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Assessment of Fusarium wilt resistant *Citrullus* sp. rootstocks for yield and quality traits of grafted watermelon

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ABSTRACT

The experiments were conducted to characterize eighteen Fusarium wilt resistant *Citrullus* accessions for their root parameters and to evaluate their performance as rootstocks for vigour, earliness, quality, yield and tolerance to sudden wilt disease of grafted watermelon in comparison with *Cucurbita* and *Lagenaria* rootstocks. Significant variations were observed among the rootstocks for all the root traits under study. The length and diameter of the rootstock hypocotyl were found to be an indicator of the robustness of the root system. A significant influence on vine length and earliness of the grafted crop was observed across different rootstocks. The grafts on *Citrullus* rootstocks could maintain the oblong fruit shape of the scion compared to those onto *Cucurbita* hybrid and *Lagenaria* rootstocks which yielded from flat-globe to spherical shaped fruits. A wide variation of TSS and carotenoid content was observed upon grafting while the pulp pH remained largely unaffected. An increase of average fruit weight by 82.37% and yield per plant by 112.15% in grafts onto RS-18 and RS-10 respectively were recorded. The grafts onto rootstocks *viz.*, RS-10, RS-21 and RS-25 also exhibited reduced severity of sudden wilt; hence are of value in regions prone to both Fusarium wilt and sudden wilt diseases. Overall, the *Citrullus* accessions *viz.,* RS-10, RS-11 and RS-18 were found to be promising in comparison to *Cucurbita* hybrids and *Lagenaria* rootstocks. Grafting onto resistant rootstocks can help in environment-friendly management of Fusarium and sudden wilt diseases in watermelon without compromising the yield and quality.

1. Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is one of the most important cucurbitaceous vegetables of the tropics and subtropics. Globally, about 118.4 million tonnes of fresh watermelon are produced annually, out of which, Asia contributes 83.69% followed by the Americas (5.86%) (FAO, 2017). Due to increasing market demand, watermelon cultivation is spreading to new areas and seasons. With such intensive cultivation, soil-borne pathogens are emerging as the major production constraints. In this context, grafting has emerged as the most popular method against the soil-borne pathogens and nematodes (Crino et al., 2007). Grafting technique is currently being adopted widely in Japan, Korea, China, Taiwan, USA, Spain, Italy and France (FAO, 2009; Lee et al., 2010; Karaca et al., 2012), while it is yet to be attempted on a commercial scale in India. Globally, *Lagenaria* and interspecific *Cucurbita* hybrid rootstocks are mostly used for grafting watermelon (Yetisir and Sari, 2003; Colla et al., 2006), of which the latter was found to be more vigourous with increased fruit weight and total yield (Davis et al., 2008; Miguel et al., 2004; Bigdelo et al., 2017). Grafting onto *Lagenaria* resulted in early flowering (Davis and Perkins-Veazie, 2005) while 40% increase in fruit lycopene content was observed in scion grafted onto *Cucurbita* hybrid (Proietti et al., 2008). Grafting was also employed in watermelon to mitigate abiotic stresses such as drought (Sakata et al., 2007), low temperature (Liu et al., 2004a), flooding (Yetisir et al., 2006), salinity (Colla et al., 2006; Yetisir and Uygur, 2010) and alkalinity (Colla et al., 2010). Further, grafting could also enhance the uptake and utilization of nitrogen (Colla et al., 2011), phosphorus (Zhang et al., 2012) and potassium (Huang et al., 2013) in watermelon.

Among the soil-borne pathogens of watermelon, Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (E.F. Sm.) Synd. & Hans., or *FON* is the most serious disease globally, causing up to 100% yield loss (Callaghan et al., 2016). The fungus is predominantly soil-borne and is difficult to manage, as it produces resilient chlamydospores (Martyn,

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Table 1
List of genotypes used as rootstocks in the current study.

Rootstock ID	Scientific name	Denomination	Biological status	Resistance*
RS-1	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	IIHR-9	Inbred line	*FON* 1 and *FON* 2
RS-2		IC0523048	Inbred line	*FON* 1 and *FON* 2
RS-3		EC794455	Inbred line	*FON* 1 and *FON* 2
RS-4		EC794421	Inbred line	*FON* 1 and *FON* 2
RS-5		EC759804	Inbred line	*FON* 1 and *FON* 2
RS-6		EC794420	Inbred line	*FON* 1 and *FON* 2
RS-7		EC794458	Inbred line	*FON* 1 and *FON* 2
RS-8		EC794461	Inbred line	*FON* 1
RS-9		IIHR-38	Inbred line	*FON* 1
RS-10		EC794460	Inbred line	*FON* 1 and *FON* 2
RS-11		IC0523059	Inbred line	*FON* 1 and *FON* 2
RS-12		IIHR-30	Inbred line	*FON* 1 and *FON* 2
RS-13		EC794429	Inbred line	*FON* 1 and *FON* 2
RS-14		EC797210	Inbred line	*FON* 2
RS-15		IIHR-12	Inbred line	*FON* 1
RS-16		EC797225	Inbred line	*FON* 1
RS-17		EC678822	Inbred line	*FON* 2
RS-18	*Citrullus mucospermus* (Fursa) Fursa	EC677147	Inbred line	*FON* 2
RS-19	*Citrullus amarus* Schrad. × *Citrullus lanatus* (Thunb.) Matsum. & Nakai derived backcross inbred line	BIL-53	Backcross inbred line (BC_1F_7)	Field tolerance to sudden wilt disease
RS-20	*Cucurbita maxima* Duch. cv. Arka Suryamukhi (AS) × *C. moschata* Duch. ex Poir.	AS × Kashi Harit	F_1 hybrid	*FON* 1 and *FON* 2
RS-21		AS × KPS1	F_1 hybrid	*FON* 1 and *FON* 2
RS-22		AS × Suvarna	F_1 hybrid	*FON* 1 and *FON* 2
RS-23	*Cucurbita maxima* Duch.	Arka Suryamukhi	Open-pollinated variety	*FON* 1 and *FON* 2
RS-24	*Lagenaria siceraria* (Mol.) Standl.	Arka Bahar		*FON* 1 and *FON* 2
RS-25		Pusa Samriddhi		*FON* 1 and *FON* 2
Non-grafted control	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Suprit	F_1 hybrid	None

* *FON* 1 and *FON* 2 implies resistance to races 1 and 2, respectively, of *Fusarium oxysporum* f. sp. *niveum*.

2014). To manage this pathogen through non-host resistance, hybrid squash and *Lagenaria* rootstocks were most frequently used for grafting. However, earlier studies reported a reduction in average fruit weight and sugar content upon grafting on *Cucurbita* and *Lagenaria* rootstocks (Davis and Perkins-Veazie, 2005; Rouphael et al., 2010; Ioannou et al., 2002; Liu et al., 2004b, 2006), delayed pulp colour development (López-Galarza et al., 2004; Soteriou et al., 2014) and variable fruit firmness on hybrid squashes (Bruton et al., 2009; Huitron et al., 2009), delayed flowering and reduction in lycopene content on *Lagenaria* rootstocks (Çandır et al., 2013; Yamasaki et al., 1994). Further, the availability of fewer commercial rootstocks (Elazar and Zoran, 2014), graft incompatibility (Andrews and Marquez, 1993) and increased production cost (Djidonou et al., 2013) are the major reasons hindering wide-scale adoption of grafting practice. A Fusarium wilt resistant and compatible *Citrullus* sp. rootstock could eliminate the problems of incompatibility and the detrimental effects on fruit quality. Earlier, *Citrullus* sp. rootstocks were employed in watermelon grafting to mitigate Fusarium wilt (Huh et al., 2002); nematodes (Theis et al., 2015; Garcia-Mendivil et al., 2019) and drought (Parsafar et al., 2019). In this direction, at ICAR-IIHR, we screened a germplasm panel of 360 accessions along with ten *Lagenaria* and five *Cucurbita* hybrids in two bioassays through artificial inoculation of spore suspension of races 1 and 2 of *FON* (unpublished data). Based on their mean survival at 28 days post-inoculation, we identified 18 resistant (survival ≥70%) *Citrullus* sp. accessions. Our main objective in this experiment was to characterize these accessions based on their root parameters and to test these as rootstocks in comparison with *Cucurbita* hybrid and *Lagenaria* rootstocks that would have a desirable impact on fruit yield and quality of grafted watermelon.

Our recent experience suggests that a sudden wilt disease with unknown etiology has emerged as another major production constraint of watermelon in India, especially in the provinces of Karnataka and Andhra Pradesh. The disease is more severe during December to March (winter season) as compared to other seasons. During our earlier varietal trials, we could isolate *Stagonosporopsis cucurbitacearum* (Fr.) Aveskamp, Gruyter & Verkley, from several samples of sudden wilt infected plants and therefore assume that it is a major pathogen of the disease complex. Therefore, another experiment was conducted to test if any of the Fusarium wilt resistant rootstocks were imparting tolerance to this disease.

2. Materials and methods

2.1. Experimental site

The experiments were conducted during late-rainy (September to December), 2018 and winter seasons (December to March), 2018–2019 at the Experimental farm of ICAR-Indian Institute of Horticultural Research, Bengaluru, India. The site is located at 13°07′41.1″N latitude, 77°29′34.1″E longitude and 890 m above mean sea level. The soil in the experimental plot belongs to Alfisol order, has loamy-sand texture and neutral pH (6.7). The available nitrogen, phosphorus and potassium content were 94.1, 44.8 and 175 mg/kg soil, estimated following the standard alkaline permanganate (Subbiah and Asija, 1956); sodium bicarbonate (Olsen et al., 1954) and flame-photometric (Black, 1965) methods respectively.

2.2. Plant materials

Seventeen *Citrullus lanatus*, one *Citrullus mucospermus*, two *Lagenaria*, three *Cucurbita* hybrids (*C. maxima* × *C. moschata* hybrids generated at ICAR-IIHR) and one *Cucurbita maxima* accession were selected as rootstocks. A *Citrullus amarus* derived prebred line (BIL-53), susceptible to Fusarium wilt, but found to be field tolerant to sudden wilt syndrome during our earlier experiments was used as a check rootstock for sudden wilt disease. The list of all the rootstocks, their biological status and reaction to *FON* is presented in Table 1. A commercial watermelon hybrid, Suprit (Known-You Seeds, India) which is susceptible to *FON* races 1 and 2 as well as sudden wilt was selected as the scion for grafting.

2.3. Observations on the root traits of the rootstock accessions

All the 25 rootstocks were studied for various root traits in comparison with that of the scion. Fourteen days old seedlings were planted in PVC pipes (60 cm length and 7.62 cm diameter) filled with coco peat on 27th August 2018 (rainy season). The experiment was laid in a Completely Randomized Design with two replications with four seedlings in each. The plants were maintained in a naturally ventilated polyhouse (NVPH) (28 ± 5 °C) for the next 60 days, after which, the root-ball was carefully removed from the pipes, washed and shade dried to record the root fresh weight (g), root depth (cm), hypocotyl length (cm), hypocotyl diameter (mm) and the number of secondary roots. These observations were recorded on 26th October 2018. The roots were then dried in a hot-air oven at 55 °C for three days for recording root dry-weight (mg).

2.4. Grafting method and agronomic practices

Considering their vigour, the rootstock lines were sown four days later than the scion to match their hypocotyl diameter to ease grafting. Eighteen days old scion and fourteen days old rootstocks were grafted employing splice grafting method for the *Citrullus* and pinhole insertion method for the *Cucurbita* hybrid and *Lagenaria* rootstocks (Lee and Oda, 2003). The protrays were shifted to dark low-tunnels (≥ 90% humidity) immediately after grafting. After three days, the grafts were gradually exposed to lower humidity for six days and thereafter the grafts were shifted to 75% shaded net house. Twelve days after grafting, the grafts were hardened in the NVPH for three days and then the grafted plants were planted in the main field on 4th September 2018 and on 10th December 2018 for the rainy and winter season crop respectively. The experiments were laid in a Randomized Complete Block Design with three replications of ten plants each at a spacing of 2 m × 0.3 m on raised beds. Standard cultural practices were followed to raise a healthy crop except for fungicidal sprays (including tebuconazole, pyraclostrobin and trifloxystrobin which are generally used for managing sudden wilt disease syndrome in watermelon) during the winter season of 2018–2019.

2.5. Observations on plant vigour, fruit quality and yield traits of the grafted plants

The length of the vine of each graft was measured (cm) using a scale after fruit harvest. For determining earliness, the node number at which the first staminate and pistillate flower appeared was recorded for each plant. The number of fruits per plant and yield per plant was recorded as an average of all ten plants in each replication. For quality assessment, five random fruits were selected from each replication. Fruit length and diameter were measured after cutting the fruits longitudinally and the fruit shape index was calculated as a ratio of length and diameter (cm). Rind thickness (cm) was measured at the middle portion of the fruit with a digital vernier caliper. The total soluble solids (TSS) (^{o}B) content was measured using a handheld refractometer (ERMA, Japan). Pulp sampling was done from four different portions of the fruit for estimating total carotenoids (mg/100 g edible portion), pulp pH and pulp colour. Seeds were separated and the pulp was homogenized for 15 s in a mixer (BUCHI Labortechnik, Switzerland) followed by filtration with a cheesecloth. Two-gram fresh sample was taken in 50 mL of 80% acetone was used to estimate total carotenoids following 'Method of Mean' (Biehler et al., 2010) using an UV-1650-PC spectrophotometer (SIMADZU, Japan); pH was measured with pH Tutor (Thermo Scientific) and pulp colour components were measured using Hunter L-a-b colorimeter. These observations were recorded at 90 days after planting i.e. on 3rd December 2018.

2.6. Observation on the severity of sudden wilt disease on the grafted plants

Another experiment was conducted during the winter season of 2018–2019 for field-screening all the grafted plants against sudden wilt disease. The grafted plants were grown in a sick-plot where consistent disease occurrence was observed during the last two years. The severity of sudden wilt was recorded for each of the plants at 85 days after planting i.e. on 5th March 2019. A scale for gummy stem blight (Dos-Santos et al., 2016) was suitably modified and used for scoring in the current study; where 0 – no symptoms, 1 – drooping, wilting and blight of the leaves at the collar region, 2 – drooping, wilting and blight of the leaves up to half of the vine, 3- severe wilt of plant and 4 – complete mortality of the plant. The per cent disease index (PDI) was calculated using the following formula of McKinney (1923):

$$\mathrm{PDI}(\%) = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Further, in this experiment, the performance of the grafted plants concerning to plant vigour, earliness, fruit quality and yield traits have also been recorded at 90 days after planting i.e. on 10th March 2019 (Supplementary Tables 4 and 5).

2.7. Statistical analysis

The data obtained for the root traits and the two graft evaluation trials were analyzed separately using OP-STAT software (Sheoran et al., 1998) (available at http://14.139.232.166/opstat/default.asp). The data for the root traits, vine length and yield per plant observed during the rainy season of 2018 was subjected to principal component, correlation and regression analysis using IBM-SPSS (v. 20) software package.

3. Results

3.1. Root traits of the rootstock accessions

There were significant differences in all the root traits among the rootstocks used in this study (Table 2). The maximum hypocotyl length was recorded in RS-6 and RS-8, while RS-21 possessed maximum hypocotyl diameter. The maximum amount of both fresh and dry weight of the roots was found in RS-20 (8.66 g and 1439.71 mg respectively). The scion cultivar recorded moderate root fresh-weight (2.23 g) and dry weight (435 mg). The *Lagenaria* rootstocks possessed the maximum number of secondary roots per plant (13.00) and root depth (99.78 cm). The scion cultivar had a moderate number of secondary roots (3.5) per plant. The majority of the *Citrullus* sp. rootstocks except for RS-4, RS-8 and RS-10 seemed to be relatively shallow-rooted and had a lower number of secondary roots as compared to the *Cucurbita* hybrids and *Lagenaria* rootstocks.

Principal component analysis of data obtained from six root traits of the rootstocks, and yield of the grafted plants onto them revealed that the first three principal components i.e. root fresh weight, root dry weight and root depth explained 96.26% of total variation (Supplementary Table 1). No significant correlation of yield of the grafted plant with these traits was found. However, a significant correlation was recorded among root fresh weight, root dry weight, root depth, hypocotyl diameter and the number of secondary roots per plant. Further, a significant negative correlation of hypocotyl length with root fresh weight, root dry weight, root depth, hypocotyl diameter, number of secondary roots per plant of the rootstocks and vine length of the grafted plants were observed (Supplementary Table 2). Hence, positive selection for hypocotyl diameter and negative selection for hypocotyl length is recommended for breeding rootstocks of watermelon.

3.2. Plant vigour and earliness traits of the grafted plants

There was a significant improvement in plant vigour, recorded in terms of vine length of grafted plants on different rootstocks. The grafts on eight *Citrullus* sp. and three *Cucurbita* hybrid rootstocks had significantly longer vines compared to the non-grafted control (288.15 cm). Among the rootstocks, RS-21 imparted maximum vine

Table 2
Root parameters of the genotypes (rootstocks and scion).

Rootstock	Root fresh weight (g)	Root dry weight (mg)	Root depth (cm)	Hypocotyl length (cm)	Hypocotyl diameter (mm)	Number of secondary roots
RS-1	2.70 fgh	410.00 ghij	54.28 hijkl	6.68 ij	4.47 def	6.00 defg
RS-2	2.70 fgh	462.50 efghi	51.15 klm	7.98 efgh	4.64 def	6.25 defg
RS-3	2.75 fgh	490.25 efgh	56.28 fghijk	8.50 def	4.38 defg	6.50 def
RS-4	2.57 fgh	470.00 efgh	64.20 e	8.60 de	4.70 de	7.75 cd
RS-5	2.39 fgh	352.18 ijk	48.98 lm	6.20 jkl	3.69 hi	6.00 defg
RS-6	4.38 d	662.50 d	51.80 jklm	12.13 a	4.75 de	5.75 defg
RS-7	1.72 h	310.00 jk	58.05 fgh	10.60 b	3.99 fghi	5.75 defg
RS-8	2.45 fgh	472.55 efgh	60.25 efg	12.10 a	4.40 defg	6.25 defg
RS-9	3.14 efg	400.00 hij	54.65 hijk	8.20 defg	4.09 efghi	4.50 fgh
RS-10	2.73 fgh	461.49 efghi	61.03 ef	9.30 cd	4.70 de	7.25 cde
RS-11	2.73 fgh	432.50 fghi	48.00 m	9.08 cde	4.25 defgh	4.75 fgh
RS-12	2.71 fgh	527.50 ef	51.60 jklm	7.03 hij	4.74 de	6.00 defg
RS-13	3.25 defg	542.50 ef	56.50 fghij	6.28 ijkl	4.79 d	4.25 gh
RS-14	3.04 fg	497.50 efgh	52.30 ijklm	5.43 klm	4.58 def	4.75 fgh
RS-15	3.52 def	512.50 efg	54.50 hijk	7.23 ghij	4.62 def	6.25 defg
RS-16	2.66 fgh	262.50 k	52.13 ijklm	6.70 ij	3.79 ghi	4.75 fgh
RS-17	3.26 defg	450.00 fghi	55.05 ghijk	6.53 ijk	4.60 def	6.00 defg
RS-18	3.51 def	432.50 fghi	52.35 ijklm	4.90 m	4.47 def	7.00 de
RS-19	4.25 de	572.50 de	57.33 fghi	7.20 ghij	4.82 d	5.50 efgh
RS-20	8.66 a	1439.71 a	81.53 cd	3.26 n	7.82 c	10.75 b
RS-21	8.31 a	1363.75 a	90.50 b	3.18 n	9.44 a	10.75 b
RS-22	6.79 bc	1193.64 b	85.68 bc	5.23 lm	8.58 b	10.75 b
RS-23	5.95 c	890.78 c	77.50 d	6.55 ijk	7.69 c	9.25 bc
RS-24	7.03 bc	1168.25 b	84.85 c	7.38 fghi	7.88 c	11.25 ab
RS-25	7.77 ab	1372.75 a	99.78 a	7.95 efgh	8.33 bc	13.00 a
Non-grafted Suprit	2.23 gh	435.00 fghi	48.20 m	9.78 bc	3.45 i	3.50 h
Critical difference ($p \leq 0.05$)	1.16	111.86	5.37	1.18	0.67	2.03
Standard error of mean (±)	0.40	38.19	1.83	0.40	0.23	0.69
Standard error of difference (±)	0.56	54.00	2.59	0.57	0.32	0.98
Coefficient of variation	14.16	8.47	4.22	7.61	6.13	14.11

Values followed by different letters indicate significant ($p \leq 0.05$) differences.

length of the grafted plants (458.70 cm) followed by RS-17 (456.68 cm) (Table 3).

All the graft combinations differed significantly for earliness as indicated by the node number at which the first staminate and pistillate flowers appeared. RS-15 produced staminate flower at the lowest node (4.27). The grafts onto the *Cucurbita* hybrid and *Lagenaria* rootstocks were statistically on par with the non-grafted control for node number bearing first staminate flower (7.53). However, RS-5 possessed pistillate flower at the lowest node (13.30). All the other grafted plants also possessed pistillate flowers on a lower node than that of the control (20.50) except for RS-21, which also recorded the longest vines (458.70 cm).

3.3. Fruit quality traits of the grafted plants

Statistical analysis showed a significant impact of graft combinations on majority of the fruit quality traits under study (Table 3). Maximum fruit shape index was recorded in RS-4 (1.87) as compared to the fruits of the control plants (1.50). Grafts onto rootstocks other than *Citrullus* sp. produced spherical or flat-globe shaped fruits (shape index ranging from 0.87 to 1.03). Besides, the fruits from grafts onto these rootstocks had significantly thicker rinds; RS-22 recorded the maximum (2.09 cm) (Table 3). There was significant variation in the TSS across the graft combinations. Maximum TSS was recorded in RS-21 (11.33°B) followed by RS-1 (10.66°B), RS-17 (10.66°B), RS-8 (10.45°B) and RS-4 (10.44°B). The remaining rootstocks performed statistically on par with that of non-grafted control (8.89°B) (Table 3). Across the graft combinations, all the fruits were slightly acidic as the pulp pH significantly varied from 5.26 (RS-23) to 5.97 (RS-8). The fruits from the grafts onto *C. maxima* and *Lagenaria* rootstocks recorded a slight reduction in pH, although not statistically different from that of non-grafted control (5.50) (Table 3). Statistical analysis confirmed significant impact of the studied graft combinations on total carotenoid content in the fruit-pulp. RS-2 (5.45 mg/100 g) recorded 34.26% increase in total carotenoids over the control. In addition, grafted plants on RS-13 (5.36 mg/100 g), RS-17 (5.21 mg/100 g), RS-21 (5.32 mg/100 g) and RS-22 (5.39 mg/100 g) showed significant improvement in its content when compared to the non-grafted control (4.06 mg/100 g) (Table 3). The flesh colour measured as chroma (c) and hue (h) component varied significantly across the graft combinations while 'L' component remained unaffected by grafting. The maximum value of chroma was recorded in the fruits harvested from grafts onto RS-22 while the minimum value of hue was recorded in those grafted onto RS-2, indicating significant desirable impact of these rootstocks for the development of intense red flesh colour of the fruits. It was evident from the results of correlation analysis that the fruits with higher carotenoid content also had higher value of chroma ($r = 0.972$) and lower hue angle ($r = -0.605$), indicating a highly significant ($p < 0.001$) positive and negative correlation respectively (Supplementary Table 3). Further, regression analysis of chroma and carotenoids was significant ($R^2 = 0.946$, $p < 0.001$).

3.4. Fruit yield and its contributing traits of the grafted plants

As presented in Table 4, the average fruit weight and fruit yield per plant showed statistically significant variation across different graft combinations. We recorded an increase in average fruit weight by 82.37% in grafted plants on RS-18 (6.62 kg). In addition to this, thirteen other *Citrullus* sp. along with RS-20 and RS-21 (*Cucurbita* hybrids) significantly improved this trait over the non-grafted control (3.63 kg). Total fruit yield per plant was also significantly influenced by grafting onto different rootstocks. The grafted plants on RS-10 (7.51 kg) outperformed others with 112.15% increase in yield. In addition, eleven other *Citrullus* sp. rootstocks along with RS-20 and RS-21 (*Cucurbita* hybrids) showed a significant and positive impact on fruit yield per plant. The remaining graft combinations performed statistically on par

Table 3
Performances of the grafting combination of watermelon concerning to plant vigour, earliness, fruit quality traits observed during the rainy season of 2018.

Rootstock	Vine length (cm)	Node number bearing first staminate flower	Node number bearing first pistillate flower	Fruit shape index	Rind thickness (cm)	TSS (°Brix)	Pulp pH	Total carotenoids(mg/100 g)	Pulp colorimetric parameters		
									L	*c*	*h*
RS-1	394.14 bcde	6.81 cdefgh	16.33 defgh	1.79 ab	1.33 cde	10.66 ab	5.50 bcdef	4.40 bcdef	29.60	14.17 bcdefg	41.57 defgh
RS-2	285.68 jk	5.75 fghijk	15.33 fghij	1.62 bc	1.04 g	10.09 abcdef	5.57 bcde	5.45 a	28.77	15.87 a	38.23 j
RS-3	273.95 k	6.72 cdefgh	16.39 cdefgh	1.82 ab	1.50 c	10.07 abcdef	5.51 bcdef	4.03 defgh	31.30	13.30 efgh	43.70 abcdef
RS-4	290.20 jk	5.68 fghijk	15.43 efghij	1.87 a	1.28 def	10.44 abcd	5.62 bcd	4.80 abcde	28.33	14.63 abcdef	42.27 cdefg
RS-5	296.28 ijk	5.45 ghijk	13.30 j	1.51 cd	1.37 cd	10.23 abcde	5.40 cdef	3.75 fghij	28.40	12.10 h	45.70 a
RS-6	295.48 ijk	5.28 hijk	13.64 ij	1.33 d	1.25 def	9.59 bcdef	5.57 bcde	4.91 abcde	28.20	14.70 abcde	41.87 cdefgh
RS-7	304.24 ijk	5.99 defghij	14.89 fghij	1.32 d	1.28 def	9.47 bcdef	5.61 bcd	4.00 defgh	28.43	13.27 efgh	43.20 abcdef
RS-8	367.40 defgh	5.13 hijk	13.58 ij	1.34 d	1.36 cd	10.45 abcd	5.97 a	3.59 fghijk	30.90	12.03 h	44.77 abc
RS-9	372.00 defg	5.80 efghijk	15.57 efghij	1.49 cd	1.14 fg	10.19 abcde	5.56 bcdef	4.19 cdefg	29.43	13.53 cdefgh	42.27 cdefg
RS-10	292.45 jk	5.00 ijk	14.28 ghij	1.42 cd	1.23 def	9.26 bcdef	5.52 bcdef	4.05 defgh	29.03	13.33 efgh	43.87 abcdef
RS-11	383.48 cdef	4.60 jk	13.93 hij	1.47 cd	1.37 cd	9.08 cdef	5.55 bcdef	3.92 efghi	30.50	13.30 efgh	42.57 bcdefg
RS-12	439.30 abc	4.43 jk	15.27 fghij	1.41 cd	1.29 def	8.97 def	5.68 abc	4.89 abcde	29.97	14.70 abcde	39.27 hij
RS-13	309.85 ghijk	4.94 ijk	15.55 efghij	1.46 cd	1.17 efg	8.82 ef	5.50 bcdef	5.36 ab	28.50	15.07 abc	39.93 ghij
RS-14	321.87 fghijk	5.73 fghijk	15.92 defghi	1.50 cd	1.14 fg	8.63 f	5.54 bcdef	3.72 fghij	29.40	12.73 gh	44.37 abcd
RS-15	347.40 efghij	4.27 k	15.20 fghij	1.42 cd	1.19 defg	9.58 bcdef	5.48 cdef	3.79 fghij	27.57	12.73 gh	43.60 abcdef
RS-16	333.10 efghijk	4.89 ijk	14.21 ghij	1.47 cd	1.18 efg	9.83 abcdef	5.79 ab	3.87 efghi	29.67	13.00 fgh	40.93 fghij
RS-17	456.68 ab	5.47 ghijk	16.26 defgh	1.32 d	1.19 defg	10.57 abc	5.62 bcd	5.21 abc	29.10	15.00 abcd	41.40 defgh
RS-18	413.00 abcd	6.53 defghi	14.45 ghij	1.38 d	1.30 cde	9.73 bcdef	5.67 abc	4.23 cdefg	28.23	13.67 cdefgh	42.57 bcdefg
RS-19	382.52 cdef	5.47 ghijk	16.64 cdefg	1.30 d	1.85 b	9.42 bcdef	5.44 cdef	4.38 bcdef	28.43	13.83 cdefg	38.40 ij
RS-20	356.57 defghi	9.23 a	19.60 ab	0.87 ef	2.02 ab	9.37 bcdef	5.45 cdef	4.09 defgh	28.50	13.40 defgh	42.30 efghi
RS-21	458.70 a	8.68 ab	20.50 a	0.94 ef	2.01 ab	11.33 a	5.40 cdef	5.32 ab	29.10	15.50 ab	41.90 cdefgh
RS-22	395.40 bcde	7.09 bcdefg	18.46 abcd	1.00 e	2.09 a	10.12 abcdef	5.49 cdef	5.39 ab	29.57	16.07 a	42.90 abcdefg
RS-23	283.07 k	8.25 abc	17.17 bcdef	1.03 e	1.84 b	7.07 g	5.26 f	4.35 bcdef	28.20	14.00 bcdefg	41.10 fghij
RS-24	305.90 hijk	7.37 bcdef	17.96 abcde	0.77 f	1.89 b	8.77 ef	5.27 ef	5.02 abcd	28.40	15.50 ab	43.00 abcdef
RS-25	293.07 jk	7.50 bcde	18.95 abc	1.00 e	1.92 ab	9.04 def	5.35 def	3.84 efghi	29.10	13.00 fgh	44.20 abcde
Non-grafted Suprit	288.15 jk	7.53 abcd	20.50 a	1.50 cd	1.26 def	8.89 ef	5.50 bcdef	4.06 defgh	28.20	13.53 cdefgh	45.27 ab
Critical difference ($p \le 0.05$)	63.29	1.71	2.59	0.23	0.18	1.51	0.30	0.98	ns	1.66	2.98
Standard error of mean (±)	22.22	0.60	0.91	0.08	0.06	0.53	0.11	0.34	1.80	0.58	1.05
Standard error of difference (±)	31.42	0.85	1.27	0.11	0.09	0.75	0.15	0.485	2.54	0.82	1.48
Coefficient of variation	11.19	16.96	9.76	10.09	7.61	9.57	3.33	14.39	10.88	7.21	4.29

Values followed by different letters indicate significant ($p \le 0.05$) differences; ns: Non-significant.

Table 4
Performances of the grafting combinations of watermelon concerning to yield and its contributing traits during the rainy season of 2018 and severity of sudden wilt disease during the winter season of 2018–2019.

Rootstock	Average fruit weight (kg)	Number of fruits per plant	Fruit yield per plant (kg)	Severity of sudden wilt disease (%)#
RS-1	6.58 a	0.93	6.05 abcde	69.25 (56.30) ijklmnop
RS-2	4.93 cdefg	1.00	4.93 cdefghij	73.75 (59.16) ghijklmno
RS-3	4.81 cdefgh	1.00	4.81 defghij	68.75 (56.03) ijklmnop
RS-4	5.30 bcde	1.08	5.71 bcdefgh	66.75 (54.83) jklmnopq
RS-5	4.37 defghij	1.00	4.36 ghij	66.75 (54.81)jklmnopq
RS-6	5.63 abc	0.87	4.54 fghij	81.50 (64.60) cdefghijk
RS-7	5.17 bcde	1.11	5.70 bcdefgh	73.50 (59.11) ghijklmno
RS-8	4.99 cdefg	1.05	5.26 bcdefghi	89.59 (76.41) bcdefgh
RS-9	5.46 abcd	1.20	6.55 ab	76.25 (61.01) efghijklmn
RS-10	5.47 abcd	1.37	7.51 a	52.50 (46.42) nopqrs
RS-11	5.64 abc	1.19	6.71 ab	93.88 (75.70) bcd
RS-12	5.59 abc	1.14	6.31 abc	90.46 (72.01) bcdefg
RS-13	6.45 ab	0.90	5.82 bcdefg	93.34 (75.12) bcd
RS-14	4.61 cdefghi	1.07	4.85 cdefghij	97.84 (83.98) ab
RS-15	4.13 defghijk	1.13	4.69 defghij	95.00 (77.05) abc
RS-16	5.07 cdef	1.17	5.85 bcdef	88.54 (70.68) bcdefghi
RS-17	4.40 cdefghij	0.85	3.77 j	92.59 (78.66) bcde
RS-18	6.62 a	1.13	7.33 a	91.88 (73.51) bcdef
RS-19	4.95 cdefg	1.08	5.32 bcdefghi	87.09 (68.99) cdefghij
RS-20	5.23 bcde	1.02	5.35 bcdefghi	77.09 (62.71) defghijklm
RS-21	4.95 cdefg	1.21	6.10 abcd	54.58 (47.61) nopqr
RS-22	3.95 efghijkl	1.17	4.66 defghij	79.78 (63.41) cdefghijkl
RS-23	3.87 fghijklm	1.09	4.22 ij	85.85 (68.09) cdefghij
RS-24	4.08 defghijk	1.03	4.24 hij	73.89 (59.31) ghijklmno
RS-25	4.20 defghijk	1.11	4.60 efghij	55.00 (47.87) nopqr
Non-grafted Suprit	3.63 ghijklmn	0.99	3.54 j	100.00 (90.00) a
Critical difference ($p \leq 0.05$)	1.30	ns	1.48	13.81
Standard error of mean (±)	0.48	0.103	0.52	4.71
Standard error of difference (±)	0.68	0.145	0.73	6.67
Coefficient of variation	16.17	0.99	16.85	10.17

Values followed by different letters indicate significant ($p \leq 0.05$) differences; #Values in the parenthesis are angular transformed; ns: Non-significant.

with the non-grafted control (3.54 kg) for fruit yield per plant. However, no significant difference for the number of fruits per plant was observed across different graft combinations (Table 4).

3.5. Severity of sudden wilt on the grafted plants

Statistical analysis confirmed significant impact of graft combination on the severity of sudden wilt disease. The least percent disease index (PDI) was observed in RS-10 (52.5%) while 100% PDI was recorded in non-grafted control (Table 4). Grafts onto the rootstocks *viz*., RS-21 (*Cucurbita* hybrid) (54.58%) and RS-25 (*Lagenaria*) (55.0%) were also found to be more tolerant to sudden wilt as compared to the non-grafted control plants.

4. Discussion

There were significant differences among the rootstocks for the root traits and among the rootstock-scion combinations for all the generative and yield traits under study except for the number of fruits per plant during the rainy season of 2018.

The *Cucurbita* hybrids and the *Lagenaria* accessions were found to be deep-rooted (> 60 cm), possessed a higher number of secondary roots, higher weight of both fresh as well as dry roots when compared to all the *Citrullus* sp. rootstocks. Our results are in line with those reported by Bertucci et al. (2018). The root vigour might be one of the most important reasons for widespread adoption of *Cucurbita* hybrid and *Lagenaria* rootstocks for grafting watermelon. Generally, an increase in the length of the taproot and the number of secondary roots enable the plant to produce a greater yield (Lee et al., 2010). Egel et al. (2008) also recorded a significant improvement in yield with a greater tap-root dominance in direct-seeded watermelon. Miller et al. (2013) recorded significantly longer roots and its density (within 0 − 30 cm depth) in watermelon grafted onto bottle gourd cv. FR-Strong and hybrid squash cv. Chilsung Shintoza.

Among the root traits, root fresh weight, root dry weight and root depth showed greater genetic variation (Supplementary Table 1) and hence rootstock selection based on these traits is recommended. However, phenotyping these traits are cumbersome requiring artificial structures for screening. In this context, we observed a negative significant association of these traits with hypocotyl length and a positive significant association with hypocotyl diameter across the twenty-five rootstocks. Hence, indirect selection for hypocotyl diameter and against hypocotyl length can be carried out instead of the tedious procedure of measuring these traits.

Generally, vigourous rootstocks tend to produce longer vines of the scion. The length of the vines varied widely across the graft combinations. Yetisir and Sari (2003) and Davis et al. (2008) also recorded longer vines imparted by the *Cucurbita* hybrid and *Lagenaria* rootstocks as observed in this experiment. However, the root and shoot vigour imparted by these rootstocks did not translate into higher yield. This is confirmed by a lack of correlation between yield and root parameters. Further, several *Citrullus* sp. rootstocks with lower root fresh and dry weight yielded on par with *Cucurbita* hybrids and *Lagenaria* rootstocks.

In the present study, we found a significant influence of the rootstocks on earliness in terms of the appearance of both staminate and pistillate flower at the lower nodes. These results are similar to Bigdelo et al. (2017). However, there was a non-significant change in earliness in terms of fruit maturity on the grafted plants.

Fruit shape is one of the major consumer appealing traits. Although, watermelon fruit shape (spherical or elongated) is a monogenic trait governed by a gene (Cla011257) located on chromosome 3 (Dou et al., 2018), the rootstocks used in the present investigation had a significant influence on the fruit shape index of the grafted scion. The grafts on rootstocks other than *Citrullus* sp., produced flat-globe to spherical shaped fruits while the non-grafted control produced oblong fruits. Deformation on external fruit shape in grafts onto *Cucurbita* rootstocks were also noted by Edelstein et al. (2014). Further, Alan et al. (2018) recorded significant fruit elongation upon grafting while Alan et al. (2007); Soteriou and Kyriacou (2015) and Fredes et al. (2017) did not notice any changes in the shape of round-fruited watermelon upon grafting.

Rind thickness determines the suitability of the watermelon fruits for long-distance transportation. The significant increase in the rind thickness by RS-3, RS-20 to RS-25 as observed in the present experiment was also observed by Turhan et al. (2012) onto hybrid squash; by Alexopoulos et al. (2007) onto bottle gourd, Early Max, Max-2 and F-14 gourd and by Fredes et al. (2017) onto *C. amarus* rootstocks.

Sweetness is the most valued singular quality trait of watermelon, deciding the consumer acceptability (Kyriacou et al., 2017). In the present study, only five rootstocks could improve the TSS which ranged from 8.63°B to 11.33°B. These findings are in line with the earlier reports of this trait in different watermelon cultivars (Perkins-Veazie et al., 2001; Quek et al., 2007; Proietti et al., 2008). However, a reduction in TSS was reported upon grafting onto *Cucurbita* hybrids (Turhan et al., 2012; Çandır et al., 2013), *Lagenaria* and onto both *C. argyrosperma* and *C. pepo* (Davis and Perkins-Veazie, 2005). Our results also corroborate Fredes et al. (2017), who reported an increase in TSS with *C. amarus* as rootstock. In addition to TSS, acidity balances the sweetness in the taste profile of most of the fruits (Kyriacou et al., 2017). In the current experiment, the pH of the fruit flesh did not vary across graft combinations and only RS-8 could significantly reduce the acidity, while the other rootstocks performed on par with the control.

Carotenoids determine the flesh colour of watermelon (Zhao et al., 2013) and flesh colour is one of the most important traits that strongly influence consumer preference (Kyriacou et al., 2017). Of all the carotenoids, lycopene constitutes the major proportion of red-fleshed watermelon (Zhao et al., 2013; Tamburini et al., 2017) containing almost 40% higher amount of lycopene than tomato (Naz et al., 2014; Soteriou et al., 2014). Grafting watermelon onto *Cucurbita* hybrid has been reported to increase the lycopene content (Turhan et al., 2012; Soteriou et al., 2014 and Kyriacou and Soteriou, 2015). However, in our study, only two out of three *Cucurbita* hybrid and three *Citrullus* sp. rootstocks could significantly improve the total carotenoid content, indicating the rootstock-scion specific effect for this trait. The slight reduction of total carotenoid content observed in grafts on *Lagenaria* in our study was also reported earlier by Çandır et al. (2013). Across the graft combinations, there was a strong positive and negative significant association of carotenoid content with chroma and hue angle respectively, which is in line with Perkins-Veazie et al. (2001).

In the present experiment, we recorded up to 82.37% increase in average fruit weight upon grafting. Soteriou and Kyriacou (2015) also recorded increased mean fruit weight on different *Cucurbita* hybrid rootstocks. Similarly, Bigdelo et al. (2017) recorded 14% increase in average fruit weight of cv. Crimson Sweet grafted onto hybrid *Cucurbita* rootstocks as compared to grafting onto *C. colocynthis* rootstock. However, Alan et al. (2007) did not find significant improvement of average fruit weight in grafts on *Cucurbita* hybrid rootstocks. Alan et al. (2007) and Colla et al. (2006) reported a significant increase in the number of fruits per plant and average fruit weight upon grafting. However, in the present experiment, there was no significant change in the number of fruits per plant across the rootstocks. Yield improvement upon grafting is attributed to synergistic rootstock-scion interaction and by imparting tolerance to biotic and abiotic stresses (Louws et al., 2010; Savvas et al., 2010; Schwarz et al., 2010). In the present study, a maximum yield increase of 112% under optimal growing conditions (rainy season of 2018; Table 2) and 36.59% under sudden wilt stress (winter season of 2018–2019; Supplementary Table 5) was recorded on RS-10. This indicates a potential for deployment of this *Fusarium* wilt resistant *Citrullus* rootstock for grafting on a commercial scale in watermelon.

The agro-climatic condition prevailing during the winter season at Bengaluru is congenial for the occurrence of sudden wilt. Among all the graft combinations evaluated during the winter season of 2018–2019, grafts onto RS-10 (*C. lanatus*), RS-21 (*Cucurbita* hybrid) and RS-25 (*Lagenaria* sp.) recorded significantly reduced disease severity. Hence, these rootstocks may be deployed in regions prone to both Fusarium wilt and sudden wilt diseases. In this regard, Jifon et al. (2008) observed that a rootstock with vigorous root system could resist the sudden collapse of watermelon vines. Similarly,a significant reduction in the severity of sudden wilt has also been reported in melons upon grafting onto *Cucurbita maxima* (Edelstein et al., 1999), hybrid squash (Cohen et al., 2004) and *Cucumis melo* ssp. *agrestis* (Fita et al., 2007).

5. Conclusion

In the present experiment, we observed several *Citrullus* sp. rootstocks which were either better or at least on par with *Cucurbita* hybrid and *Lagenaria* rootstocks for various generative and yield parameters of the grafted plants. Grafting on *Cucurbita* hybrid and *Lagenaria* rootstocks negatively affected the fruit quality parameters *viz*., fruit shape, carotenoid content and TSS. Overall, the *Citrullus* accessions *viz.,* RS-10, RS-11 and RS-18 were found to be promising for yield and quality traits of the grafted plants in comparison to those on *Cucurbita* hybrids and *Lagenaria* rootstocks. Hence, in addition to imparting resistance to Fusarium wilt, grafting watermelon onto these rootstocks can potentially improve productivity without compromising the fruit quality traits. The rootstocks *viz*., RS-10, RS-21 and RS-25 can further be tested and used for watermelon grafting in areas that are prone to both Fusarium and sudden wilt diseases.

Author contributions

ESR, SSH and SS conceptualized the experiment. SP performed the screening and carried out grafting and graft evaluation trials with guidance from SSH, ESR and SS; SP and ESR performed the statistical analysis and wrote the manuscript; MP and VKR made crucial corrections to the manuscript. All authors have read and commented on the manuscript.

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Research involving human participants and/or animals

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Informed consent

Not applicable.

CRediT authorship contribution statement

Saheb Pal: Investigation, Formal analysis, Writing - original draft. **Eguru Sreenivasa Rao:** Conceptualization, Methodology, Supervision, Writing - review & editing. **S. Shankara Hebbar:** Conceptualization, Methodology. **Subbaraman Sriram:** Conceptualization, Methodology. **M. Pitchaimuthu:** Methodology. **V. Keshava Rao:** Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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