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Original Research Paper

Effect of container size and types on the root phenotypic characters of *Capsicum*

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ABSTRACT

***Capsicum* genus comprised of several cultivars is considered as an important spice crop worldwide. Roots play a vital role in a plant to mine water from the deeper layers of the soil. Although, characterisation for root traits have been made using different containers in many crops, such efforts for phenotyping root characteristics in *Capsicum* species are limited. Therefore, the experiment was initiated to find out the influence of container size on root characteristics and also to identify the appropriate container for high throughput phenotyping of *Capsicum* species for desirable root characteristics. Nine genotypes belonging to different *Capsicum* spp. were grown in three types of containers having different dimensions. Among the three types of containers, the bucket type container with dimension of 32 cm height 30 cm diameter with 23 kg soil media capacity was most suitable for phenotyping root characteristics compared to PVC pipe and pot type. Subsequently, 18 genotypes were phenotyped for plant growth and root characteristics in the bucket type container. The genotypes IHR 4517, IHR 3529, IHR 4501, IHR 4550, IHR 4491 and IHR 3241 with better root characteristics were identified.**

Key words: Capsicum, container, root characteristics and plant growth

INTRODUCTION

The genus *Capsicum* comprises several cultivars that are grown worldwide. In addition to their use as spices and food vegetables, *Capsicum* species have also been used in pharmaceutical industries. The genus *Capsicum* has five domesticated species, *Capsicum annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz and Pav. However, among them, *Capsicum annuum* L. is distributed world over with greatest economic importance and is part of many dishes mainly because of its spicy taste, pungency, appealing colour and flavor. India is the world's largest producer and exporter of chilli, contributing about 25% of world's chilli production (National Horticultural Board, 2017).

Several abiotic stresses during critical stages of crop growth and development severely affect the productivity of *Capsicum* sp. inadequate water availability is a major abiotic stress which adversely affects growth and productivity of chilli crop (Bhutia *et al*., 2018). The major growing areas in India experience water limiting conditions due to limited water resources. In India in some parts, chilli is grown under rainfed conditions. The sporadic water stress is a common feature that causes considerable reduction in productivity of chilli, through modification in various morpho-physiological and bio-chemical processes (Singh, 1994). The antagonistic effects of water deficit stress have been studied by several workers in chilli (Cantore *et al.,* 2000; Kirnak *et al*., 2003; Antony and Singandhupe, 2004; Khan *et al*.,2008; Gunawardena and De-Silva 2014; R'Him and Radhouane, 2015; George and Sujatha, 2019).

Some of the plant's adaptive strategies under deficit water stress situations are; deep root system, higher water use efficiency (WUE) and tissue water retention through modifications in leaf, stomatal and cuticular characteristics (Basu *et al*., 2016). These adaptive features help plants to maintain higher tissue water content under deficit moisture stress and facilitate them to delay the imminent adverse effects of water stress. Roots play a major role under water deficit conditions by acquiring water from the deeper layers of the soil.

They also communicate with above ground parts through signaling pathways. The growth and development of plants is controlled through the alterations in root morphology and physiology. Modifications were noticed in root to shoot transport of signaling molecules including hormones, proteins, RNAs and mineral nutrients (DoVale and Neto, 2015).

The restricted growth and development of plants by limited water availability could be overcome through root morphological plasticity at different soil moisture levels (Forde 2009). Under water limited conditions, roots improve the ability of crop plants to maintain water relations by exploring available water in the soil profile. Identification of root characteristics that enhance the plant's capability to mine soil water and sustain productivity is very essential.Several workers have attempted studies on various root characteristics and have elucidated the role of root characteristics like deep root system (Sashidhar *et al*.,2000; Sinclair and Muchow 2001; Venuprasad *et al*., 2002), thick root system (Chang *et al*., 1986), root to shoot ratio (Fukai and Cooper 1995), enhanced root system (Price and Tomos, 1997), root penetrating ability (Ray *et al*., 1996) and higher number of roots in the crown region (Kinyua *et al*., 2003).

Understanding the role of roots in improving tolerance and maintenance of water relations under water limiting conditions is very important. In this direction quantification of the root characteristics and their role in enhancing water stress tolerance is of primary relevance. Conventional crop improvement approaches have played a principal role in many crops for enhancing drought tolerance (Sreenivasulu *et al*., 2007). The desirable root characteristics like, deeper root length, large root volume, high root dry weight, and higher root-to-shoot ratio coupled with thick lateral roots were observed to confer water stress tolerance in chilli germplasm IIHR 4502 (*Capsicum chinense*) (Naresh *et al*., 2017). Since, phenotyping root characteristics under field conditions are highly cumbersome and challenging, researchers have been relying on assessing the desirable root characteristics in container grown plants. Studies have also shown relationships between controlled-environment root vigor and field root vigor, indicating that evaluations at early stage are predictive of future root performance (Wasson *et al*., 2012). Using containers for measurement of root systems reduces the growing medium volume and enables proper removal of the root system as compared to plants grown in field (Neumann, 2009). There is a need for identification of suitable container type and size that provide congenial growing conditions for expression of genetic potential and also enable easy extraction of root system to phenotype root characteristics.

Though studies have been conducted to characterize root characteristics using different containers in many crops, such efforts for phenotyping root characteristics in *Capsicum* species are very much limited (Kulkarni and Phalke, 2009; Naresh et *al*., 2017). Hence, the objective of the study was to identify appropriate container and size for high throughput phenotyping of root characteristics which facilitate selection of genotypes having desirable root characteristics for water mining.

MATERIAL AND METHODS

Experiment was carried out during 2018-2019 at the Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The experimental site is located at 13°58' N latitude, 78°E longitude and 890 m above mean sea level. Seeds of *Capsicum* sp. genotypes used in the study were obtained from the Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru.

In order to achieve objectives of the study, two experiments were conducted. First experiment was carried out using three different containers to identify appropriate container for high throughput phenotyping of root characteristics. Second experiment was conducted to phenotype for desirable root characteristics using 18 genotypes belonging to different *Capsicum* sp. in the suitable container identified in the first experiment.

Identification of appropriate container for high throughput phenotyping of root characteristics

In order to identify appropriate container for high throughput phenotyping of root characteristics, nine genotypes belonging to different *Capsicum* sp. IHR 3226, IHR 3455, IHR 3575, IHR 4517, IHR 3476 (*C. annuum*) IHR 3240, IHR 3241, IHR 4491(*C. baccatum*) and IHR 3529 (*C. chinense*) were selected. The genotypes were evaluated in three types of containers having different dimensions and soil media holding capacity. The containers used were: (i) bucket type container (Empty paint container, 30 cm diameter, 32 cm height having capacity to hold 23 kg soil), (ii) PVC pipe container (20 cm diameter, 64 cm height having capacity to hold 26 kg soil) and (iii) pot type container (18 cm diameter, 27 cm height having capacity to hold12 kg soil). The containers were filled with soil, Farm Yard Manure (FYM) and sand (2:1:1 v/v). The experiment was laid out in a factorial completely randomized block design with five replications.

Phenotyping of *Capsicum* sp. genotypes in appropriate container for desirable root characteristics

Eighteen genotypes belonging to different *Capsicum* sp. were evaluated for root characteristics in the bucket type container (30 cm diameter, 32 cm height having capacity to hold 23 kg soil). The experiment was laid out in a completely randomized block design with five replications.

Seedling raising and crop care: The seeds of genotypes used in both the experiments were sown in pro trays filled with coco peat as a growing medium. The seedlings were maintained in the shade net nursery for 45 days and recommended cultural practices were adopted to maintain plant health status and population. Forty-five-day old seedlings were transplanted into the containers. The plants were provided with recommended dose of fertilizer and crop protection measures. The plants were irrigated regularly to maintain 100 per cent field capacity.

Growth parameters: The observations in both the experiments were recorded at peak flowering stage (50 DAT). Plant height was measured using graduated scale and expressed in centimeters. The number of primary branches were counted manually at the point of initiation. The plant shoot parts were excised and the leaf and stem portions were separated. The entire root portion was carefully extracted from the soil medium using water jet to clean the soil. Soon after extracting the roots, observations on root parameters like root length (using graduated scale), root volume (water displacement method), number of primary roots and fresh and dry weights were recorded. Fresh weights of the root and shoot samples were measured immediately after extraction by using a Sartorius BSAZZAS-CW balance. The root, stem and leaf parts were dried in oven separately at 80°C for 72 h to achieve stable weight. The dry weight was recorded as total biomass accumulated and expressed as gram per plant.

To quantify the leaf area, representative sample of 20 leaves from each plant was taken and the leaf area was determined using leaf area meter (Biovis, PSM-L2000, India). Then the leaves were kept in oven at 70ºC for five days and leaf dry weight was measured using Sartorius BSAZZAS-CW balance. The ratio of leaf area to the leaf dry weight was computed as specific leaf area (SLA). The leaf dry weight of each plant was multiplied with SLA to arrive at the total plant leaf area (TLA).

Root: shoot ratio: It was arrived by dividing root dry matter with shoot dry matter.

Statistical analysis

ANOVA: The data obtained in different experiments was analyzed in factorial completely randomized block design and completely randomized block design for first and second experiment, respectively using two factors statistical package OPSTAT developed by CCSHAU (Sheoran *et al.*,1998).

RESULTS AND DISCUSSION

Plants manifest physiological and morphological modifications in response to change with soil volume. The container size and type influence root volume and in turn determine the dry matter distribution between above and below ground parts. Studies have shown that with doubling in pot size there is an average increase of 43% plant mass (Poorter, 2012). Container size is known to influence morphological and physiological changes in crops like tomato (Oagile *et al.*, 2016), bell pepper (Weston, 1988), squash (Nesmith, 1993) and cabbage (Csizinszky and Schuster, 1993). Alterations in container size leads to changes in available rooting volume which subsequently affects plant growth.

Identification of appropriate container for high throughput phenotyping of root characteristics

The container size plays a major role in plant root and shoots growth. The root length was not significantly influenced by the container type. However, among the three containers, higher root length was observed in PVC pipe container compared to bucket type and pot type containers. The root volume in bucket type container was 35.8% and 72.4% higher compared to pot type and PVC pipe containers, respectively (Figure 1). The studies conducted in bell pepper have shown that the container size has influence on the root volume and plant growth (Weston, 1988; Nesmith *et al.*,1992). In this experiment, among the three types of containers, the plants grown in bucket type container produced significantly a greater number of primary roots (44.8) compared to pot type (33.1) and PVC pipe (25.4) containers (Figure 1). Studies conducted by Cantliffe, (1993) and Kharkina *et al*., (1999) have shown that there is a strong positive correlation between container size and root biomass. In the present study, significantly higher root fresh weight and dry weights were observed in bucket type container compared to other two types of containers (Figure 1). The genotypes IHR 4491, IHR 3241, IHR 4517 and IHR 3529 produced significantly higher root fresh weight as compared to remaining genotypes (Figure 1). Plants grown in bucket type container recorded 73.14 % (4.32 g) and 40.86% (5.31 g) higher root dry weight compared to PVC pipe and pot type containers (Table 1).

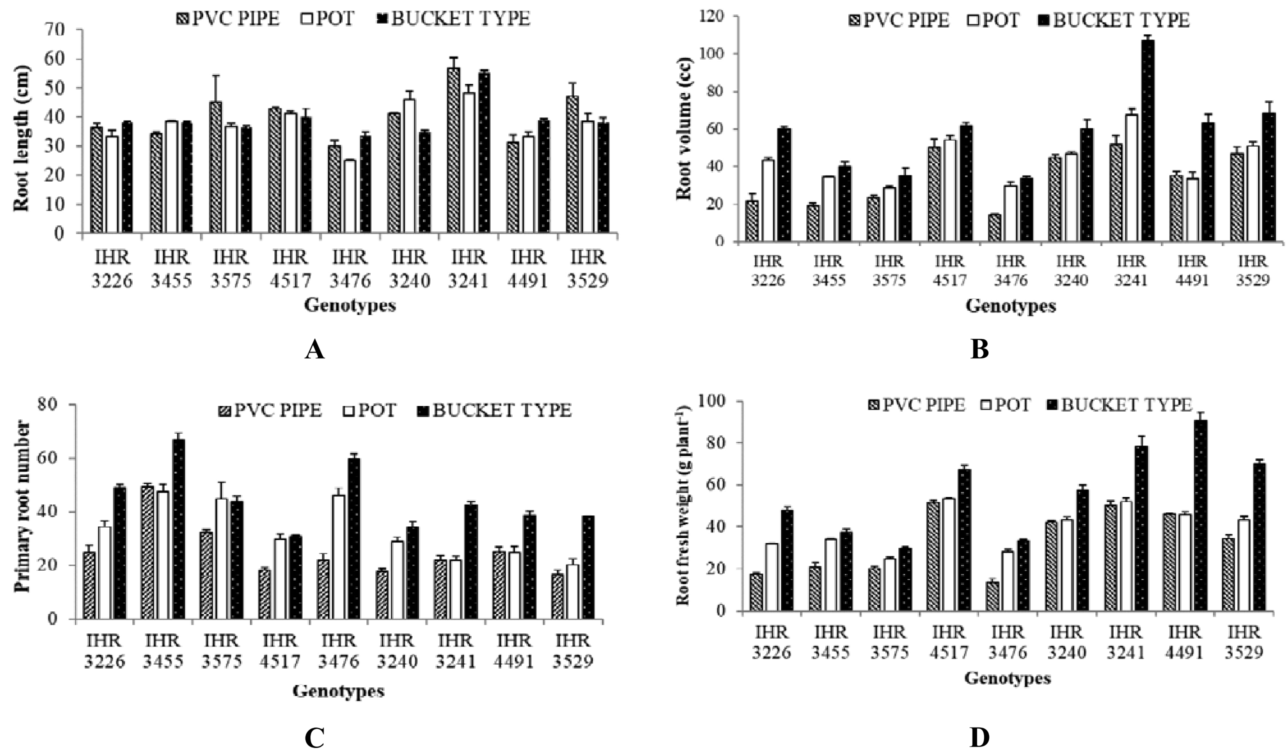

Figure 1: Influence of containers on root length (A), root volume (B), primary root number (C) and root fresh weight (D) of *Capsicum* sp.

Healthy root system growth promotes better above ground canopy growth. Hence, providing appropriate space for adequate root growth is essential. It is observed that the shoot growth is greatly impacted by varying container size and root restriction (Poorter, 2012). The plant height was significantly higher in bucket type container compared to remaining types of containers. Genotypes, IHR 3241 (68.1 cm) and IHR 3226 (57.2 cm) recorded significantly higher plant height compared to rest of the genotypes (Table 2). Tomato plants when grown in containers with low volume showed reduction in shoot height and biomass (Peterson *et al*., 1991). Hence, providing better rooting space helps the plants to produce higher above ground biomass with increased shoot height. Among the three types of containers, plants grown in bucket type produced significantly a greater number of branches compared to remaining two types of containers (Table 2). In bell pepper (*Capsicum annum* L.), root restriction caused reduction in number of branches (Nesmith *et al*.,1992). In container grown bell pepper plant, reduction in leaf area was observed mainly due to smaller and fewer leaves per plant (Weston, 1988; Nesmith *et al.,* 1992). With the increase in container size, the leaf area and shoot biomass has increased (Cantliffe, 1993). In this experiment, the leaf area was significantly higher in plants grown in bucket type container (5690 cm^2) as compared to pot (3797 cm^2) and PVC pipe (2690cm^2) containers (Table 2).

Table 1: Influence of containers on root dry weight and shoot dry weight in *Capsicum* sp.

Genotype	Root dry weight (g plant^{-1})			Shoot dry weight (g plant^{-1})		
	PVC PIPE	POT	BUCKET TYPE	PVC PIPE	POT	BUCKET TYPE
IHR 3226	1.91	3.47	5.28	9.6	15.3	33.0
IHR 3455	3.06	5.49	6.26	15.6	32.9	45.0
IHR 3575	3.30	3.89	5.02	18.2	21.4	26.6
IHR 4517	6.92	7.11	8.60	34.3	39.5	51.1
IHR 3476	1.71	4.04	4.43	6.2	17.1	28.3
IHR 3240	5.14	5.19	6.82	26.2	18.7	49.9
IHR 3241	6.49	7.21	10.44	31.1	44.7	53.5
IHR 4491	5.52	5.31	10.81	27.4	27.1	54.1
IHR 3529	4.79	6.10	9.63	14.8	33.5	51.7
Mean	4.32	5.31	7.48	20.4	27.8	43.7
Factors	G	C	GxC	G	C	GxC
C.D@0.05	0.65	0.38	1.13	3.2	1.85	5.54
SE (m)	0.23	0.13	0.4	1.12	0.65	1.95
CV (%)	10.8			11		

Table 2. Influence of containers on plant height, leaf area and number of branches in *Capsicum* sp.

Genotype	Plant height (cm plant^{-1})			Leaf area (cm^2 plant^{-1})			Branch number (no. plant^{-1})		
	PVC PIPE	POT	BUCKET TYPE	PVC PIPE	POT	BUCKET TYPE	PVC PIPE	POT	BUCKET TYPE
IHR 3226	67.7	57	57.3	1363	2221	4526	12	9	13
IHR 3455	40	44.3	54.7	2186	4510	6015	7	9	10
IHR 3575	45.7	41.7	53.3	2706	3179	3977	9	10	12
IHR 4517	49.3	38.3	47	4656	5263	6737	8	5	8
IHR 3476	29.3	25	41.7	1161	3092	4797	3	4	7
IHR 3240	46	52.7	52.3	2901	2209	5240	9	10	10
IHR 3241	54.3	51.5	84.7	4389	6058	8365	9	9	11
IHR 4491	32.3	29	71.3	2074	2040	4089	5	7	10
IHR 3529	25.7	27	55.7	2773	5600	7467	4	5	6
Mean	43.4	40.7	57.6	2690	3797	5690	7	8	10
Factors	G	C	GxC	G	C	GxC	G	C	GxC
C.D. (0.05)	3.17	1.83	5.49	669	386	1159	0.88	0.5	1.52
SE (m)	1.11	0.64	1.93	136	235	408	0.31	0.18	0.53
CV (%)	6.8	17.4	10.8						

Table 3. Variability in root and shoot growth characteristics among 18 *Capsicum* sp. genotypes

Genotype	RL (cm $plant^{-1}$)	RV (cc $plant^{-1}$)	PRN (no. $plant^{-1}$)	RFW (g $plant^{-1}$)	RDW (g $plant^{-1}$)	SDW (g $plant^{-1}$)	Root: Shoot ratio	PH (cm $plant^{-1}$)	BN (no. $plant^{-1}$)	LA (cm^2 $plant^{-1}$)
IHR 3240	38.0	50.0	32	54.0	6.90	60.6	0.118	54.7	10	6284
IHR 3241	55.0	100.0	43	69.2	10.63	51.2	0.211	79.7	10	8837
IHR 4491	39.3	73.3	36	88.6	11.81	48.2	0.249	75.6	10	4455
IHR 4550	54.3	110.0	45	127.7	15.38	42.9	0.412	68.0	6	6583
IHR 4501	65.0	125.0	33	110.8	12.56	44.2	0.29	68.0	8	7630
IHR 3529	48.7	71.3	36	76.0	10.23	36.9	0.268	54.7	7	7012
IHR 4658	42.3	38.3	34	49.9	5.80	47.1	0.122	69.3	9	4724
IHR 3982	33.3	18.3	14	24.6	2.01	19.7	0.346	48.3	13	3872
IHR 3983	45.7	31.7	29	38.6	7.55	46.1	0.169	95.3	15	3302
IHR 3226	35.0	38.3	47	30.5	3.78	40.0	0.096	58.0	12	5176
IHR 3455	37.0	32.7	63	32.4	5.17	57.7	0.091	54.3	11	6922
IHR 3575	33.3	30.0	41	29.5	4.13	25.2	0.164	52.0	12	3689
IHR 4517	44.0	61.7	30	75.1	8.93	52.0	0.176	44.7	10	7936
IHR 3476	30.7	35.0	60	32.2	4.45	29.8	0.15	41.3	7	4463
IHR 3447	28.0	16.7	25	20.1	2.30	8.7	0.264	38.0	10	1854
IHR 4108	42.7	44.0	46	35.1	3.40	54.1	0.065	71.7	10	4955
Chikkaballapur Local	42.0	30.0	19	28.6	3.13	15.8	0.206	52.0	11	1517
Guntur Local	42.3	29.3	31	41.0	6.70	53.6	0.125	72.7	14	6865
C.D. (0.05)	4.6	18.5	6.5	14.5	1.79	12.4	0.06	8.79	2.35	2914
SE (m)	1.6	6.4	2.2	5	0.6	4.3	0.021	3.05	0.82	1012
CV (%)	6.5	21.3	10.6	16.1	15.35	18.2	18.6	8.63	13.8	32.9

RL: Root length, RV: Root volume, PRN: Primary root number, RFW: Root fresh weight, RDW: Root dry weight, SDW: Shoot dry weight, PH: Plant height, BN: Branch number and LA: Leaf area Phenotyping of *Capsicum* genotypes for desirable root characteristics

Shoot growth is greatly impacted by varying container size and root restriction in tomato (Kemble *et al*., 1994) and soybean (Krizek *et al*.,1985). In this study, among the three types of containers, plants grown in bucket type container produced significantly higher amount of shoot biomass compared to remaining two types of containers. Plants in bucket type container produced 57.1% (15.9 g) and 114.2% (23.3 g) higher shoot biomass than plant grown in pot type and PVC pipe containers, respectively (Table 1). Therefore, the bucket type container with higher soil volume and area enabled the *Capsicum* spp. genotypes to express their genetic potential with higher shoot and root growth.

Roots, stems and leaves are functionally interdependent and these three systems maintain a dynamic balance in biomass production and distribution. It is clearly evident from the study that the bucket type container provided enough rooting space for *Capsicum* spp. genotypes to express their genetic potential in terms of shoot and root biomass production. Hence, the bucket type container was chosen for further studies on phenotyping *Capsicum* spp. genotypes for desirable root characteristics.

The importance of plant phenotyping based on specific root characteristics like root length, number of primary roots and root volume are of practical value for crop improvement (Garcia, 2015). Genetic potential of a genotype for root characteristics plays a critical role during growth and metabolic aspects of the plants. In this study, to know the genetic potential and behavior of each genotype under optimal moisture condition *Capsicum* sp. genotypes were evaluated for desirable root characteristics and shoot growth. The results clearly indicated that genotypes, IHR 4501, IHR 4491, IHR 3241, IHR 4550, IHR4517, IHR 3529 exhibited desirable root characteristics such as root length, root volume, primary root number, root fresh and dry weight. The genotypes, IHR 3982 and IHR 3447 showed poor root characteristics (Table 3). Studies have indicated that root length, root volume and root dry weight have strong positive correlation with total dry matter production (Lakshmamma *et al*., 2014). The genotypes which showed higher root length and volume also produced higher biomass because of adequate water and nutrients uptake from deeper layers of the soil and maintained the tissue water potential (Khan *et al*., 2008).

Under ample supply of water and nutrient, the plant height, leaf area, branch number and shoot biomass production are dependent on the size of the root system (Zakaria *et al*., 2020). Our results clearly demonstrated that genotypes, IHR 3241, IHR 4501, IHR 4491, IHR4517 and Guntur Local exhibited better shoot growth in terms of plant height, number of branches, leaf area and shoot biomass. The genotypes, Chikkaballapur Local, IHR 3447 and IHR 3982 showed poor shoot growth (Table 3). In fact; leaf area determines the light interception capacity of a crop and is often used as a surrogate for plant growth and above ground biomass. From the results it is clear that the genotypes having higher leaf area showed better shoot biomass. Concurrently, our results suggested that number of branches in a plant is independent with plant height. The branching pattern in a plant depends on the genetic makeup of each genotype and it is not linked with plant height and other characteristics. Similar observations were made in chilli (Bijalwan *et al*., 2018) and tomato (Malaker *et al*., 2016).

At optimal moisture condition, shoot and root dry weights are interred linked (Brdar-Jokanovic *et al*., 2014). Root to shoot ratio is an important index and it reflects the plant health status. In this regard our results confirms that genotypes, IHR 4550, IHR 4501, IHR 3529 and IHR 4491 recorded significantly higher root to shoot ratio compared to other genotypes. The genotypes, IHR 4108, IHR 3455 and IHR 3226 showed significantly lower root shoot ratio (Table 3).

Though enough rooting space was available in the bucket type container only few genotypes had higher shoot and root growth. This could be due to the genetic potential of the genotypes exhibiting higher root and shoot biomass (Chowdary *et al*., 2015). Based on the growth pattern with respect to root and shoot characteristics, six genotypes, IHR 4517 (*C. annuum*), IHR 3241 (*C. baccatum*), IHR 4491 (*C. baccatum*), IHR 4550 (*C. chinense*), IHR 3529 (*C. chinense*), IHR 4501 (*C. chinense*) were identified having desirable root characteristics and IHR 3447 (*C. annuum*) and IHR 3982 (*C. chacoense*) were identified having poor root characteristics.

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