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Application of Phenomics to Elucidate the Influence of Rootstocks on Drought Response of Tomato

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Abstract: The cultivation of nutritionally and economically important crops like tomato are often threatened by dry spells due to drought as these crops largely depend on an assured water supply. The magnitude and intensity of drought is predicted to intensify under climate change scenarios, particularly in semi-arid regions, where water is already a scarce resource. Hence, it is imperative to devise strategies to mitigate the adverse effects of drought on tomato through improvement in the plant's efficiency to utilise the moisture in the growth medium. Since the root is the entry point for water, its intrinsic structure and functions play a crucial role in maintaining the soil–water–plant continuum during moisture deficit at the rhizosphere. Grafting offers a great opportunity to replace the root system of the cultivated tomato plants with that of wild species and hence provide a rapid solution to modulate root system architecture in contrast to the time-consuming conventional breeding approach. However, the success in developing the best graft combination of cultivated tomato and rootstock depends on the source of rootstock and selection methods. In this study, we used a high throughput phenomics facility to assess the efficiency of tomato, grafted on the rootstocks of different genetic backgrounds, at different levels of moisture in the soil. Rootstocks included tomato cultivars and the hybrids, derived from the crosses involving wild relatives, as donor parents. Among the rootstocks, an interspecific (*Solanum lycopersicum* × *S. pennellii*) derivative RF4A was highly efficient in terms of productive use of water. The RF4A rootstock-grafted plants were more conservative in water use with higher plant water status through relatively better stomatal regulation and hence were more efficient in generating greater biomass under water stress conditions. These plants could maintain a higher level of PSII efficiency, signifying better photosynthetic efficiency even under water stress. The distinct response of interspecific rootstock, RF4A, to water stress can be ascribed to the effective root system acquired from a wild parent (*S. pennellii*), and hence efficient water uptake. Overall, we demonstrated the efficient use of a phenomics platform and developed a protocol to identify promising rootstock–scion combinations of tomato for optimization of water use.

Keywords: phenomics; *Solanum lycopersicum*; rootstocks; drought; wild species; water use index

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1. Introduction

Tomato is one of the most sought vegetable crops due to its high nutritive value and multiple uses worldwide [1]. As a crop dependent on an assured water supply, it is often vulnerable to water scarcity arising from drought, which is one of the abiotic stresses threatening global food and nutritional security. Especially in water scarce arid and semi-arid regions, the impact of drought on sensitive vegetable crops becomes aggravated due to less productive soil [2]. The water shortage during the drought period has significant implications on the production, leading to a 50% loss in tomatoes [3]. Hence, it is imperative to develop strategies to mitigate the adverse effects of drought on tomato through improvement of the plant's capacity to utilise the moisture in the growth media based on access to water and sensitivity of growth stages of plants. The early growth stage of the tomato is sensitive to drought as it coincides with the establishment of roots in the rhizosphere [4]. The roots acquire water and nutrients for optimal growth and the development of plants; moreover, they anchor plants in the ground [5]. Since it is the entry point for water, the root's intrinsic structure and functions play a crucial role in maintaining soil-water-plant balance during moisture deficit at the rhizosphere [6].

Root systems of tomato vary widely in terms of both architecture and function at intra and inter species levels, and they play a crucial role in coordinating plant responses to different biotic and abiotic stresses [7]. It is apparent from studies that the roots of wild relatives outperform those of cultivated tomato under very harsh environments due to their tolerance to several biotic and abiotic stresses [7–9]. Grafting offers a great opportunity to replace the root system of the cultivated tomato plants with that of wild species and thus provide a rapid solution to modulate root system architecture in contrast to time consuming conventional breeding approaches [10].

In an intensive production system, grafted tomatoes are increasingly becoming a prerequisite for the efficient use of resources [8,11]. A root system architecture that can impart stress tolerance in commercial tomatoes can be effectively and sustainably deployed through surgical and efficient means of grafting [12]. The literature suggests that the substantial improvement in intrinsic tolerance to stress in tomato grafts could be attributed to root traits acquired either directly or as crosses involving wild parents utilized as rootstocks in vegetable plants [2,13]. This was reflected by more efficient water and nutrients uptake due to positively modulated shoots' physiological and biochemical processes.

The selection and development of the line or heterotic F_1 hybrid has been a successful approach for developing rootstocks to address the issues related to using wild species, such as a rootstock that retains beneficial traits in the tomato [14]. The wild species of tomato, including *S. pimpinellifolium* and *S. pennellii* and even others, are tolerant to various abiotic stresses, including drought [15]. Furthermore, these hybrid rootstocks and their derivatives are grafted to F_1 hybrid scions to create a grafted plant with different interacting genomes [16]. In tomato, *S. lycopersicum* × *S. habrochaites* has been the most commonly used combination in developing commercial interspecific hybrid rootstocks. However, there is little information available on the use of other wild species as a donor parent or their derivatives as a rootstock in the public domain [17,18]. Thus, many wild species of tomato provide a wide range of options for graft combinations to improve root system and water use [11,19]. This gap can be bridged through the development of efficient screening tools that can accelerate the screening process.

Phenomics is a facility where plant growth and water use can be closely and precisely monitored and leverages an efficient screening at early crop stage when compared to cumbersome field trials [20,21]. To understand the morphometric changes and functional developments in plants, a computational framework for image-based plant phenotyping has been devised [22]. In other words, phenomics facilitates to decode the genetic information of a plant into phenotypic characters like growth architecture, rates and biomass [23,24]. Image-derived phenotyping of plants offer enormous potential for understanding functional features and estimating intricate traits, like yield, and their interaction with the unfavourable environmental conditions [25]. Throughout the past decade, phenomics has been a highly sought tool to precisely decipher the responses of field crops like wheat, barley, rice, mungbean and safflower for abiotic stresses [26–30]. However, there is little information regarding protocol and screening methodologies in the phenomics conditions for the tomato [31].

In the present study, our hypothesis was that it is possible to differentiate the water stress responses of graft combinations derived from cultivated and wild derivative of tomatoes on the basis of image-derived parameters.

2. Materials and Methods

2.1. Plant Materials

In the present study, four previously screened lines, including one wild species, were continuously selfed to obtain pure inbred lines. The genotypes and wild species like Arka Vikas (AV), Punjab Chhuhara (PC), RF4A (RF) and IIHR-1939 (39) were used alone and or in hybrid rootstocks in the study, as presented in Table 1. The lines Arka Vikas and IIHR-1939, and a derivative line RF4A [32], were sourced from the Indian Institute of Horticultural Research (IIHR), Bengaluru. The variety Punjab Chhuhara was provided by the Punjab Agricultural University (PAU), Ludhiana. The F_1 hybrid rootstocks AV39, PC39 and RF39 were developed at the Central Arid Zone Research Institute (CAZRI), Jodhpur. The ruling commercial hybrid (6242, Syngenta seeds, India) of semi-determinate growth habits was used as a scion and non-grafted control.

Table 1. List of genotypes and species used as rootstock and scion in the study.

Denomination	Species/Cross	Biological Status	Source
	Rootstock		
Arka Vikas (AV)	*Solanum lycopersicum* L.	Variety	IIHR, Bengaluru
P. Chhuhara (PC)	*S. lycopersicum* L.	Variety	PAU, Ludhiana
RF4A (RF)	*S. lycopersicum* × *S. Pennellii*	Derivative line	IIHR, Bengaluru
IIHR-1939 (39)	*S. pimpinellifolium*	Wild species	IIHR, Bengaluru
AV39	Arka Vikas × IIHR-1939	F_1 hybrid	CAZRI, Jodhpur
PC39	P. Chhuhara × IIHR-1939	F_1 hybrid	CAZRI, Jodhpur
RF39	RF4A × IIHR-1939	F_1 hybrid	CAZRI, Jodhpur
	Scion		
6242 (62)	*S. lycopersicum* L.	F_1 hybrid	Syngenta Seeds

2.2. Grafting Conditions

The rootstock and scion seeds were sown in 52 mm cell plug-trays filled with cocopeat:perlite:vermiculite in a ratio of 2:1:1 v/v per portion. At first true leaves opening, tube grafting (splice) was performed on the seedlings. Immediately after grafting, seedlings were placed in a growth chamber where the temperature was 25–27 °C and relative humidity was 85 to 95%. In the growth chamber, relative humidity (95%) with the dark condition was maintained for initial 36 hrs, then light intensity was increased gradually with a slight decrease in relative humidity to 85% for 5–6 days. Grafted seedlings were hardened by shifting them to full light inside the greenhouse for 4–5 days.

2.3. Plant Growth Conditions

In the greenhouse, grafted tomato seedlings were transplanted into plastic pots (12″ diameter) filled with clay loam soil (14 kg). Four pots per graft combination (i.e., four replicates) in each irrigation treatment and a total of 96 pots were arranged randomly in four lanes in the phenomics facility. The soil used for experiments comprised 72% clay, 24% sand and 4% silt; pH 8.4, EC 0.24 dSm^{-1}, organic carbon 6.3 g, nitrogen 85 mg, phosphorus 7.85 mg and potash 70 mg per kg of soil. The pots were maintained in the greenhouse for 20 days after transplanting (DAT) for the proper establishment of grafted seedlings. Then pots were shifted to the plant phenomics facility at the ICAR-National Institute of Abiotic Stress Management, Baramati, for phenotypic study. In the phenomics facility, relative air humidity ranged from 50 to 65% and PAR ranged from 450 to 750 µmol $m^{-2}s^{-2}$, while day/night temperatures were maintained at 32 ± 2/24 ± 2 °C.

2.4. Field Capacity Estimation

Field capacity (FC) and water holding capacity of soil were estimated as per Canavar et al. [33]. Briefly, the dried soil was ground and passed through a 5 mm sieve at ambient conditions. The soil-filled pots were kept in a water-filled flat tub to allow soil to absorb water through drainage holes at the bottom of the pots through capillary movement overnight. The soil saturation up to FC was assessed on the appearance of the uniform wetting of the top layer of the soil as an indicator of completion of capillary action. Then pots were shifted to empty trays to allow a complete drain of excess water. The FC was calculated based on the initial dry and final weights of the pots. The pot weights were recorded every day, and the reduction in pot weight was used to calculate relative water losses. Nearly 80%, 60% and 40% of water at FC were maintained in well-watered (WW), moderate water stress (MWS) and severe water stress (SWS) treatments, respectively.

2.5. Watering and Weighing

It was ensured that all the plants were watered uniformly until they were transferred to the plant phenomics facility. In the phenomics facility, an automated system was used to impose desired soil moisture levels at 80%, 60% and 40% of water at FC by regulating the application of water through gravimetric measurement. The decrease in soil moisture was gradual and the desired levels of moisture stress were obtained only a week after imposing stress. Hence, days after treatment refers to the day + initial 7 days at which difference between well-watered and water stress became conspicuous.

2.6. Image Acquisition

A Scanalyzer 3D imaging system (LemnaTec GmbH, Aachen, Germany) was used to image plants on a regular basis. Plant images were captured from the top view and side views (0° and 90°). High-resolution cameras (piA2400-17 gc CCD cameras, Basler, Ahrensburg, Germany) were used to capture images in the visible range (400–700 nm) of the electromagnetic spectrum. Only images within the field of view of the camera were analysed.

2.7. Image Analysis and Data Mining

Image analysis was carried out with LemnaGrid software (LemnaTec GmbH, Aachen, Germany). A region of interest was defined to acquire the complete plant's image, omitting visible components of the imaging hardware (e.g., lifter/turner), in order to obtain the best segmentation of the plant image. Using a nearest-neighbour colour classification, plants were separated from the backdrop. Before combining all sections classified as a plant into one item, the noise was eliminated by erosion and dilation steps. Data mining software was used to turn the image analysis result into a dataset. For each of the three images captured (top and two side views), we generated data on 14 different parameters. As a result, there were 42 distinct parameters for predicting the biomass.

2.8. Measure of the Actual Shoot and Root Parameters

Plants were harvested 43 days after transplanting (20 days after imposing water stress). The fresh weight of the leaf and the stem were determined on a single-plant basis by harvesting the above-ground shoot manually. A medium-scale balance (Model Ohaus R21PE30) was used for this purpose. Then leaves were separated to measure leaf area with a LI3100c leaf area meter (LICOR Bioscience, Lincoln, NE, USA). The sum of leaf and stem weight was considered as total shoot biomass. The roots from the pots were extracted without disturbing the plants' root ball, washed gently with water and carefully separated from the soil. A dry weight of shoot and roots was recorded after drying the harvested material in a hot air oven at 65 °C till constant tissue weights were obtained.

2.9. Growth Rates and Water Use Index

In order to assess plant growth through the time scale, we applied partial least square regression (PLS), using machine learning algorithms. Actual measured fresh biomass under stress (MWS, SWS) was used as a reference to predict the biomass from all the geometric parameters of each of the images acquired from three different views of the plants. This model was chosen based on the previous phenomics experiments [30].

Growth rates were calculated for the entire experimental period or at different phases of plant growth by using the following formulae, as reported earlier [30]:

Absolute Growth Rate (AGR) [gd^{-1}]

$$AGR_{(t2,t1)} = \frac{PFB_{t2} - PFB_{t1}}{t2 - t1} \quad (1)$$

Relative Growth Rate (RGR) [$gg^{-1}d^{-1}$]

$$RGR_{(t2,t1)} = \frac{\ln(PFB_{t2}) - \ln(PFB_{t1})}{t2 - t1} \quad (2)$$

where 'PFB' refers to predicted fresh biomass, '$t2$' and '$t1$' are the final day and the initial day, respectively, of the time interval for which the growth rate was computed.

Water use index (WUI) mg PFB mL^{-1} was computed using following formulae:

$$WUI\_BM_{t1} = \frac{PFB}{WUCum_{t1}} \quad (3)$$

$$WUI\_AGR_{(t2,t1)} = \frac{AGR_{(t2,t1)}}{WU_{(t2,t1)}} \quad (4)$$

$$WUI\_RGR_{(t2,t1)} = \frac{RGR_{(t2,t1)}}{WU_{(t2,t1)}} \quad (5)$$

where the cumulative 'WU' refers to the total amount of water utilised for both the transpiration and evaporation process during the interval between '$t2$' and '$t1$'. WUCum and PFB refer to the cumulative use of water and predicted fresh biomass on a particular day. AGR and RGR refer to absolute growth and relative growth rates during the interval between $t2$ and $t1$.

2.10. Physiological Parameters

2.10.1. Chlorophyll Fluorescence (PSII)

Nine days after imposing the water stress in phenomics, the quantum efficiency of PSII was measured from the active growing leaf of the plant with an imaging fluorometer (Handy FluorCam, P.S.I., Brno, Czech Republic) using the process described in [34]. Leaf sampling was carried out at around 09:00 h, and then samples were adapted and stabilized for the next 3 h under darkness. Dark-acclimatized and stabilized samples were taken out for recording.

2.10.2. Stomatal Conductance, Relative Water Content and Canopy Temperature

The stomatal conductance (g_s) of the actively growing, fully expanded upper leaves was measured by a leaf porometer (Model SC-1, Decagon Devices, Inc., Pullman, WA, USA) between 10:00 and 11:00 am. Leaf relative water content (RWC) was measured by following a method described by Khare et al. [35]. For each measurement, 12 leaf discs were weighed to determine the fresh weight (FM) and the same were rehydrated in water for 6 h. The turgid leaf discs were surface dried and reweighed to obtain turgid weight (TM). The same discs were oven dried at 80 °C for 24 h to record dry weight (DM). The RWC was calculated by the equation: RWC (%) = [(FM − DM)/(TM − DM)] × 100. The canopy temperature was recorded between 10 h and 12 h by using a thermal imager (Vario CAM hr. inspect 575, Jenoptic, Jena, Germany). The areas of interest (canopy) for analysis were outlined manually while selecting the images for analysis using IRBIS® software (Jenoptic, Jena, Germany).

2.11. Statistical Analysis

The data for growth and physiological traits were analysed using the IBM-SPSS (v. 16) software package. For assessing the treatment and graft combination effects on biomass accumulation and growth-related traits, analysis of variance (ANOVA) was carried out with general linear models (GLMs) using the 'Agricolae' package in R [36]. Data were tested for normality and log-transformed, if necessary, to satisfy the assumptions of the statistical methods. Duncan multiple range test was implemented for a pairwise comparisons of means. Any pair of means annotated with the same letters in graphs indicate the absence of a significant difference at a 95% confidence interval.

3. Results

3.1. Soil Moisture Stress

The imposition of the desired soil moisture stress was possible through automated gravimetric measurements, as evident from the nearly constant soil moisture for all the treatments throughout the experiments in the plant phenomics facility. It took almost a week to stabilize the soil moisture to the desired field capacity. The impact of stress was evident during this period, although it was gradual. Hence, the days after treatment referred to in the subsequent figures indicate the day when the desired moisture levels were stabilized and the day on which water application was stopped. Initially, soil moisture treatments were maintained at 80, 60 and 40% field capacity; however, the plant-weight-adjusted soil moisture levels ranged from 76–77%, 58–59% and 39–40% for well-watered (WW), moderate water stress (MWS) and severe water stress (SWS) treatments, respectively.

3.2. Effect of Treatment on Biomass and Growth Rate

There was continuous increase in the predicted biomass in well-watered and moderate water stress treatments but not in severely water stressed conditions. In well-watered conditions, the increase in biomass was nearly twofold after 11 days of treatment, whereas moderate water stress reduced the biomass accumulation to approximately 50% relative to well-watered plants (Figure 1A). Similarly, the absolute growth rate (AGR) was nearly three- to sevenfold higher in moderately stressed and well-watered plants, respectively, relative to severely stressed plants (Figure 1B). This increase plateaued at six days after stress, and it later decreased in well-watered plants. There was a significant treatment effect on the trends of relative growth rate (RGR), which was seven- and threefold higher in well-watered and moderately stressed plants, respectively, relative to the severely stressed plants at initial phases; however, there was gradual decrease in the difference between the RGR of well-watered and that of moderately stressed plants (Figure 1C).

3.3. Effect of Grafting on Biomass and Growth Rates

The fresh biomass accumulation exhibited significant differences among different graft combinations across well-watered, moderate and severe water stress conditions. Biomass produced in severe water stress conditions was constant among the graft combinations, though it was slightly reduced after nine days of treatment (Figure 2A). However, the water use index per unit biomass was significantly higher in the 62/RF graft combination under severe water conditions (Figure 2B). The AGR revealed significant differences among the graft combinations across well-watered and moderate water stressed conditions; however, this parameter was not effective in differentiating the graft combinations under severe water stress conditions (Supplementary Figure S1). A similar trend was observed in RGR; however, the differences between the graft combinations were more conspicuous in well-watered plants as compared to moderately stressed plants. Furthermore, in moderate water stress, the RGR was higher in graft combination 62/RF than in non-grafted plants at nine days after the initiation of stress (Supplementary Figure S2).

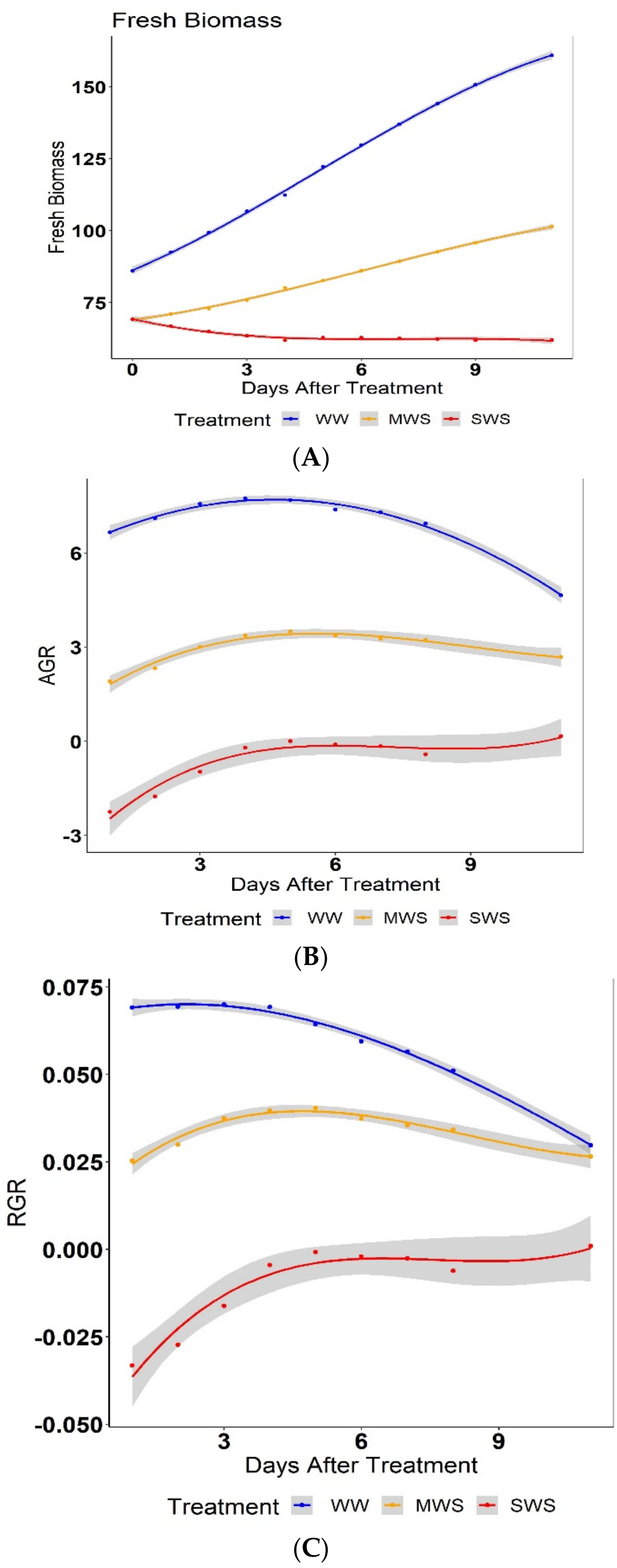
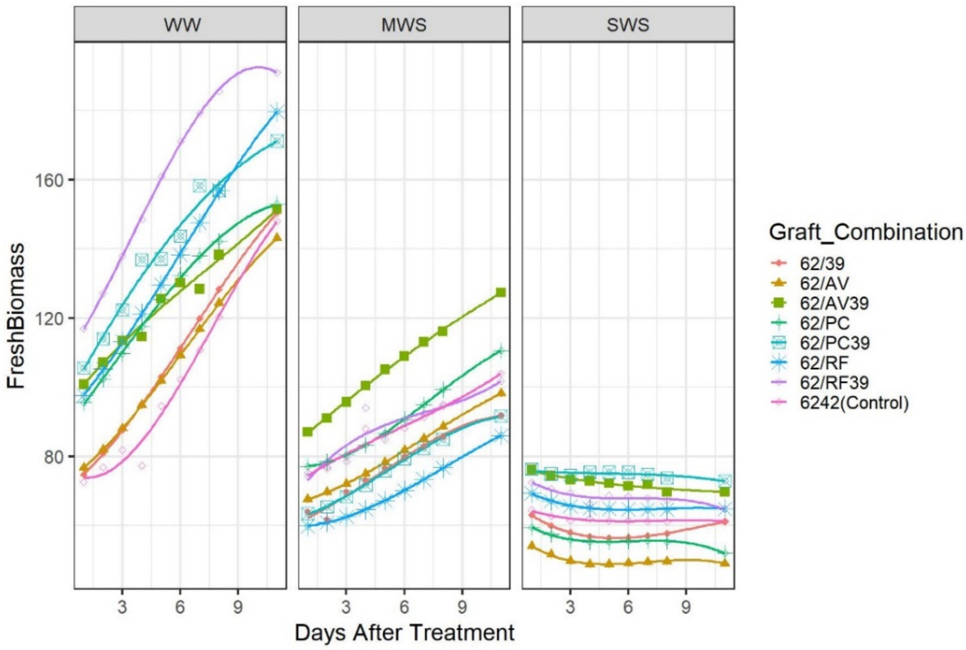
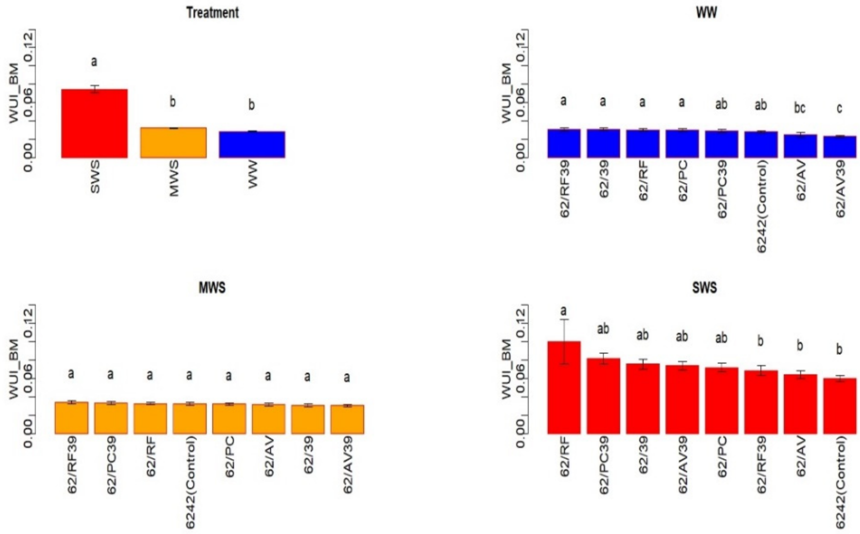


Figure 1. Fresh biomass and growth rates in response to different levels of water stress. (**A**) fresh biomass (g plant^{-1}) was predicted by PLS, based on different image parameters; (**B**) smoothed AGR values (g day^{-1}) and (**C**) smoothed RGR values (g g^{-1}day^{-1}) were obtained from the predicted biomass. The solid line represents the grand average of well-watered conditions (blue), moderate water stressed conditions (orange) and severe water stressed conditions (red). Shaded part along the curve displays CI of 0.95.



(**A**)



(**B**)

Figure 2. Effects of water stress and graft combinations on fresh biomass and WUI_BM. (**A**) Variations in fresh biomass in response to water stress levels represented by an average of four replications in well-watered (WW), moderately water stressed (MWS) and severely water stressed (SWS) plants. (**B**) Each bar in each figure represents the mean values of four replications for WUI_BM in the graft combinations. Letters represent the significance of differences among mean values, as computed by the DMRT at 0.95 CI. Graft combinations with common letters are not significantly different from each other.

3.4. Water Use Index (WUI)

The cumulative water use (WUCum) was sixfold higher in the well-watered treatment than in severely water stressed plants nine days after imposing the water stress treatments (Figure 3A). The water use index (WUI_BM) denoting biomass accumulation per unit of water used was higher in water stressed plants than well-watered plants, and significant differences were observed across the water stress levels (Figure 3B).

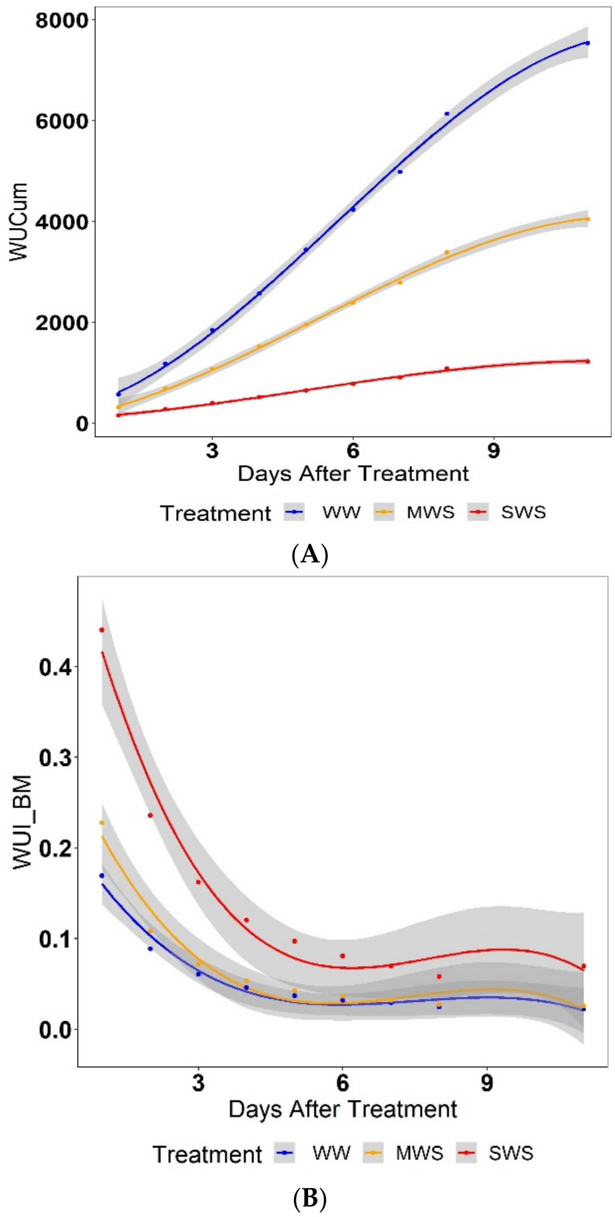


Figure 3. Water use indices at different levels of water stress. (**A**) cumulative water use (WUCum), (**B**) Biomass based water use index (WU_BM) in well-watered (WW), moderate water stress (MWS) and severe water stress (SWS) treatments; WUCum represents the cumulative water use in mL plant^{-1} at particular time of growth, while WUI_BM computed as the predicted biomass per unit of water use (mg mL^{-1}) as presented in material and methods. Solid lines represent the means of all genotypes while the shaded part indicates the CI at 0.95.

3.5. Stomatal Conductance, RWC, Canopy Temperature, and PSII Efficiency (F_v/F_m)

There was a significant difference in stomatal conductance among the graft combinations and 62/RF had the lowest values (Table 2, Figure 4). RWC was higher in 62/RF and 62/RF39, which had lower canopy temperatures relative to other graft combinations. The graft combinations 62/RF, 62/39, 62/PC39 and 62/RF39 recorded low canopy temperature, indicating that the hybrid rootstock and wild species have different mechanisms to maintain canopy cooling under severe water stress (Figure 5). The graft combinations 62/RF and 62/RF39 maintained significantly higher PSII efficiency relative to others (Table 2). Both moderate and severe water stress significantly reduced PSII efficiency.

Table 2. Influence of grafting and water stress levels on stomatal conductance, leaf relative water content (RWC), canopy temperature and F_v/F_m.

	Stomatal Conductance (g_s) (mmol $m^{-2}s^{-1}$)	**RWC (%)**	**Canopy temperature (°C)**	**PSII (F_v/F_m)**
	Graft combination (G)			
62/AV	300.54 ab	84.08 abc	30.91 bc	0.817 abc
62/PC	308.57 ab	84.68 ab	31.18 ab	0.819 abc
62/RF	270.42 b	84.34 abc	30.16 d	0.823 a
62/39	326.85 a	81.95 de	30.44 d	0.814 c
62/AV39	341.65 a	82.43 cd	30.56 cd	0.816 bc
62/PC39	304.51 ab	83.15 bcd	30.34 d	0.820 abc
62/RF39	327.40 a	85.76 a	30.26 d	0.822 ab
6242 (Control)	328.43 a	80.10 e	31.48 a	0.813 c
	Irrigation regime (S)			
WW	411.38 a	89.58 a	29.91 b	0.825 a
MWS	350.02 b	83.80 b	30.18 b	0.820 b
SWS	179.24 c	76.56 c	31.85 a	0.809 c
	Significance			
G	*	***	***	*
S	***	***	***	***
G × S	*	***	***	NS

Each value for the factor G is the mean (n = 9) of three replications of factor S, while for factor S, each value is the mean (n = 18) of three replications of factor G. For each factor (G or S) values within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT. The significance of difference between the means is designated as * for $p \leq 0.05$, *** for $p \leq 0.001$ and NS for no significant difference.

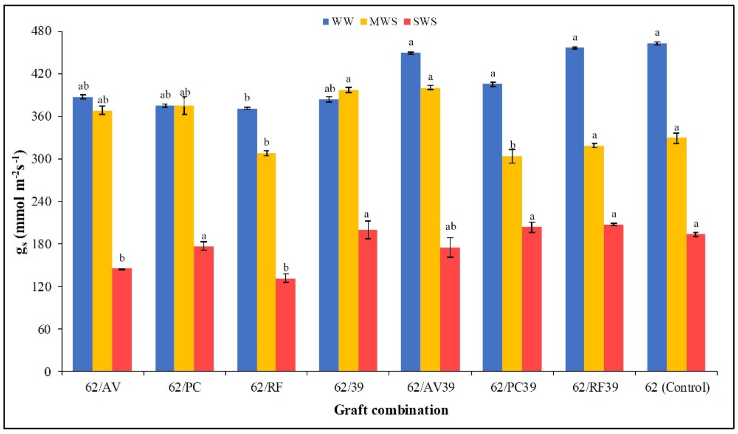


Figure 4. Stomatal conductance (g_s) in graft combinations in different levels of water stress. Values followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

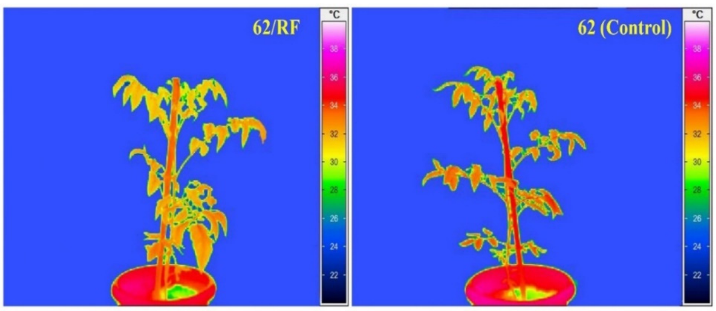


Figure 5. Thermal images of graft 62/RF and control in severe water stress (SWS) conditions.

3.6. Shoot and Root Parameters

Significant differences were observed in graft combinations and water stress levels for the actual shoot and root parameters recorded using the conventional approach 20 days after stress imposition (43 days after transplanting) (Table 3). The fresh and dry biomasses were significantly higher in 62/RF39 than in 62/AV, 62/PC and 62/39 as well as non-grafted plants. The dry biomass of graft combination 62/RF39 was on par with that of 62/RF and 62/AV39. The leaf area and root length of 62/RF was on par with that of the non-graft control; however, they had significantly different root dry mass (Supplementary Figure S3). There was a significant association (R^2 = 0.86) between the fresh and dry biomass of plants under stress conditions (MWS, SWS), as indicated by Supplementary Figure S4)

Table 3. Influence of grafting and water stress on shoot and root parameters (plant^{-1}).

	Fresh Biomass (g)	Dry Biomass (g)	Leaf Area (m^2)	Root Length (cm)	Root Dry Mass (g)
Graft combination (G)					
62/AV	120.22 bc	16.07 bcd	0.14 d	37.06 bc	33.68 b
62/PC	143.61 b	17.78 bcd	0.19 abc	36.67 bc	32.22 c
62/RF	136.49 bc	21.42 ab	0.18 bcd	56.41 ab	36.54 a
62/39	106.44 c	12.33 d	0.15 cd	52.06 ab	34.41 b
62/AV39	154.43 ab	21.81 ab	0.20 ab	51.50 ab	36.58 a
62/PC39	150.49 ab	20.13 bc	0.22 a	44.21 bc	36.62 a
62/RF39	183.05 a	25.85 a	0.23 a	66.43 a	36.47 a
6242 (Control)	105.85 c	15.48 cd	0.17 bcd	48.18 bc	33.80 b
Irrigation regime (S)					
WW	217.13 a	27.95 a	0.30 a	47.77	36.08 a
MWS	128.06 b	19.08 b	0.17 b	49.47	34.97 b
SWS	67.53 c	9.55 c	0.09 c	49.96	34.06 c
Significance					
G	***	***	***	**	***
S	***	***	***	NS	***
G × S	NS	NS	NS	NS	**

Each value for the factor G is mean (n = 9) of three replications of factor S, while for factor S each value is the mean (n = 18) of the three replications of factor G. For each factor (G or S) values within a column followed by the same letter are not significantly different at $p \leq 0.05$, according to DMRT. The significance of difference between the means is designated as ** for $p \leq 0.01$, *** for $p \leq 0.001$ and NS for no significant difference.

4. Discussion

Drought tolerance is a complex polygenic trait, and hence developing a drought tolerant variety of a crop is a highly challenging task, particularly when the magnitude of drought stress is highly influenced by the plant growth stage, duration and severity [2]. With fruit yield and quality as the target during domestication, modern tomato varieties must have lost those traits contributing to abiotic stress tolerance [37–39], though some landraces possess those traits and are tolerant to abiotic stresses [40,41]. However, such traits continue to exist in wild species. Nonetheless, it has previously been successfully demonstrated that tomato grafted on rootstocks of wild species can be resilient to abiotic stresses [9,42,43]. This can be attributed to the fact that plant roots play numerous roles that range from providing support and anchorage to facilitating water and nutrient uptake and nutrient assimilation and transport [6]. However, undesired traits, such as reproductive incompatibility [44], poor germination of seeds and the reduction in stem girth of plants may limit the scope of using wild species directly as rootstock. Hence, instead of simply using wild species as a rootstock, the hybrids or derivatives of wild species can be successfully used as rootstocks to obtain desired results [14,45]. Several reports suggest that drought-induced growth inhibition in tomatoes can be circumvented by grafting [46,47]. Several wild species of tomato exist [44], and very few of them have been evaluated for their potential to contribute to drought tolerance in a plant through graft combination due to constraints in phenotyping protocols. Though robust high throughput phenotyping platforms have demonstrated and assessed the response of large numbers of plants under water stress and interaction of complex traits like yield with environment [25,30]; so far, there are no reports on employing phenomics for differentiating water productivity of graft combinations of tomato. Hence, the present study was planned to optimise phenotyping protocol for identification of the best rootstock in tomato.

The focus of this study was primarily on water productivity as a trait for selecting promising graft combinations in tomatoes. Like many other vegetables, the tomato is grown under assured irrigation, but water saving is one of the major requirements in both protected and open field conditions [1,48]. Water productivity, in the present study, represents the capacity of plants to produce biomass per unit of water applied in a conventional approach. The biomass of a plant is one of the most critical variables that responds to environmental stimuli, such as soil moisture level, and thus is frequently used as a reliable indicator of plant performance [49].

Deviating from the conventional approach, we attempted to assess water productivity based on the growth rates that require frequent measurements of biomass. Since conventional approaches for biomass estimation are invasive and cumbersome, we employed high throughput phenomics and developed a protocol to predict biomass and derive growth rates (Figure 6). There are several reports on an estimation of biomass based on shoot system architecture [27,30,31]. Many of them are very efficient in providing a precise estimation of biomass, particularly when plants are healthy. However, there is a possibility of the low efficiency of prediction models when plants show wilting symptoms under severe soil moisture deficit conditions. The biomass prediction models use geometric features of the shoot. Nevertheless, in the present experiment, the water use index derived from biomass was efficient in differentiating responses of graft combinations to all the levels of soil moisture in contrast to indices derived from AGR and RGR, which were reliable only under sufficient or moderate soil moisture deficit conditions. This can be attributed to the fact that the growth of plants tends to cease under severe soil moisture stress and that the biomass predicted during the previous day was more than the next day, as shoot geometry changed due to the wilting and drooping of leaves, which ultimately resulted in negative values of growth rates. Hence, to interpret water productivity, we focused more on the WUI values derived from biomass even under severe conditions and growth rates of graft combinations under moderate stress.

Protocol for WUI developed through this experiment clearly differentiated the responses of rootstocks to levels of water stress and also helped in assessing the water productivity. It was evident that the grafted plants with rootstock RF4A outperformed non-grafted plants in achieving maximum biomass accumulation even under severe water stress. This could be attributed to a relatively better root system of RF4A, which enabled better uptake of soil moisture and nutrients. Distinctness in root traits of RF4A can be traced back to its original parents (*S. lycopersicum* × *S. pennellii*) that involve a wild relative of tomato, and others have also reported the importance of using wild species in rootstock development [42,50]. The support for the interpretation of better water uptake by RF4A and other rootstocks can be derived from higher relative water content (RWC) in leaves and the lower canopy temperature relative to non-grafted plants. However, it is interesting to note that plants raised on rootstock, RF4A had canopy temperatures similar to those raised on other rootstocks, such as PC39 and RF39. This can be supported from the data on stomatal conductance, which was substantially lower in RF4A, suggesting that it has better mechanisms to save water through stomatal regulation, which was then utilised for more extended periods during severe water stress conditions to accumulate more biomass (Figure 1 and Table 3). A similar water conservation strategy to avoid drought tolerance in tomato plants was observed when *S. habrochaites* introgressed line 'LA3957' was used as a rootstock [51]. It was observed that, in ABA-deficient mutants of tomatoes, stomata can close independently of the leaf water status, suggesting that there is a chemical signal produced by the roots that controls stomatal conductance under water stress [52]. Therefore, it is suggested that an efficient tissue water conservation or dehydration avoidance strategy allowed higher dry biomass and hence relatively higher WUI in RF4A relative to other grafts or non-grafted plants.

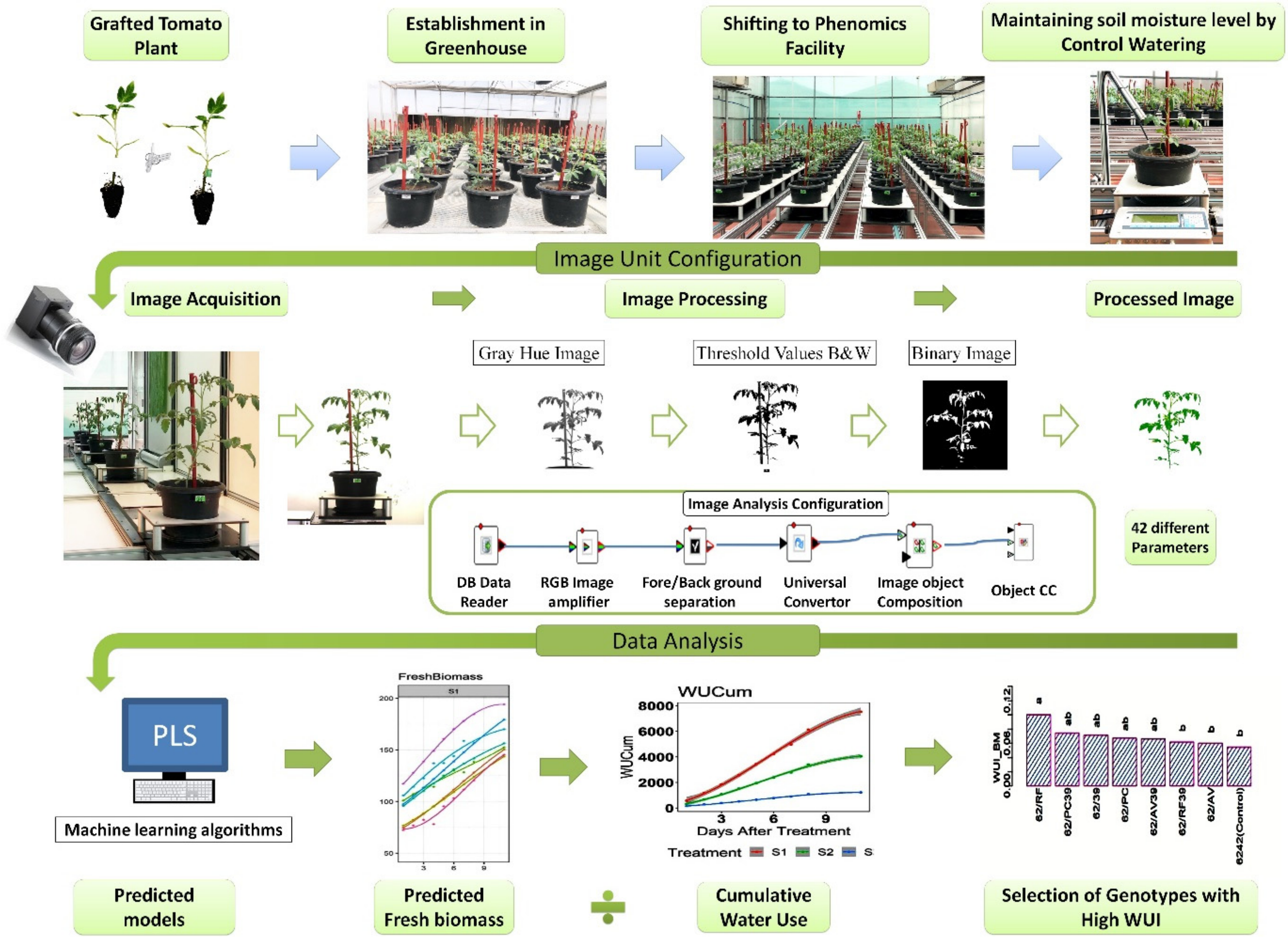


Figure 6. Protocol for phenotyping of grafted tomato plants under different soil moisture regimes in phonemics facility.

As PSII is highly sensitive to drought and its efficiency, as indicated by maximum quantum yield (F_v/F_m), it provides a hint about the health of the photosynthesis system under abiotic stresses such as soil moisture deficit [53,54]. Better tissue water levels facilitated by the efficient water uptake by roots of wild species have been attributed to relatively higher photosystem II efficiency under water stress conditions in *S. pennellii* [50] and *S. pimpinellifolium* [55]. A recent study on the screening of tomato genotypes in high-throughput phenotyping reveals that physiological functioning is directly linked to digital characteristics under water deficit conditions [56]. Several other reports also suggest that selected rootstock-based grafted tomato plants under water stress can maintain better PSII activity [42,57]. However, in our study, the differences in water productivity between tomato grafts and non-grafted plants was not due to a variation in their PSII efficiency as plants were able to retain sufficient water in their leaves even under deficit soil moisture conditions. Though there were significant differences in F_v/F_m between graft combinations, values were above 0.70, which is considered normal [58].

The graft combination 62/RF4A outperformed others because it is a derivative cross from the wild species, consisting of *S. pennellii* as a donor parent in this derivative rootstock (RF4A). Our result is in concurrence with the findings by Gur et al. [50] and Nilsen et al. [42], who reported that tomato grafting on *S. pennellii* rootstock conferred drought tolerance, which is attributed to root-mediated signalling under water stress conditions. The heterotic effect of the hybrid rootstock PC39 and AV39 for WUI could have facilitated grafted plants to acquire water more efficiently as there was an interaction between different genomes and it was also observed by Ryder et al. [16]. However, rootstock (RF4A) outperformed significantly the non-grafted (control) or those grafted on RF39 and AV. In addition to this, some reports suggest that *S. pennellii* exhibits an adaptive mechanism by altering the morphophysiological and anatomical traits under water deficit conditions [38,59]. As reported previously, *S. pennellii* tends to have lower stomatal conductance for preventing leaf water loss as compared to the cultivated tomato [60].

We propose that tolerance to deficit soil moisture in the rootstocks was associated with better physiological functions under optimum leaf RWC, facilitated by lower canopy temperature or the efficient regulations of stomata. Relatively lower canopy temperature in these graft combinations suggested that their root system had better capacity to acquire water and to maintain a relatively better plant–water status even in severe water stress conditions. This was evident from relatively higher dry biomass recorded by roots of 62/RF39 and 62/RF. The higher RWC and lower stomatal conductance in grafted plants on RF4A rootstock might be attributed to a higher root to shoot ratio, indicating that this rootstock had improved the intrinsic water use efficiency of tomato plants. Our results concur with earlier research on tomato grafting by Yao et al. [61]. In fact, RF4A was highly efficient for water use per unit of biomass and growth rates in contrast to hybrid rootstocks, which maintained high leaf RWC and lower canopy temperature by spending more water. Thus, the superiority of graft combination 62/RF4A resulted from rootstock mediated processes, which led to a reduction in stomatal conductance and high leaf RWC. Several experiments have demonstrated that the wild species as a donor parent in rootstock breeding can be highly beneficial for improving vigour and tolerance to abiotic stresses [62–64]. The hybrid vigour in these rootstocks has contributed to an accumulation of higher biomass and efficient conservation of water [42]. *S. pimpinellifolium* was one of the parents in the hybrid rootstock that also favoured the vigorous root and shoot growth in these graft combinations, as has been previously reported [55,64]. The yield advantage in grafted tomato plants has been explored from the interspecific hybrid rootstock *S. lycopersicum* × *S. pennellii* [45]. Our study suggests a more significant advantage of using the *S. lycopersicum* × *S. pennellii* cross combination in contrast to using *S. pimpinellifolium* as a male donor parent or their F_1 hybrid rootstocks under water stress conditions.

The drought tolerance mechanism of *S. Pennellii* is due to low stomatal conductance and less reduction in leaf water loss and, furthermore, the genes involved in the amino acid metabolism, together with genes linked to ET/JA, seem to be key actors in drought tolerance [60]. Moreover, a recent study revealed that RabGAP proteins, such as RabGAP18 or RabGAP22, could be associated with different intracellular vesicular trafficking pathways. The genes that encode them can change their expression, depending on both endogenous and environmental stimuli. A comparative study revealed that the expression of many of the RabGAP genes in roots was significantly lower in *S. pennellii* than in *S. lycopersicum* [65]. The relevance of such interspecies differences for root system architecture and functions under depleting soil moisture remain to be explored, particularly in the context of the relatively efficient stomatal regulations and desiccation avoidance exhibited by RF4A in our study. This may help in establishing the hypothesis that the stomata regulators like ABA may be efficiently operating from root to leaves for the conservation of tissue moisture at a level sufficient to maintain normal physiological functions for a longer period during soil moisture deficits, and this can help in enhancing the water productivity of a tomato grafted on a rootstock derived from wild species.

5. Conclusions

Our study reveals that the water use index developed in our research can differentiate the efficiency of tomato rootstocks in facilitating biomass accumulation under a restricted soil moisture regime. The RF4A-derived rootstock from the cross *S. lycopersicum* × *S. pennellii* effectively contributes to productive use of water through efficient stomatal regulation and a robust root system that facilitated optimum RWC in leaves essential for normal physiological functions even under severe soil moisture stress. For the first time, to the best of our knowledge, we were able to demonstrate the efficient use of a phenomics platform and a protocol to identify promising rootstock–scion combinations of tomatoes for water use optimization.

Supplementary Materials: The following supporting information is available online at https://www.mdpi.com/article/10.3390/agronomy12071529/s1, Figure S1. Effects of water stress and graft combinations on ARG and on WUI_AGR; Figure S2. Effects of water stress and graft combinations on RGR and on WUI_RGR; Figure S3. Roots of rootstock RF4A and 6242 (control) in severe water stress conditions. Figure S4. Significant association between the fresh and dry biomass of plants under stress conditions.

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