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Phenotyping Deficit Moisture Stress Tolerance in Tomato Using Image Derived Digital Features

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ABSTRACT

Evaluation of tomato genotypes for the response to water stress using digital features assists in high-throughput phenotyping. A study was conducted on imaging tomato genotypes maintained at field capacity and water deficit stress at flowering stage during 2016 at the plant phenomics facility, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India. Image derived digital features were obtained through image analysis. Physiological parameters, relative water content, water potential and quantum efficiency of PSII were measured at field capacity and water deficit stress conditions. Study established the relationship between physiological functionality and digital features of tomato plants. Transition of plants from field capacity to water stress caused reduction in digital biomass and canopy top area (CTA) among nine tomato genotypes. Deficit moisture stress resulted in lower relative water content, water potential and quantum efficiency of PSII. We observed an apparent relationship between physiological functionality and digital features, convexhull area and compactness. Two genotypes, IIHR 2843 and IIHR 2195 displayed contrasting response under 100% and 50% FC moisture regimes. At 50% FC, the genotypes IIHR 2843 and IIHR 2195 showed lower and higher water use efficiency, respectively. Genotype IIHR 2195 not only continued to sustain biomass production under deficit moisture stress but showed lower water consumption and higher water use efficiency compared to IIHR 2843. Apparent relationship established between physiological functionality and the digital features in this study clearly indicated that the digital features could be employed to capture the response of tomato genotypes to water deficit stress.

KEYWORDS: Digital biomass, genotypes, moisture stress tolerance; phenomics, tomato

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1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown worldwide with production of about 186.82 million tons from 5.05 m ha (Anonymous, 2020). India ranks second next to China with 20.50 million tons from an area of 0.0.81mha (Anonymous, 2020). Tomato is produced under assured irrigation; however in field situations due to limited water availability, crop encounters deficit moisture stress during critical growth phases. The morphological and anatomical characteristics of tomato genotypes associated with drought tolerance have been studied by Maiti et al. (2014) and Dash et al. (2015) worked out water use efficiency based on fruit yield. Under climate change conditions, sustaining tomato production with the available water resources becomes a great challenge. Though cultural adaptations have been identified (Singh et al., 2014) there is need for identification of physiological strategies of novel tomato genotypes (Francesca, 2022). Hence, in the long run there is need for evolving tolerant cultivars for sustainable tomato production.

Tomato genomic resource database with genetic information, micro RNAs, simple sequence repeat and genetic map that integrates genomic and trait information are presently available (Suresh et al., 2014). However, there exists a wide gap between genomic data and phenotypic information. The evaluation and identification of tolerant lines requires thorough phenotyping. Traditional phenotyping approaches require destructive sampling, more labour and time. Whereas, high-throughput phenotyping with machine vision system is an admirable technology to assess the desirable traits. A computational framework has been proposed for image based plant phenotyping to understand the morphological structure and functional developments in plants (Das Choudhury et al., 2019). Image-based shape descriptors for dynamic quantification of drought responses using RGB images help in functional genomics (Duan et al., 2018). Water relations under dynamic environmental conditions could be monitored to provide detailed physiological response profile for each plant from few minutes to the entire growing season (Halperin et al., 2016). Image derived phenotyping data have great potential in understanding functional traits and predicting complex traits like yield, their interaction with environment (vanEeuwijk et al., 2019). In maize, leaf relative water content (RWC) was estimated through visible spectrum reflectance (Zygielbaum et al., 2009). In rice under salt stress conditions, non-invasive phenotyping using infrared imaging showed a negative correlation between the average plant canopy temperature and relative water content, stomatal conductance, photosynthesis performance index and dark-adapted quantum yield (Siddqui et al., 2014). The extracted lines reflecting inclinations of rachises of tomato plants from the whole canopy image correlated well with water potential of tomato plants (Kurata and Yan, 1996) and leaf tip tracking enabled early detection of wilting (Seginer et al., 1992). Chlorophyll fluorescence and multispectral imaging were used to distinguish the type of stress (Wang et al., 2018).

Colour imaging technology is highly efficient in identifying early vigour traits and utilise germplasm to contribute to the genetic improvement (Nyugen et al., 2018). Image-based methods can be enabled for the estimation of biomass accumulation and growth dynamics (Vasseur et al., 2017). Camargo et al. (2014) explained the rosette shape variation and shape descriptors change during time using computer vision in *Arabidopsis thaliana*. Limited studies have been made to characterize water stress response employing some digital features in tomato (Kacira et al., 2002; Kurata and Yan, 1996; Petrozza et al., 2014; Seginer et al., 1992). However, studies establishing relationship between digital features and physiological parameters that depict the functional status have not been attempted. Hence, the present study was aimed to assess the response of tomato genotypes to deficit moisture stress through digital imaging and identify suitable digital features for further high-throughput phenotyping. The study also aimed at assessing performance of two tomato genotypes using digital biomass under two water regimes.

2. MATERIALS AND METHODS

The experiment was conducted during the year 2016 in the plant phenomics facility at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

2.1. Plant material

The tomato cultivars used in the study were sown in portrays filled with coco peat as the growth medium. The seedlings were grown in greenhouse up to 25 days with regular watering and nutrient supply at an average 30°C day and 18°C night temperatures. Subsequently the seedlings were transplanted to 20 L capacity plastic containers filled with 12 kg mixture of soil, farmyard manure (FYM) and sand in the ratio of 2:1:1. In experiment 1, nine tomato genotypes (Table 1) were selected for the study.

In experiment 1, water stress was imposed on nine tomato genotypes with four replications by withholding irrigation for three days at 45 days after transplanting (DAT) which coincided with peak flowering stage of all the genotypes. For experiment 2, two tomato genotypes, IIHR 2195 and IIHR 2843 were chosen based on the distinct response to water stress in the first experiment. The genotypes, IIHR 2195 and IIHR 2843 with three replications, were maintained at two irrigation regimes, 100% Field capacity (FC) and 50% FC from 30 DAT till 60 DAT.

Table 1: Tomato genotypes used in the study

Genotype	Characteristics
Abhinav	Semi determinate, vigorous, firm fruits with good keeping quality, tolerant to Toma-to yellow leaf curl virus (TYLCV)
Arka Meghali	Suitable for rainfed cultivation
IIHR 2190	Water stress tolerant line, green shoulder medium size fruits
IIHR 2195	Water stress tolerant line, resistant to TYLCV, Bacterial wilt and TMV
IIHR 2336	Water stress tolerant line
IIHR 2338	Water stress tolerant line
IIHR 335	Hybrid with tolerance to high temperature
IIHR 2843	Water stress tolerant and wilt resistant line
SH1 (IIHR 2843 ×IIHR 2101)	Tolerant to water stress

2.2. Leaf area

The leaves were separated from the plants and total leaf area was recorded using the Leaf Area Meter, Biovis PSM L2000.

2.3. Chlorophyll fluorescence kinetics

Chlorophyll fluorescence was measured with Modulated chlorophyll fluorometer (OPT1-SCIENCES, OSIp, USA). Leaves were dark adopted for 30 minutes using leaf clips to obtain fully oxidized PSII centres. The ratio of variable fluorescence (Fv) to maximum fluorescence (Fm), given as Fv/Fm, was measured to estimate photochemical efficiency of intact leaves.

2.4. Plant water relations and lipid peroxidation

The relative water content (RWC) of the fully expanded leaf was estimated as per (Lokesha et al., 2019). A portion of the leaf was frozen and thawed for extraction of sap for determination of total osmolyte content using vapour pressure osmometer (Wescor: VAPRO-Vapor Pressure Osmometer, USA). The extent of lipid peroxidation was analyzed by measuring the leaf malondialdehyde (MDA) content by the thiobarbituric acid reaction as described by (Lokesha et al., 2019). Leaf water potential (Ψ_L) of the third fully expanded leaf was determined using pressure bomb apparatus (ARIMAD-3000 MRC) as described by Mamatha, 2014).

2.5. Plant imaging and image analysis

The Plant Phenomics Platform (Scanalyzer 3D, LemnaTec, GmbH, Wuerselen, Germany) set up at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India was used for obtaining the plant images. In experiment 1, plants were imaged initially when the pots were maintained at field capacity. During stress and recovery, imaging was done after withholding irrigation for two days and after 24 hours of re-watering, respectively. In experiment 2, the imaging was done for 30 days at two irrigation regimes. Lemna Grid, a grid based graphical programming environment software developed by LemnaTec, was used for analysing the images and to derive various digital features of the plant. The plant image was parted from the background using nearest neighbour foreground/background colour separation grid. All colours of an image were assigned to either the object colour class or the background colour class to separate them. Each single pixel was assigned to one or the other class by the nearest neighbour rationale in the RGB space. A simplest approach of thresholding was adopted over the image, where the plant portion was extracted by assigning intensity value T (Threshold) so that each pixel is either classified as plant or a background point. Erosion, dilation, opening and closing morphological operations were executed in the binary image to correct the border pixels and remove noise. The resultant image was made into one single object by summing up all the objects within a region of interest. The digital plant features were calculated from this extracted image (Table 2). The area of three view images were used to calculated the digital biomass using the formula:

Digital biomass=$\sqrt{}$Side0°×Side90°×Top

Where, *Side0°* and *Side90°* are the projected areas from side (at 0° and 90° angles) and the *Top* view image area.

Table 2: Digital features employed for assessing response of tomato genotypes to water stress

Canopy top area (CTA)	Plant canopy area calculated using the top view image
Convex hull area	Smallest polygon that encloses the plant perimeter describes the canopy closure of the plant
Compactness	Ratio between the plant area and the convex hull area, gives the information about the canopy density of the plant

2.6. Water use efficiency

Containers were weighed regularly and the amount of water lost was quantified gravimetrically. The complete replenishment of the water lost was made to the containers maintained at 100% FC and only 50% of the water lost was replenished to the containers maintained at 50% FC. The cumulative water consumed by each plant during the course of experimentation was arrived at by adding the daily amount of water applied to each plant. Plants were harvested at 60 DAT and different parts were separated. Subsequently the plant parts were dried in the oven at 80°C for 72 hours. The total dry weight of the plants was recorded. The water use efficiency of two tomato genotypes, IIHR 2195 and IIHR 2843, replicated thrice was quantified in terms of dry matter produced per total amount of water consumed.

2.7. Statistical analysis

All data were analysed using Excel for the mean and standard error, and the charts were prepared using Excel 2010. The data pertaining to physiological parameters were analysed in factorial CRD. The Pearson correlation analysis was done using Excel 2010.

3. RESULTS AND DISCUSSION

Nine tomato genotypes showed variations in digital biomass at field capacity, early stress and recovery. The differences in digital biomass among the genotypes were evident during early stress i.e., on day three after withholding irrigation. In figures 1 and 2, the response of tomato genotypes in terms

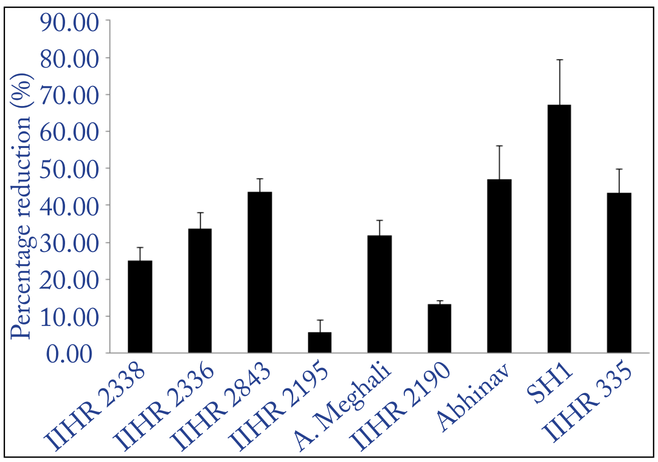

Figure 1: Differences in digital biomass percentage reduction among nine tomato genotypes under deficit water stress. Values are the means±SEM (n=4)

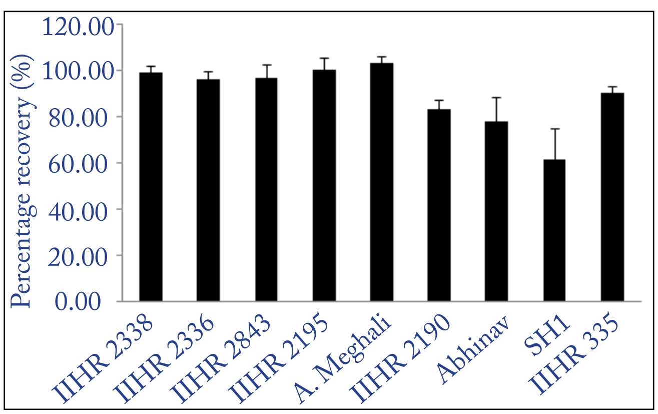

Figure 2: Differences in digital biomass percentage recovery among nine tomato genotypes under deficit water stress. Values are the means±SEM (n=4)

of percentage reduction in digital biomass under water deficit stress condition and percentage recovery, respectively are clearly depicted. The genotypes showed higher digital biomass initially at field capacity and under water stress showed reduction in digital biomass as plants lost higher amount of water. Upon re-watering, the genotypes, SH1, IIHR 335 and Abhinav did not show complete recovery in digital biomass. Among rest of the genotypes, IIHR 2843 and IIHR 2195 exhibited distinct response. The genotype, IIHR 2843 exhibited considerable reduction in digital biomass under deficit water stress. Whereas genotype IIHR 2195 did not show much reduction in digital biomass under deficit water stress condition. However, both the genotypes showed higher recovery upon re-watering.

In visual imaging the quantification of digital biomass is based on the pixels obtained from plants. The development of water stress in plants alters the leaf water status leading to reduction in leaf turgor. Such changes in the leaf water status and plant canopy could be captured employing some of the digital features through visual imaging. The reduced leaf turgor under water deficit condition causes drooping of leaves and the whole leaf area of the plant is not entirely captured during plant imaging process. High-throughput and plant image analysis has been used to dissect vegetative biomass accumulation in response to two different watering regimes (Neumann et al., 2015). Joshi et al. (2021) observed high correlations between quantified plant traits with digitally derived traits like estimated shoot biomass, convex hull area, caliper length and minimum area rectangle in safflower. Their association with drought indices facilitated the classification of diverse genotypes and established the possibility of image-based phenotyping for drought stress tolerance. Due to drooping of leaves the pixels obtained from stressed plants through imaging would be lower compared to the plants grown under field capacity conditions. Petrozza et al. (2014) opined that, though weight of the plant could be correlated with digital biomass nevertheless it is influenced by the projected area in the two-dimensional images. The reduced digital biomass observed in the plants is because of loss in turgor pressure of drought stress-imposed plants. However, they noticed the Megafol, (a mixture of amino acids, glycosides, polysaccharides, organic nitrogen and organic carbon) treated tomato plants exhibited delayed response in terms of reduction in digital biomass. Under water deficit stress condition as compared to untreated plants,Megafol treated plants were able to withstand drought. Hence, in the present study also it was possible to capture the differential response of tomato genotypes to deficit water stress by monitoring percent reduction in digital biomass through visual imaging employed inplant phenomics platform.

Under water stress, along with reduction in digital biomass, considerable reduction in canopy top area (CTA) was also observed in the present study (Figure 3 and plate 1). Though the digital biomass (calculated using area from two sides and one top view of the plant), captures genotypic response to water deficit stress, previous studies have also underlined the importance of canopy top area. Kacira et al. (2002) emphasized on the early detection of plant water stress from top-projected canopy area (TPCA) in New Guinea Impatiens plants before the observance of wiltingsymptoms. Hence, in this study we observed that obtaining only top view images of the plants and assessing reduction in CTA would suffice to capture the response of tomato genotypes to deficit water stress. Further, the relationship between reduction in canopy top convex hull area and amount of water lost was also observed (Figure 4). The lower leaf turgor due to transpirational water loss under deficit water stress results in reduced convex hull area. Hence, greater reduction in convex hull area represents higher water lost in plants under deficit moisture stress conditions. The digital feature, compactness, which depicts canopy density, also exhibited strong relationship with leaf area (Figure 5).

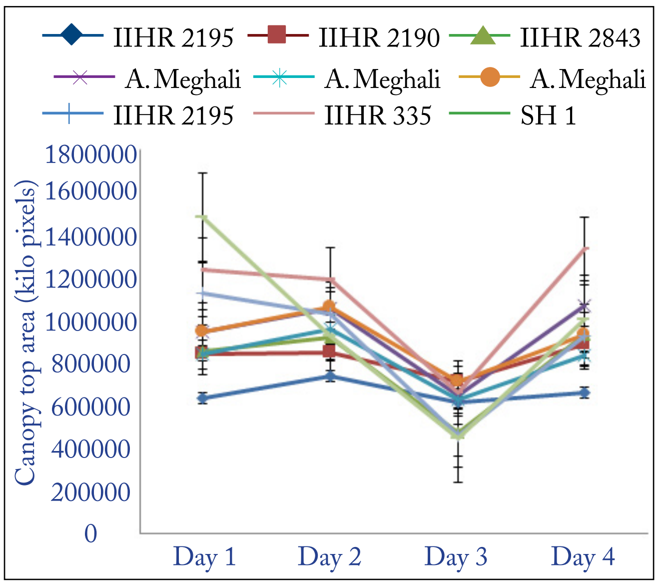

Figure 3: Top view area of tomato genotypes under stress and recovery conditions. Values are the means±SEM (n=4)

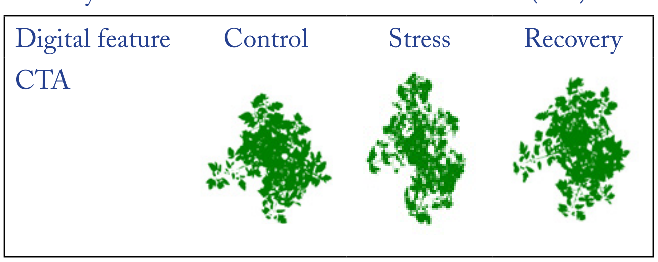

Plate 1: Top view images of tomato genotype IIHR 2843 depicting digital features under control, stress and recovery conditions

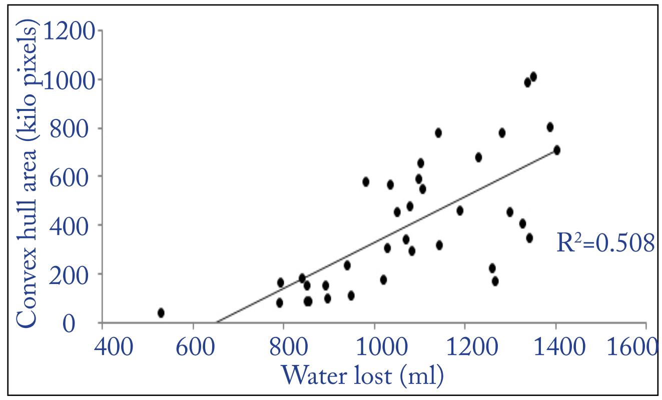

Figure 4: Relationship between convex hull area and water lost in tomato under deficit water stress

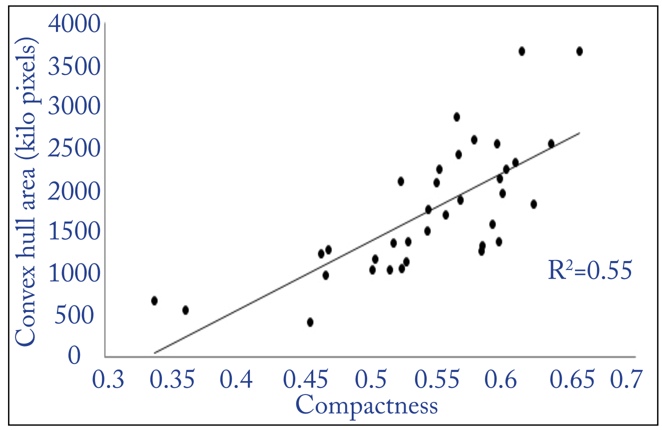

Figure 5: Relationship between leaf area and compactness in tomato under deficit water stress

Quantitative relationship between image-based features and plant biomass accumulation in control and stress treatments was established by Chen et al., 2018. Neilson et al. (2015) suggested that the digital features, convex hull area and compactness can be used as proxies for LAI. In a study on *Arabidopsis*, under deficit moisture stress conditions, Acosta-Gamboa et al. (2017) emphasized the importance of visual image derived features, convex hull area, compactness and projected shoot area.

Further in this study the relationship between digital biomass and other digital features were worked out using Pearson's r method and we observed a positive relationship under control, stress and recovery conditions (Table 5). We further wanted to study the relationship between digital features and few physiological parameters. The image derived digital features are further supported by the physiological parameters. The tomato genotypes showed significant reductions in relative water content, water potential and quantum efficiency of PSII under deficit moisture stress (Table 3). Simultaneously, the increase in total osmolyte and MDA contents were observed in response to moisture stress (Table 4).

Table 3: The variations in relative water content, water potential andquantum efficiency of PSII in nine tomato genotypes under two water regimes

Genotype	Relative water content (%)			Water potential (MPa)			Quantum efficiency of PSII		
	Control	Stress	Mean	Control	Stress	Mean	Control	Stress	Mean
IIHR 2338	86.510	66.952	76.731	1.050	2.050	1.550	0.824	0.755	0.789
IIHR 2336	86.193	66.650	76.422	1.150	1.925	1.538	0.815	0.716	0.766
IIHR 2843	85.423	67.354	76.389	0.900	2.000	1.450	0.837	0.775	0.806
IIHR 2195	89.154	71.104	80.129	0.725	1.750	1.238	0.828	0.68	0.754
A. Meghali	84.618	74.724	79.671	0.900	1.700	1.300	0.825	0.697	0.761
IIHR 2190	86.549	76.586	81.567	0.975	1.625	1.300	0.814	0.704	0.759
Abhinav	87.600	66.446	77.023	1.100	1.700	1.400	0.823	0.651	0.737
SH1	87.505	64.505	76.005	1.200	1.725	1.463	0.818	0.669	0.743
IIHR 335	86.697	66.408	76.552	0.700	2.000	1.350	0.827	0.711	0.769
Mean	86.694	68.970		0.967	1.831		0.823	0.706	
Factors	T	G	T*G	T	G	T*G	T	G	T*G
SEM±	0.97	2.02	2.09	0.058	0.123	0.174	0.006	0.014	0.019
CD (p=0.05)	2.495	5.294	7.486	0.164	0.349	0.493	0.018	0.038	0.054

Table 4: The differences in total osmolyte and MDA contents in nine tomato genotypes under two water regimes

Genotypes	Total osmolytes (mmol kg^{-1})			MDA content (nmol g^{-1})		
	Control	Stress	Mean	Control	Stress	Mean
IIHR 2338	256.00	379.75	317.88	11.26	25.45	18.36
IIHR 2336	244.50	319.25	281.88	10.87	24.10	17.49
IIHR 2843	284.75	350.25	317.50	14.94	26.49	20.72
IIHR 2195	210.00	296.50	253.25	14.19	22.72	18.46
A. Meghali	259.50	348.00	303.75	12.97	26.14	19.56
IIHR 2190	208.50	300.25	254.38	18.55	25.32	21.94
Abhinav	280.25	425.75	353.00	21.77	32.75	27.26
SH1	245.00	455.50	350.25	16.90	32.10	24.50
IIHR 335	231.00	389.75	310.38	11.23	37.03	24.13
Mean	246.61	362.78		14.74	28.01	
Factors	T	G	T*G	T	G	T*G
SEM±	4.564	9.682	13.692	0.515	1.093	1.55
CD (p=0.05)	12.25	25.99	36.76	1.58	3.36	4.75

In this study a good correlation between RWC and digital biomass (r^2=0.50), compactness (r^2=0.58) and CTA (r^2=0.57) was observed during water stress conditions. A positive relationship was also observed between water potential and the digital parameters like convex hull area (r^2=0.73), and CTA (r^2=0.63). Further, the quantum efficiency of PSII had a positive correlation with convex hull area (r^2=0.58), and CTA (r^2=0.55). The studies have shown that the image derived digital traits could be utilized very effectively to represent plant architecture (Knecht et al., 2016). The reductions in aboveground biomass, compactness, caliper length and convex hull area enabled the identification of changes in two common bean cultivars underwater deficit conditions (Padilla-Chacon et al., 2019).Under different water levels in arid land shrub, Escos et al. (2000), observed changes in the plant geometry. In tomato, the lines reflecting inclinations of rachises in the whole canopy image depicted the water potential (Kurata and Yan, 1996). Hence, the image derived digital features could be employed efficiently to assess the plant's physiological functionality in a high-

Table 5: Relationship between digital biomass and other digital features under control, stress and recovery conditions

	Convex hull area	Compactness	CTA
Control	0.94	0.73	0.99
Stress	0.82	0.63	0.80
Recovery	0.87	0.67	0.92

throughput manner.

From the first experiment, among the nine genotypes, two genotypes showing distinct response were selected for further studies. The genotypes, IIHR 2195 and IIHR 2843 showed the least and highest reduction in digital biomass, respectively during stress condition. However, both exhibiting complete recovery after re-watering. Both the genotypes, IIHR 2195 and IIHR 2843 when supplied with 100% FC, though had differences during initial stage, maintained almost equal digital biomass in the later stages, consuming 11.34 L and 12.61 L water, respectively during the course of one month.

However, under 50% FC, the genotype IIHR 2195, exhibited reduced growth as observed in lower digital biomass and moreover consumed less water (4.36 L) compared to IIHR 2843 (7.17 L). The genotypes, IIHR 2195 and IIHR 2843 had almost same water use efficiency at 100% FC, whereas, at 50% FC the genotype, IIHR 2195 exhibited higher water use efficiency compared to IIHR 2843. Under water limiting conditions, the genotype IIHR 2195 not only continued to maintain higher digital biomass but also consumed less water with higher water use efficiency compared to IIHR 2843 (Figure 6). Hence, the genotype, IIHR 2195 showed its uniqueness through its response under water limiting conditions.Joshi et al. (2021) observed the differences in estimated shoot biomass of safflower plants in response to water supply levels. Under drought treatments estimated

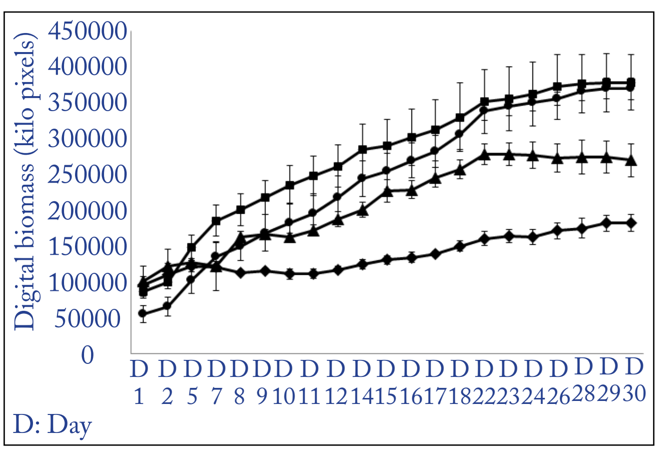

Figure 6: Digital biomass of IIHR 2195 and IIHR 2843 at 100% and 50% FC. Values are the means±SEM (n=3)

shoot biomass was lower as compared to control. The reductions in fresh and dry weights of tomato plants are observed under deficit water stress (Ors et al., 2021) and studies have shown that tomato genotypes exhibit wide variability in drought tolerance (Conti et al., 2019).

4. CONCLUSION

Digital features facilitate non-invasive high-throughput phenotyping of tomato genotypes to deficit water stress. Digital features, digital biomass, convex hull area, compactness and CTA depict the response of tomato genotypes to deficit water stress. They also revealed the plant's functionality due to their significant correlation with physiological parameters. It was evident that the growth performance of contrasting tomato genotypes at different water regimes could be monitored through imaging and quantification of digital biomass non-invasively.

5. FURTHER RESEARCH

The high-throughput phenotyping of diverse tomato genotypes using the identified digital features, digital biomass, convex hull area, compactness and CTA to identify deficit water stress tolerant genotypes is envisaged.

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