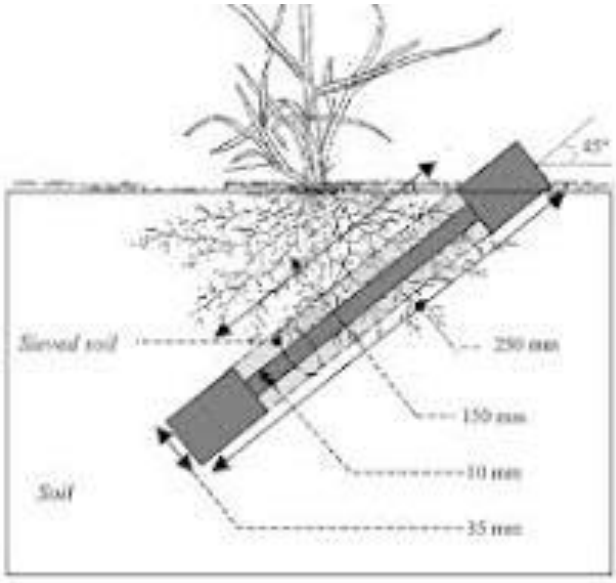
Applications: Suitable for fine roots and provides information on vertical distribution.

Soil Monoliths: The monolith method requires removal of a soil block and separation of roots contained in that block. Sometimes a trench is dug for access to the profile. Blocks of soil are removed from successively deeper layers until the bottom of the root zone is reached. Each block is soaked in water and soil is washed through a mesh grid leaving the roots. Alternatively, some type of container is forced into the soil deep enough to enclose the deepest roots. The container is pulled from the soil and either the monolith is cut into depth increments or small rods are forced horizontally through the soil. Soil is then washed from the roots.

Applications: Useful for studying root-soil interactions and for preserving root structure.

Ingrowth Cores: Insert porous tubes filled with fresh soil into the ground for roots to grow into. Ingrowth cores are very effective in studying ecosystems with rapid fine root growth and extremely suitable for comparing fine root production between sites or treatments. However, the reliability

of this method has been impaired by many limitations: (i) the core installation causes great disturbance to roots and the rooting environment, (ii) physical and chemical characteristics of soil are altered when the root-free soil is placed in the cores.



Procedure: Bury ingrowth cores, allow roots to colonize, and later extract cores for analysis.

Applications: Quantifies root production and turnover rates.

Pit Excavation: General architecture of the root system and extent of rooted volume of soil can be determined by excavating the entire root system or a segment of it. A trench is first dug around the plant. A support is provided for the plant top. Soil is removed from around the roots by using needles, air, or water pressure. The location and extent of the root system can be drawn on a plastic sheet, photographed, or mounted (Fig. 2). This technique may be the only feasible one in a rocky or gravelly soil or if the rooted volume of soil is not symmetrical. Many small roots are lost during excavation, and the technique is time-consuming. Loss of soil and water.

Fig. A thin layer of soil is washed and scraped from the prepared face of an Ida silt loam soil profile. A square grid (left) is then placed on the soil face to allow root location and numbers to be determined in the trench profile method.

Trench Profile Method: The trench profile method is used to observe the root distribution underground by making a rectangular hole close to the crop, providing informative images of the root distribution compared to other root phenotyping methods. However, much effort is required to segment the root area for quantification.

Applications: Useful for studying root architecture and interactions with soil layers.

Shovelomics: By simply using a shovel combined with imaging technology, this method was effective in phenotyping the top part of a crown root system of mature maize. For that purpose, the method was defined as high throughput field method. For each sampled plant, the root was excavated by extracting a soil cylinder of about 40 cm in diameter and 25 cm deep with the plant crown in the middle. This off course will not work in a very dry and hard soil. The soil was shaken off the roots after which it was dipped in water and a detergent to remove the remaining soil. The exposed root was measured for various trait of significance to this study and crop, such as number of crown root wholes above and below ground, crown root number etc.' The extracted roots can also be stored under refrigeration and phenotyped over time.

Non-Destructive Methods

Rhizotron Systems: Transparent or semi-transparent chambers that allow non-destructive root observation.

Procedure: Install rhizotrons in the soil and periodically observe roots without disturbing them.

Applications: Ideal for continuous monitoring of root growth and development.

a. Mini-Rhizotrons: The method has gained popularity as a leading qualitative and quantitative field observation system. It is based on a transparent glass or plastic sleeve inserted at an angle (usually 45^0 to the vertical) into the soil before roots become very developed. The sleeve is inserted into a borehole made manually or with hydraulic assistance. The procedure is important since good contact must be achieved between the sleeve surface and the soil. An imaging device, whether a digital camera, a video camera or an endoscope is inserted into the sleeve in order to record images of roots seen through the sleeve walls. The images are then recorded and processed by an aboveground unit. Depending on the system and model, images can be processed within this unit or downloaded to a computer equipped with the appropriate image processing software. In many cases, images are processed for root length, thickness and other parameters by the WinRhizo software as one example. Free and open-source software for analyzing minirhizotron images is available also, such as the from Clemson University. It is off course understood the protocol for acquiring the images is linked to the specific image analysis software used. Root images seen on the sleeve wall are not a perfect representation of the actual root distribution at that soil horizon and the software attempts to simulate real root data from the images.

b. Minirhizotron Tubes: Transparent tubes inserted into the soil for visualizing and photographing roots.

Procedure: Install tubes and use a camera to capture images of roots at different depths.

Applications: Provides non-destructive monitoring of root dynamics.

Hydroponics: Roots can be easily observed, sampled or measured non-destructively when the plant is grown in hydroponics. Root development may not be comparable to that grown in soil but for certain applications this system might be acceptable. A suitable container for root hydroponics is again the tube. In this case a sidearm with a fitted graduated pipette at the top can serve well to measure solution level (see photo). Aeration of the solution is necessary. In certain cases, root-

compatible fungicides may be added to the solution in order to avoid bio-contamination. It is however recommended to replace the solution occasionally, depending on root size and solution volume.

The advantage of hydroponics is the accessibility to the root for measurements. While most forms of root measurements are possible, the main advantage is in being able to measure also root volume. This is a simple method based on classical water displacement. It has been shown that root volume was highly correlated with total root length as estimated by the WinRHIZO method.

Techniques used for root length estimation:

1. **Root Scanners:**
    - **Description:** These devices use imaging technology to capture high-resolution images of roots. Root length is then determined through image analysis software.
    - **Advantages:** Non-destructive, provides detailed spatial information.
    - **Limitations:** Limited to visible roots near the soil surface.
2. **Grid Line Intersect Method:**
    - **Description:** A grid is placed over soil cores or images, and the intersections of roots with the grid lines are counted to estimate root length density.
    - **Advantages:** Simple and cost-effective.
    - **Limitations:** Labor-intensive and may be less accurate for complex root systems.
3. **Root Image Analysis Software:**
    - **Description:** Software tools designed to analyze images of roots captured using various methods, providing quantitative data on root length.
    - **Advantages:** Objective and efficient.
    - **Limitations:** Relies on the quality of images and may require some manual verification.
4. **Root Length Meters:**
    - **Description:** Handheld devices designed to measure root length directly in the field or lab by inserting a probe into the soil.
    - **Advantages:** Portable, relatively quick measurements.
    - **Limitations:** Limited to accessible areas and may not capture the full root system.

- **Advantages:** Provides information on vertical distribution.
- **Limitations:** Destructive, labor-intensive.

5. **WinRHIZO Software:**
   - **Description:** Software used with images of roots to analyze various root parameters, including length, surface area, and diameter.
   - **Advantages:** Efficient and provides detailed root morphology data.
   - **Limitations:** Requires high-quality images.
6. **Automated Root Tracking Systems:**
   - **Description:** Utilizes advanced computer vision and machine learning to track and measure roots in real-time.
   - **Advantages:** High precision, continuous monitoring.
   - **Limitations:** Equipment cost and technical expertise may be barriers.

When selecting a root length estimation method, consider the specific requirements of your study, the characteristics of the root system, and the available resources. Combining multiple methods may provide a more comprehensive understanding of root length and distribution.

There are several software tools available for plant root image analysis, each with its own set of features and capabilities. These tools are designed to help researchers and scientists analyze and quantify various aspects of root systems. Here are some popular plant root image analysis software options:

1. **WinRHIZO:**
   - *Developer:* Regent Instruments Inc.
   - *Features:* WinRHIZO is a widely used software for root image analysis. It provides tools for measuring root length, diameter, and other morphological parameters. The software is suitable for both field and laboratory studies.
2. **ImageJ:**
   - *Developer:* National Institutes of Health (NIH)
   - *Features:* ImageJ is an open-source image processing program that can be customized with plugins. Several plugins, such as RootNav and SmartRoot, have

been developed for plant root analysis. ImageJ is versatile and can be used for various image analysis applications.

3. **SmartRoot:**
    - *Developer:* Cirad, INRAE, and the University of Nottingham
    - *Features:* SmartRoot is an ImageJ plugin specifically designed for analyzing plant root systems. It provides tools for automatic and manual root tracing, length measurement, and branching analysis.
4. **RootNav:**
    - *Developer:* University of Nottingham
    - *Features:* RootNav is another ImageJ plugin tailored for automated analysis of root systems. It offers features such as root tracing, length measurement, and branching analysis. It is suitable for various plant species.
5. **Root System Markup Language (RSML) and RootReader3D:**
    - *Developer:* French National Institute for Agricultural Research (INRAE)
    - *Features:* RSML is a standardized markup language for describing root architecture. RootReader3D is a software that uses RSML files for 3D root system reconstruction and analysis.
6. **EZ-Rhizo:**
    - *Developer:* Armengaud et al.
    - *Features:* EZ-Rhizo is a user-friendly software for measuring root system architecture. It allows for the analysis of parameters such as root length, surface area, and volume.

Chapter-12

ROOT TRAITS AND PHENOTYPING STRATEGIES FOR GENETIC ENHANCEMENT OF CROPS

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Roots play pivotal role in plant development, serving crucial functions such as water and nutrient uptake, acting as storage organs, anchoring plants to the soil, and facilitating interactions within the rhizosphere. The dynamic spatial distribution of root parts collectively known as 'root system architecture' (RSA) responds to external factors like soil moisture, temperature, nutrients, pH, and microbial communities, presenting opportunities to explore natural variations for beneficial root traits that enhance plant productivity.

As the global population expands, there is an increasing demand for agricultural systems and cultivars capable of thriving in an unpredictable weather pattern and efficiently extracting resources from the soil. Conventional breeding programs have traditionally emphasized aboveground plant parts, but there is a growing interest in "root breeding" to identify underground traits for efficient water and nutrient utilization.

Root traits, especially under stress conditions like drought, are linked to crop productivity. Understanding the genetic basis of root phenotype contributing to higher yields and stress tolerance provides breeders with tangible targets for hybridization and selection. However, breeding success depends on factors like trait heritability, accurate phenotyping, farming systems, soil properties, and target environments.

Roots also play a crucial role in soil health, sustaining ecosystems that support plants, animals, and humans. Strategies like crop rotation enhance nutrient availability and soil health, while the interaction between legume roots and nitrogen-fixing bacteria reduces the need for chemical fertilizers. Plant roots contribute to soil conservation, minimizing water leaching, soil erosion, and participating in phytoremediation.

Despite the importance of root systems, breeding efforts focusing on specific root traits are limited. Genetic gains in grain production could be further enhanced by understanding root traits contributing to overall plant performance. The challenge lies in developing non-destructive root

phenotyping systems accurately reflecting and capturing RSA for 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.

Root System Architecture Impacting Crop Productivity

The distribution of nutrients across different layers of the soil and the availability of these nutrients in different environments make the RSA, a fundamental trait for efficient resource acquisition. In situations where soil nutrient availability is restricted, growers often resort to intensive fertilization to optimize biomass and yield. However, this approach is not only costly but can also lead to nutrient leaching into ecosystems, contributing to the contamination of rivers, lakes, and coastal waters. To mitigate these negative impacts, there is a need for more effective fertilizer uptake, achievable by employing genotypes with roots that exhibit enhanced efficiency in capturing nutrients from the soil. Roots sense and respond to various abiotic and biotic stresses, and are able to communicate with the aboveground plant parts *via* signaling pathways. Plants respond to stress, such as drought, by adjusting their root traits through morphological changes. This adaptive strategy enhances the soil's water-exploring capacity, ensuring sustained crop productivity in limited water conditions. Root traits targeted for crop improvement under drought and nutrient limitation conditions have been the subject of research for crop production systems. Understanding how root systems develop in response to stress, like water and nutrient shortages, can reveal connections between the mechanisms that sense and transmit signals in plants, mediating these adaptations. Field observations, even when focused on a part of the root system, can use indicators like root weight or length densities to gain insights into this distribution. For instance, achieving higher root length density in deeper soil may result from increased growth angles of certain nodal roots, or from elongating the primary root and developing more extended lateral roots. Therefore, understanding the development of individual roots is crucial for constructing an ideal root system adaptable to diverse conditions. Crops cannot freely place their roots in the soil because crops have the evolutionary constraint of sequentially developing roots one-by-one from the seed. The individual root is defined as the basic unit of root development. Root system consists of many individual roots of different types and ages. Each individual root has specific traits, which change with the growth.

Root Types: Roots in plants are classified as seed-borne (primary and seminal roots), shoot-borne (nodal, basal, and adventitious roots), and root-borne (lateral roots). Each type plays a distinct role

in resource acquisition, with nodal roots crucial in gramineous crops like rice and basal roots significant in leguminous crops. Adventitious roots, formed in response to soil disturbance, may play a role in nutrient uptake. Lateral roots, known as root-borne roots are the most abundant and essential for resource acquisition across all crops.

Growth Direction: The growth direction of roots is primarily determined by the root emergence angle and gravitropism (also known as geotropism, is the process by which plants grow in a specific direction in response to gravity). For instance, in rice, the emergence angle of nodal roots changes with the time of emergence and root diameter, influencing vertical root distribution. Growth direction affects the overall depth and structure of the root system, with deeper distribution linked to increased emergence angles.

Root Elongation: Root elongation is governed by cell division and elongation in the root apical meristem. Environmental conditions such as water deficit and soil compaction can significantly impact the rate of root elongation. The elongation zone is a small region, and changes in this process can affect the overall growth of the root system.

Age: As individual roots progress from emergence to death, various events occur, including lateral root and root hair formation. Anatomical transformations, like root cortical aerenchyma formation also take place. These changes influence resource acquisition capacity, with factors such as root surface area, hydraulic conductivity, and the release of root exudates altering during root development.

Individual Root Traits: Several traits contribute to the forms and functions of individual roots. Root length, determined by the elongation rate and growth duration, and root hair characteristics impact nutrient acquisition. The number of individual roots is crucial, and these traits can be measured in laboratory experiments to understand and optimize plant root systems.

Traits	**Remarks**
Type	Primary root, seminal root, nodal root, basal root, adventitious root, and lateral root
Elongation rate	Cell division x cell elongation at the root apical meristem
Emergence angle	Vertical and horizontal angles of the root generated from the seed, shoot, and root
Gravitropism	The curvature per unit time in gravity

Root thickness	The size of radial direction *i.e.,* diameter
Growth duration	The time from emergence to cessation of elongation
Longevity	The time from emergence to root death
Lateral root density	The number of root-generated roots per unit of the parent root length
Root hair properties	Density, length, and longevity
Resource acquisition capacity	The activity of ion transporters etc.

Rooting Depth: Rooting depth, a key trait influencing crops' access to water and nutrients, is frequently evaluated, particularly nitrogen, prone to leaching into deeper soil layers. While, soil properties strongly affect rooting depth, recent studies reveal additional factors that can be leveraged in crop breeding. Anatomical traits, like reduced cortical cell number and large cell size in maize are correlated with deeper roots under water stress. Root cortical aerenchyma presence in various crops is associated with enhanced exploration and reduced metabolic costs, especially in hypoxic conditions. Specific root traits, such as longer stolon roots in potatoes, also indicate drought tolerance in field conditions. Gravitropism, guided by the *DRO1* gene in rice, influences rooting depth by promoting a deeper root system. Understanding molecular components related to root gravitropism, particularly those involving auxin transport, cell walls, and the cytoskeleton, could offer strategies for enhancing rooting depth in crops. Focusing on selecting faster-growing and deeper roots may enhance water access in deeper soil layers, crucial for maintaining yield in limited rainfall conditions. Despite variations in environmental conditions, certain root traits associated with crop productivity under drought have been identified, such as the size of the root system relative to aboveground parts. However, complexities arise, as increased grain yield in maize under drought stress has been linked to a decrease in root mass, suggesting a potential trade-off between aboveground and belowground resource allocation influenced by metabolic costs and plant lifespan.

Root Hairs: Root hairs, tiny single-cell projections from root epidermal cells, play a crucial role in plant water absorption, contributing to nearly 50% of the total root surface area. This significance was evident when comparing wild-type Arabidopsis plants with root hairless mutants, which exhibited reduced water absorption capacity and increased sensitivity to drought, salinity, and heat

stress. In barley, root hairs are essential for penetrating hard soils and acquiring phosphorus (P), crucial for plant establishment. Recent findings in common beans showed that combining long root hairs with shallow basal roots resulted in a remarkable 300% increase in biomass, highlighting synergistic benefits for crop productivity. Wheat demonstrates the multifaceted roles of root hairs, with rhizo-sheath formation (soil adherence to roots) correlating with root hair tolerance to aluminum (Al). The molecular machinery governing root hair growth is well-established, and modifying certain genes controlling root hair development has produced plants with longer and highly branched root hairs. However, the extent to which these morphological changes enhance water and nutrient uptake efficiency in plants remains to be determined.

Root Branching: The development of lateral roots is a crucial aspect of RSA as it significantly impacts overall root biomass, length, and surface area. Recent studies highlight the need to tailor lateral root density to soil nutrient and water availability rather than assuming higher density always leads to increased nutrient and water uptake. In nitrogen (N)-limiting soils, maize lines with longer, fewer lateral roots show a 30% increase in yield, reducing metabolic costs associated with maintaining intricate root structures. Conversely, genotypes with more lateral roots prove advantageous in low phosphorus (P) soils, emphasizing the intricate balance between lateral root traits, soil conditions, and metabolic costs.

Studies on *PHOSPHORUS STARVATION TOLERANCE 1 (PSTOL1)* alleles in rice and sorghum explained the significance of total root length and surface area for P acquisition in low-P soils. Exploration of lateral roots in crops like wheat and maize revealed various root types adapting to environmental conditions. Hydropatterning, observed in lateral root, root hair, and aerenchyma formation, presents a unique adaptive strategy where roots respond to soil moisture through lateral emergence. The potential of hydropatterning in improving water use efficiency and enhancing plant yield under drought conditions warrants further exploration for agricultural benefits.

Technologies for Phenotyping Root System Architecture (RSA) Traits

Assessing root traits in field conditions presents challenges, impeding the comprehensive evaluation of RSA traits for breeding selection. Field-based techniques are labor-intensive and necessitate plot destruction for sample collection. Soil structure and composition heterogeneity within a field introduces variability, impacting RSA in field-grown plants and complicating the interpretation of genetic and environmental interactions. To circumvent these challenges, an alternative approach to field root phenotyping involves examining roots in plants cultivated under

controlled conditions. Methods employed for assessing plant root architecture must ensure an accurate portrayal of root growth, mitigate the influence of extraneous environmental factors on root development, exhibit sufficient throughput to phenotype numerous genotypes routinely screened in plant breeding programs, and facilitate the extrapolation of root phenotypes from controlled environments to those of biological relevance in field conditions.

Numerous software packages have been created to capture root images and extract quantitative data, enhancing root phenotyping efforts. Examples of these tools include RootScan, RootNav, DART, GiARoots, IJ Rhizo, RootSystemAnalyzer, RootReader, RootReader3D, and RooTrak. The growing array of root image analysis tools has led to the recent introduction of the Root System Markup Language (RSML) format. RSML facilitates the sharing of root architectural data among various software packages, establishing a standardized format for centralized repositories housing root trait data.

The choice of plant cultivation method is crucial for downstream root image analysis. For understanding root development, artificial gel-based media, soil-filled containers, and rhizotrons are employed under controlled conditions. For high-throughput root trait selection in breeding, methods range from screening seedlings on germination paper to excavating field-grown plants, each with its advantages and disadvantages. Gel-based systems offer real-time, non-destructive imaging but pose challenges in physiological relevance. Soil-filled containers provide more physiologically relevant data but have limitations in throughput and resolution. Laboratory and greenhouse-based methods, like GLO-Roots and X-Ray Computed Tomography, allow controlled evaluations. Destructive assays, such as shovelomics and rhizolysimeters offer practical and physiological relevance but are labor-intensive. Field-based methods are subject to environmental variabilities. The selection of cultivation method depends on the research objectives, balancing control, relevance, and throughput considerations.

The choice of plant cultivation method for root imaging depends on factors such as the specific root trait of interest, desired sampling timescale, and infrastructure capacity and costs. Various methods for growing plants for subsequent root imaging and phenotyping are outlined as follows.

Strategies and approaches for growing plants prior to root phenotyping

Approach	Advantages	Disadvantages

Laboratory methods	☑ Evaluate root growth in real time ☑ Non-destructive ☑ A large number of controlled growth conditions can be tested ☑ Repeatable conditions ☑ Large space for plant growth is not required ☑ Easy to handle and clean roots	➢ RSA may be affected by the growth container ➢ Sterile conditions for evaluation exclude effect of possible interactionwith beneficial microbes ➢ Plants are not exposed to environmental conditions and therefore physiological relevance of root phenes should be further evaluated
Greenhouse methods	☑ Intermediate system between lab and field ☑ Enables control of certain conditions such as soil type and moisture,light intensity, temperature, pot sizes and water and nutrient inputs ☑ Evaluate genetic potential of plant RSA without intraspecific competition	➢ Labor intensive to process and clean bigger roots ➢ Plants could be exposed to some disease/insect pressure ➢ RSA continues to be affected by the growth container ➢ Plant performance evaluated in the absence of other plants and/ormicroorganisms in the soil unless experimental design includes it
Field methods	☑ Physiological and practical relevance	➢ Labor and time intensive ➢ Challenges due to variability in the field, particularly for soil conditions ➢ Intensive root clean-up ➢ Destructive assays ➢ Permits are required for evaluation of transgenic plants

Root Phenotyping Strategies and Their Application in Crop Improvement

To improve a trait in new varieties, including root systems, breeders typically need three fundamental inputs:

1) access to donor germplasm harboring favorable alleles linked to the target trait,
2) a method for trait identification, achieved through either visual characteristics (phenotypic selection) or the presence of a specific allele (marker-assisted selection),
3) the required human and capital resources for executing the selection and breeding processes.

Advancing the development of plants capable of thriving in challenging environments, characterized by marginal soils and reduced water and fertilizer availability, stands as a key objective in global crop breeding programs. While, the identification of root traits and phenotypes that support efficient water and nutrient utilization is crucial for achieving these breeding goals.

The progress in crop breeding programs has led to increased yields through the selection of traits such as large shoot biomass, optimized ratios between harvested grain and shoot biomass, enhanced disease resistance, and extended growing seasons. Notably, the unintentional selection for more efficient root systems, evidenced by earlier flowering and reduced days between germination and harvest has contributed to these yield gains.

Understanding the variability and contributions of specific root traits within a given species becomes paramount for identifying traits that can more effectively enhance root system efficiency, ultimately resulting in higher plant productivity. Successful efforts to improve nutrient and water acquisition involve the identification and selection of root ideotypes tailored to specific soil and environmental conditions. This can be achieved through a combination of root phenotyping strategies, encompassing laboratory, greenhouse, and field evaluations.

Leveraging prior knowledge of RSA among different genotypes or breeding lines allows for comparisons of productivity under nutrient and water deficits. It is essential to recognize that desirable root traits may vary among different crops (annuals vs. perennials), and the integration of traits must consider potential trade-offs in resource allocation between various plant organs, such as the root-to-shoot ratio.

Considerations of heritability in root traits, genotype-environment-management (GEM) interactions, and temporal variations in root types due to changing conditions (e.g., rainfall) significantly influence breeding decisions. Additionally, the capabilities of root phenotyping

contribute to a deeper understanding of the variation in root systems across multiple species in response to diverse stress factors. This understanding aids in assessing the impact of these systems on the soil ecosystem and, in turn, informs the development of strategies to modify RSA and enhance overall soil health

Ensuring the reproducibility and accuracy of phenotyping is crucial for identifying quantitative variations in RSA among plant materials and uncovering the underlying genetic mechanisms, such as Quantitative Trait Loci (QTL) and genes associated with root traits. This knowledge is instrumental in implementing genomics-assisted breeding strategies. The efficacy of molecular breeding strategies relies on phenotypic data, which determines the estimated breeding value of a specific allele within a given genetic background and set of environments.

Numerous traits influencing root depth are known to be governed by multiple genes, indicating the potential for enhancement or modification through selective breeding. The existence of natural variation in root morphological traits within a target crop species underscores the feasibility of selecting for specific root traits. While, several genes associated with RSA are known, either through gene mutants exhibiting quantifiable changes in primary root length, root branching, or root hair formation, or from QTL studies, the mechanistic details of how these QTL influence root phenotypes, their effects in diverse genetic backgrounds, and their role in different soil types and environments remain relatively limited.

In the laboratory, root phenotyping strategies primarily serve basic research activities during the discovery phase, identifying genetic variation for RSA and elucidating the genetics of root anatomy. The evaluation phase involves understanding RSA dynamics and how root anatomy and physiology respond to various abiotic and biotic stresses in controlled single-variable experiments. Validation experiments assess plant performance with specific root traits, enabling the evaluation of biomass/yield production and the identification of associations between traits and molecular markers. In the utilization stage, molecular markers are employed to select desirable individuals as parents for crossing and population development, track desirable root traits throughout the breeding pipeline, and enhance our understanding of root plasticity across multiple soil types under varying cultivation and crop management practices. The consideration of key root traits such as tap root prevalence, branching patterns, growth rate, and direction relative to limiting resources holds significance in breeding programs aiming to introduce new cultivars that outperform existing ones in practical agricultural settings. While progress has been made through phenotype-based selection,

a deeper understanding of the underlying mechanisms is essential. For instance, in alfalfa, populations selected for enhanced fibrous or lateral roots exhibited greater biomass yield compared to those selected for minimal fibrous or lateral roots. Genetic gains from two cycles of divergent selection in alfalfa for root traits were realized, emphasizing the impact of root morphology on the persistence and productivity of perennial crops.

In rice, positive correlations were observed between root traits (length, diameter, dry weight, and total absorbing surface area) and grain yield. The introgression of the *DEEPER ROOTING 1 (DRO1)* QTL controlling root growth angle through backcrossing in a shallow-rooting rice cultivar resulted in deeper roots and improved yield performance under drought conditions. Diverse root traits play vital roles in different types of drought at various developmental stages. For example, the major P starvation tolerance QTL *Pup1* in rice led to the identification of the *Pup1*-specific protein kinase gene, *PSTOL1* enhancing early root growth and improving nutrient acquisition in P-deficient soils.

Breeding strategies for crops with deep roots, aimed at sustainable water, nutrient, and carbon sequestration have been explored. To incorporate root phenes enhancing water and nutrient acquisition as well as disease resistance into new germplasm, continuous screening of a large number of individuals is necessary, with different phenotyping systems serving complementary roles. The identification of early root development traits from seedlings grown in controlled environments for initial selections, followed by the evaluation of individuals for root traits in the greenhouse to select parents is important for generation of breeding materials. Multi-location field trials assess the performance of advanced breeding lines, focusing on traits like yield. This holistic approach aims to accelerate the development of new cultivars with improved root systems, accounting for deeper roots, branched root redistribution, and enhanced hydraulic conductivity. Ultimately, the relationship between young root systems evaluated in controlled conditions and mature root system vigor, water and nutrient uptake, and higher yields is critical. While field-based selection systems for mature root system traits are challenging, the identification of strong relationships between controlled-environment root vigor and field root vigor could provide valuable insights.

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Chapter-13

CLIMATE CHANGE IMPACT ON VARIOUS CROPS AND ITS IMPLICATION ON CROP ROOT SYSTEM

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Increasing evidence especially over the past few decades indicate that significant changes in climate are taking place worldwide as a result of enhanced human activities that lead to global warming. The change in atmospheric concentration caused by the anthropogenic Greenhouse Gases (GHGs) was observed to affect the plant metabolic activity and also the production directly. The synopsis of the dry mass production and yield increase of the world's ten most important crop species in response to elevated CO_2 revealed that in some species the relative increase of total biomass and in others that of economic yield is greater.

It is well known that elevated CO_2 stimulates photosynthesis. Doubling of CO_2 concentration may increase the photosynthetic rates by as much as 30-100 per cent in C_3 plants such as wheat, rice, and soybean and will become more water efficient as they quickly grow. While the response in C_4 plants such as maize, sorghum, sugarcane, millets etc., may not be spectacular. For a C_4 crop like sorghum, the stimulation was only about 9% under well-watered condition, while larger response of about 23% was reported under water stress, probably due to the effect of elevated CO_2 on plant water relations. Elevated CO_2 causes partial stomatal closure, which reduces the conductance for the exchange of gases and transpiration. It was reported that due to increasing CO_2 from 330 to 660 ppm, reduction in transpiration was about 34%. When there was no stress, elevated CO_2 reduced stomatal conductance by 21.3 and 16.0% for C_3 and C_4 species respectively. The pulse crops responded better than other crops to elevate CO_2 condition. Plants grown with elevated CO_2 were taller and attained greater leaf area along with more biomass than ambient CO_2 levels under irrigated and stress condition. The OTC studies indicated that under increased CO_2 levels proportioning of assimilate of black gram was greater to the roots than to the shoots under moisture stress. Atmospheric CO_2 enrichment often stimulates plants to develop more robust root systems to probe greater volumes of soil for scarce and much needed moisture. Atmospheric CO_2 enrichment increases plant water acquisition by stimulating root growth, while it reduces plant water loss by reducing stomatal

conductance, and these dual effects typically enhance plant water use efficiency, even under conditions of lower optimal soil water content, hence delay in the onset of the water stress.

The enhanced CO_2 response of root characters such as root length, root volume and root dry weight was higher under moisture deficit as compared with well-watered condition in both C3 (Sunflower) and C4 (Maize) crops. A significant improvement in root volume with eCO_2 was evident with both the crops and both conditions and the response was more prominent with maize under stressed condition. It was observed that elevated CO_2 concentration significantly increased the soybean root volume and root shoot ratio as a result of increase in root diameter, length, volume and weight.

The response of root length of black gram genotypes was positive with elevated CO_2 of 550ppm during all three seasons- summer, winter and rainy and maximum values were recorded in summer season at ambient and 550ppm while in winter season at 700ppm. The response of root volume was positive and highly significant at both elevated CO_2 levels of 550ppm and 700ppm during winter season. Maximum values for root volume were recorded in rainy season at ambient condition, while in winter season at 550ppm and in summer season at 700ppm. Plants grown at higher concentration of CO_2 were reported to partition more carbon to supporting structures such as stems, petioles and roots. This mechanism could be for balancing the supply and demand for carbohydrates needed by the whole plant for balanced growth. Increased partitioning of biomass to roots, increased root length and volume at elevated CO_2 condition was reported in soybean and cotton.

Roots exude a variety of organic molecules into the soil, modifying its structure and physical properties, affecting availability of soil minerals, and facilitating plants to interact with soil microorganisms. Plant roots are highly adaptable to the environment, and its growth is often described as “plastic”. Root growth plasticity responds to the availability of water and minerals, oxygen level, temperature, heavy metals, salinity, and mechanical properties of the soil. The projected rise in atmospheric CO2 may result in changes in shoot and root structure, higher rate of photosynthesis and increased plant productivity. In a changing climate with rising CO_2 concentration in the air, roots play an important role in the global C cycle, by transferring atmospheric C fixed via photosynthesis into the soil. Coarse roots have been predicted to accrue greater biomass under elevated CO_2 and serve as large C storage sites. Fine roots are also an

important component of the global C cycle, and as much as one-third of the total global primary productivity is allocated to fine root construction and maintenance. Thus, healthy and functional plant roots are critical to the production of food and many other resources human prosperity depends on, as well as to the health of natural ecosystems and the environment. Alterations in root growth, development, and deployment represent major plant responses to elevated CO_2 concentrations and adapts to elevated CO_2 is vital for human food security and the environment.

Enhanced photosynthesis due to high atmospheric CO_2 allows plants to allocate more fixed C belowground, often leading to increased root growth and longer, thicker, and more branched root system. As a result, root-to-shoot mass ratio often increases. High CO_2 also stimulates the production of fine roots and root hairs and interacts with other factors such as soil N availability to affect fine root distribution within the soil. The changes in root growth and architecture involve complex interplay of photosynthate, plant hormones such as auxin, ethylene, and cytokinin, as well as nitric oxide (NO), and are dependent on species. High CO_2 concentration affects root growth positively across most species studied. Under non limiting water and nutrient conditions, elevated CO_2 increased root growth and root dry weight of many crop plants and promoted the formation of lateral roots, possibly using NO as a signal. These changes to the root system may lead to greater soil exploration in both horizontal and vertical directions. Plants strategically allocate biomass between above- and below-ground tissues to optimize growth in fluctuating environments. Under elevated CO_2, root: shoot (R/S) ratios are often altered, suggesting a shift in the functional relationship between these organs. Increased R/S ratio under higher CO2 is more pronounced under abiotic stresses, such as high light and deficiencies of nutrient or water.

The distribution of fine root density per unit volume of soil, which determines overall root architecture, increased both vertically and horizontally with increased CO_2. Plant growth can benefit greatly from development of root hairs, which are important in increasing root surface area for nutrient acquisition under limited nutrient supply. Elevated CO_2 increases the production of carbohydrates, which trigger the auxin or ethylene responsive signal transduction pathways and subsequently the accumulation of intracellular NO. These endogenous signals then modulate the expression of specific gene set and the initiation of the root hairs.

With increased atmospheric CO_2 concentration along with other associated abiotic stresses, the ability of the root system to adapt for better acquisition of water and nutrient is critical. Understanding of the mechanisms involved in root architectural development under this complex interacting environmental conditions is needed to develop climate resilient crop varieties.

Chapter-14

SUPER ANNIGERI-1: COMBINE APPROACH TO IMPROVE FUSARIUM WILT RESISTANCE AND ROOT TRAITS IN A-1 VARIETY OF CHICKPEA (*Cicer arietinum* L.)

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Chickpea variety Annigeri-1, elite high yielding, drought tolerant, early maturity duration and most preferred desi cultivar by gram industry which is extensively cultivated in Karnataka. The Cultivar, in recent years, has become susceptible to race 4 of Fusarium wilt (FW). To improve Annigeri-1 for wilt resistance by keeping all other traits was a challenge. By deploying molecular breeding, a genomic region conferring resistance against Fusarium wilt race 4 (*foc4*) was introgressed through marker-assisted backcrossing using WR 315 as the donor parent. Multilocation evaluation and farm trials across the state evaluation for more than five years provided one superior line referred to as Super Annigeri-1 (MABC-WR-SA1) with 10.64 % increase in yield, enhanced disease resistance, root traits and quality over Annigeri-1. MABC-WR-SA1 (Super Annigeri-1) showed improved quality, 18.45 % protein; 64.62 mg/kg Zn and 85.14 mg/kg Fe over A1 which has 14.03 % protein, 49.22 mg/kg Zn and 59.71 mg/kg Fe. MABC-WR-SA1 (Super Annigeri-1) showed improved root traits 99.5 cm over A1 which has 91.5 cm of root (Length). The genotype was submitted to All India Coordinated testing programme during the same period from 2016-17 to 2018-19 and it has consistently out-performed among check varieties under National (AICRP) testing. This variety has the average yield potential of 1864 Kg/ha in central and south zones of India and has portrayed a yield advantage of 12 % over recurrent parent (Check). MABC-WR-SA1 recorded highest resistance among all the genotypes in 7 locations out of 9 locations tested across nation 2017-18. Considering three year's performance, this variety was identified for central and south zones by Variety Identification Committee (VIC) during *rabi* Annual Group Meet of All India Coordinated Research Project on chickpea held from 27-08-2019 to 29-08-2019 at Ranchi (Jharkhand). Subsequently, based on VIC approval this variety was released by the Central Sub-Committee on Crop Standard, Notification and Release of Varieties

for Agricultural Crops (CVRC) and notified by the Gazette Notification S.O. 99(E) dated 6 January 2020 for central Zone comprised of Maharashtra and Gujarat and South zone comprised of Andhra Pradesh and Karnataka. MABC-WR-SA1 is a wilt resistant, drought tolerant and high quality (Protein, Fe and Zn) variety with average maturity of 102 day. It may be good alternative of existing varieties under wilt prone areas of central and south zones of India. Further, the said variety is formally adopted at UAS, Raichur in June 2020.

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