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Comprehensive evaluation of abiotic stress tolerance and graft compatibility of *Citrus junos* cv. 'Shuzhen No.1'

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Abstract

Citrus is one of the world's most economically important fruit crops cultivated by grafting. To support the growth of scion cultivars, rootstock is the primary source of resistance to various abiotic stresses. Herein, seedlings of two genotypes of *Citrus junos* Sieb. ex Tanaka (the novel rootstock 'Shuzhen No.1' and commonly used rootstock 'Ziyang Xiangcheng'), as well as three commonly used rootstocks including citrange (*Citrus sinensis* Osbeck. × *Poncirus trifoliata* Raf.), trifoliate orange (*P. trifoliata*), and red tangerine (*Citrus tangerine* Hort. Ex Tanaka), were used as testing materials. The seed characteristics were evaluated, and the rootstock seedlings were subjected to flooding, drought, alkaline, and freezing treatments. Over time, the contents of chlorophyll, soluble sugar, proline, malondialdehyde, and the activity of superoxide dismutase, peroxidase, and catalase in the leaves under different treatments were examined. Furthermore, five citrus varieties were grafted as scions onto one-year-old seedlings from the four rootstocks. Graft success, shoot growth, and leaf greenness were measured and compared. The physiological and biochemical changes in 'Shuzhen No.1' were found to be similar to those in 'Ziyang Xiangcheng'. 'Shuzhen No.1' exhibited greater tolerance to flooding, alkaline, and freezing stress compared to the other four widely used citrus rootstocks, as indicated by physiological and biochemical indexes and principal component analysis. Moreover, the five citrus varieties grafted onto 'Shuzhen No.1' demonstrated vigorous growth and tree vigor. These findings provide valuable insights for the application of 'Shuzhen No.1' and future research on citrus rootstock.

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Introduction

Citrus is the world's most economically important fruit crop, and the majority of citrus is grown in mountainous regions with barren soil[1]. Citrus productivity can be greatly affected by environmental changes, such as brief periods of flooding, drought, or cold[2−4]. Moreover, citrus is susceptible to alkaline soils and exhibits leaf/shoot chlorosis, limiting its geographical distribution[5,6]. Citrus is cultivated through grafting, and rootstock can modify scion architecture and act as the core source of resistance to various stresses, allowing the upper section growth of scion cultivars to thrive[7,8]. With growing interest in perennial crops as valuable components of sustainable agriculture, rootstocks provide an approach for improving and expanding citrus perennial cultivation under various environmental conditions[9].

Generally, rootstock selection and use are mainly determined by compatibility, orchard soil conditions, and local citriculture practice[10]. Germplasm with polyembryonicity, which can develop one or more somatic embryos that are genetically identical to the mother tree, is often selected for citrus rootstock due to genetically uniform rootstocks which can feasibly be prepared solely by sowing seeds[11,12]. Many rootstocks are used in citrus cultivation. Trifoliate orange (*Poncirus trifoliata* (L.) Raf) is widely used in citrus breeding owing to its cold hardiness and disease resistance[13]. However, trifoliate orange is sensitive to alkalinity and mineral deficiency and is incompatible with some citrus cultivars[5,14−16]. Citrange (*Citrus sinensis* × *P. trifoliata*) is drought tolerant yet susceptible to salt and alkalinity[17,18]. Red tangerine (*Citrus reticulata* Blanco) is resistant to B-deficiency and citrus exocortis viroid (CEVd); however, the fruit quality of scion degrades when red tangerine is used as a rootstock[19]. *Citrus junos* Sieb. Ex Tanaka is an iron-deficient, alkaline-, cold- and acid-tolerant citrus rootstock native to southwest China[5,17,20]. Abiotic stresses can alter osmotic equilibrium and induce oxidative stress in plants through excessive generation of reactive oxygen species (ROS)[21,22]. Plants neutralize these ROS through different mechanisms, which can be classified as non-enzymatic and enzymatic antioxidant systems, including antioxidant enzymes superoxide dismutase (SOD), peroxidases (POD), and catalase (CAT)[23−25]. Tolerant species or genotypes exhibit higher antioxidant enzyme activities than sensitive genotypes. Investigating tolerance to different abiotic stresses is critical for identifying the genetic resource for abiotic stress tolerance. Although rootstock can influence the agronomic performance of citrus trees, some widely used rootstocks may still demonstrate graft incompatibility in the orchard[9, 10]. Graft compatibility of intergeneric and intrafamilial species represents a tremendous agronomic potential for genetic improvement and improved crop management by combining unique traits from wild relative rootstocks with commercial citrus scion varieties[7].

In our previous citrus rootstock breeding effort, we reported a novel rootstock cultivar, *C. junos* cv. Shuzhen No.1, with vigorous growth, spherical crown, upright and dense hard branches, cold resistance, and robust adaptation to basic soil conditions[26, 27]. Therefore, this study compared the differences in seed germination characteristics, abiotic tolerance (drought, flooding, alkaline, and freezing), and grafted plant performance between 'Shuzhen No.1' and other common citrus rootstocks. This study determined the polyembryony and seedling emergence traits of five citrus rootstocks, comprehensively evaluated the tolerance responses of different genotypes of citrus rootstock, and provided information on the performances of five citrus varieties on rootstocks. Our findings provide insights into rootstock selection and promote the utilization of the new citrus rootstock 'Shuzhen No.1'.

Materials and methods

Plant materials and culture conditions

Mature fruits of 'Shuzhen No.1' (abbreviated CjSz), trifoliate orange (Pt), red tangerine (Ct), citrange (Cp), and 'Ziyang Xiangcheng' (CjZy) were harvested for collecting seeds from the Citrus Germplasms Repository of Sichuan Province, Chengdu, China. Isolated seeds were surface sterilized using 0.5 M NaOH as described previously[17]. Uniform-sized seedlings were selected and grown in a growth chamber in perlite-filled pots. All seedlings were cultured for approximately six months with normal watering and fertilization.

Determination of polyembryony and seedling emergence traits

Two hundred viable seeds (not replicated) were selected from each rootstock, and the following parameters were assessed: (1) cumulative seedling number, (2) percentage of single seed emergence, and (3) polyembryony. *CitRWP* plays a principal role in regulating somatic embryogenesis in citrus nucellar tissues, and its alleles were divided into two types and polyembryonic alleles with a MITE insertion[11,12]. The absence or presence of the MITE insertion was evaluated with genomic PCR using the following primer set: forward 5'-GTTACTTGGA GACGGCCTAACG-3' and reverse 5'-TCGATCATGTAATGCTGACT C-3'[11].

Stress treatments

Abiotic stress treatments included flooding (roots submerged in water with only stem and leaves exposed to the environment for 6 and 7 weeks), drought (20% soil water content for 1 and 2 weeks), alkalinity (watering distilled water with pH 8.0 and 9.0 for 8 weeks), and freezing (–10 °C for 1 and 2 h) were performed. Three biological replicates (five seedlings per replicate) were set randomly for each treatment. All leaves were sampled from five rootstocks, frozen in liquid nitrogen, and stored at –80 °C.

Determination of physiological and biochemical indexes

The total chlorophyll and carotenoid contents were measured using the method reported by Lichtenthaler & Buschmann[28]. Fresh leaves (1 g) were ground in a freezing mortar and pestle with 10 mL of 80% acetone. Following filtering, the pigment solution's optical density (OD) was measured at 470, 645, and 663 nm to determine carotenoid, chlorophyll (Chl) *a*, Chl *b*, and total Chl content, respectively. The assessed photosynthetic pigments were presented in mg/g fresh weight (FW). Antioxidant enzyme activities of SOD (EC 1.15.1.1), guaiacol peroxidase (POD, EC 1.11.1.7), and CAT (EC 1.11.1.6) were determined as previously described[29]. Malondialdehyde (MDA) content was measured using the thiobarbituric acid (TBA) method[30]. Soluble sugars and proteins were analyzed as previously described[31]. To minimize the differences between different genotypes, the data were expressed as ratios relative to the values of control groups.

Performances of different graft combinations

'Chunjian' (*C. reticulata* × (*C. reticulata* × *C. sinensis*)), 'Buzhihuo' (*C. unshiu* × *C. sinensis*), 'Mingrijian' ((*C. unshiu* × *C. hassaku*) × *C. sinensis*), 'Dafen' (*C. unshiu*), and 'Tarocco' (*C. sinensis*) were grafted onto four rootstocks, including CjSz, CjZy, Ct, and Pt. Ninety seedlings were cultured for 1 month with normal watering and fertilization, and their survival rates were measured on March 30th, 2019. Tree growth and leaf greenness were assessed in 5–10 grafted trees in each cultivar. Stem thicknesses below and above the graft joint were measured using a vernier caliper on October 30th, 2020. Shoot length and longitudinal and horizontal growth of trees were recorded using a tape measure from summer shoots on October 30th, 2020. Ten mature leaves from summer shoots were selected from each tree to measure soil-plant analysis development (SPAD) with a SPAD-502 chlorophyll meter.

Statistical analysis

Microsoft Excel was used to prepare the collected data. Significant differences between grafted combinations were analyzed using Tukey's method, and Pearson correlation and principal component analyses were performed using SPSS 20.0 software. All figures were drawn using GraphPad Prism (v. 7.04).

Results

Polyembryony and seedling emergence traits among tested rootstocks

Compared with CjZy, CjSz has a spherical crown and upright and dense hard branches (Fig. 1a, b). CjSz and CjZy had solitary flowers (Fig. 1c, d). However, the lateral petals of CjSz are purple from bud to bloom, with a flower diameter of 2.0 cm (Fig. 1c). The fruits of both CjSz and CjZy were orange in color at maturity (Fig. 1c, d) and both were polyembryonic (Fig. 1c, d). Citrus rootstock seeds germinated 40–45 d after seeding and stopped germinating 95–100 d later (Fig. 1e). CjSz had the greatest emergence rate and polyembryonic ratio among the tested rootstocks, reaching 160.77% and 70.69%, respectively (Fig. 1e, f). The germination potential was 25.50%, which was lower than that of trifoliate orange (29.00%) but higher than that of CjZy, citrange, and red tangerine (Fig. 1e). According to statistics on single seed emergences, CjSz had the most single seed emergences (two, up to 44.83%), slightly lower than CjZy (46.76%) but higher than Pt, Ct, and Cp. The proportion of CjSz was highest among the five tested rootstocks, at 18.72% (three seedlings), 4.39% (four seedlings), 1.48% (five seedlings), and 0.49% (six seedlings). Additionally, the maximum number of seedlings per grain was seven (Fig. 1f). These results are

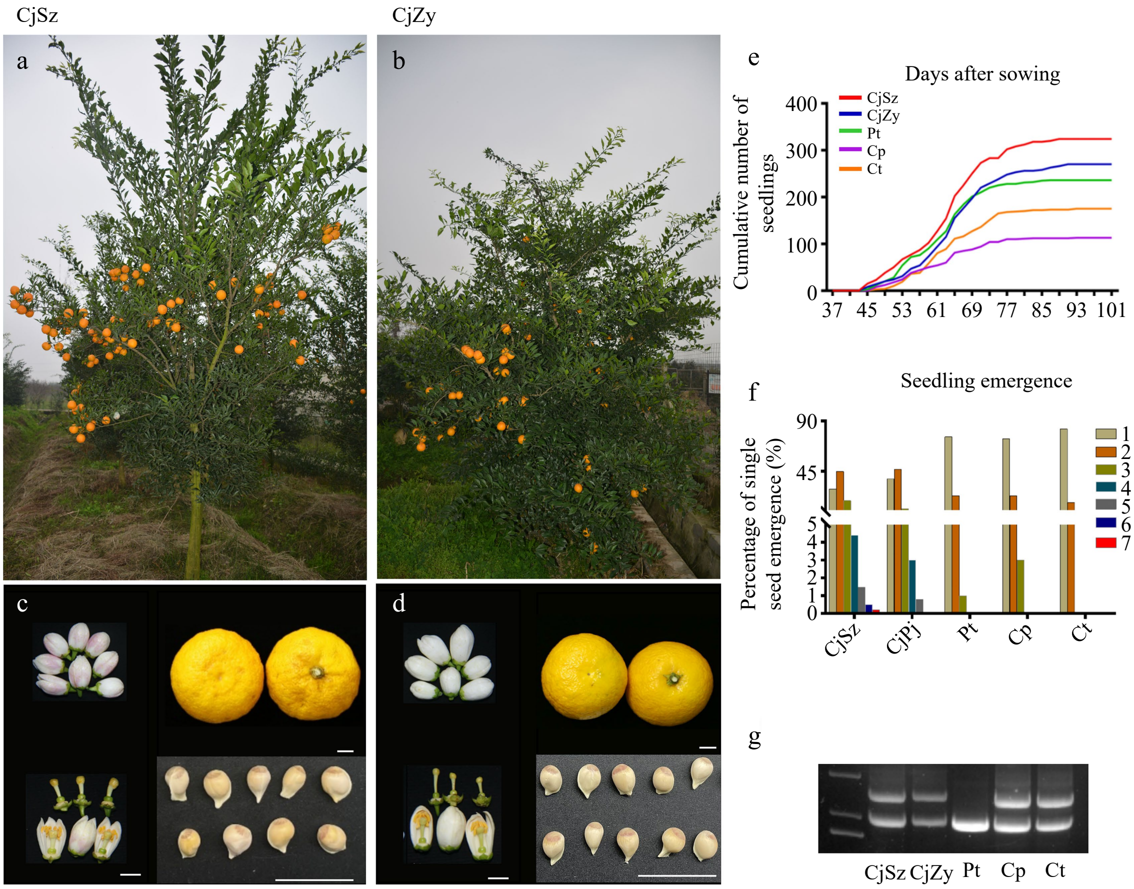

Fig. 1 Comparison of morphology between two genotypes of *Citrus junos*. (a), (b) Six year old trees; (c), (d) flowers, fruits and seeds; (e) cumulative number of seedlings; (f) percentage of single seed emergence; (g) MITE insertion in five rootstock germplasms. CjSz: Shuzhen No.1 (*Citrus. junos* Sieb. Tanaka); CjZy: Ziyang Xiangcheng (*C. junos* Sieb. Tanaka); Cp: citrange (*C. sinensis* Osbeck. × *Poncirus trifoliate* Raf.); Pt: trifoliate orange (*P. trifoliate* [L.] Raf) and Ct: Red tangerine (*C. tangerine* Hort. Ex Tanaka). Scale bars = 1 cm.

consistent with the MITE insertion detection results (Fig. 1g). Apomixis in citrus is sporophytic and highly stable across commercial varieties. *Citrus junos* fruits were densely seeded, with most of the seeds being plump and polyembryonic, which can generate large numbers of uniform rootstocks from seeds[12,31].

Leaf photosynthetic pigments under abiotic stresses

All genotypes developed leaf chlorosis at the end of the abiotic stress treatments (Fig. 2). Almost all treatments reduced the content of leaf photosynthetic pigments in all rootstocks (Table 1). Comparing the pigment content data among the different rootstocks, it is evident that CjSz demonstrated better adaptability to alkaline and freezing stresses (Table 1, Fig. 2). Specifically, under alkaline treatment with a pH of 8.0, CjSz exhibited the smallest decrease in leaf photosynthetic pigments content. In the case of alkaline treatment with a pH of 9.0, CjSz displayed similar levels of leaf chlorina compared to Cp, followed by CjZy, Pt and Ct. Similarly, under freezing treatment for 1 h, the ratios of Chl *a*, total Chl and total carotenoids in CjSz were higher than in other rootstocks, although the differences were not statistically significant. More specifically, among the different treatments, CjSz experienced the greatest decrease in Chl *a*, Chl *b*, and total Chl under 2 weeks of drought treatment, with ratios of 0.48, 0.64, and 0.53, respectively. Conversely, CjSz demonstrated the least decrease under freezing treatment for 1 h, with ratios of 0.96, 1.05, and 0.99, respectively (Table 1). The total carotenoid content in CjSz experienced the most significant decrease after 2 weeks of drought treatment, while the least decrease occurred under alkaline treatment with a pH of 8.0 (Table 1).

MDA, soluble protein, and soluble sugar contents and SOD, POD, and CAT activities under abiotic stresses

The levels of MDA, and the activities of SOD, POD, and CAT were significantly influenced by abiotic stresses (Table 2). CAT activity decreased under flooding, alkaline, and freezing stress, but slightly increased under drought stress. MDA levels and SOD and POD activities increased under flooding, drought, alkaline, and freezing stresses in all citrus rootstock genotypes (Table 2). However, CAT activity decreased under abiotic stress. Among the genotypes, CjSz exhibited the highest increase in MDA levels during 7 weeks of flooding treatment, and the lowest increase during 1 h of freezing treatment. SOD activity in CjSz showed the greatest increase after 7 weeks of flooding treatment and the smallest increase after alkaline stress treatment at pH 8.0. CjSz had the highest POD activity ratio of 1.62 under freezing stress, and the lowest value of 1.25 under 2 weeks of drought treatment. In comparison to the other four rootstocks, CjSz had the highest SOD ratio value (1.71) under 7

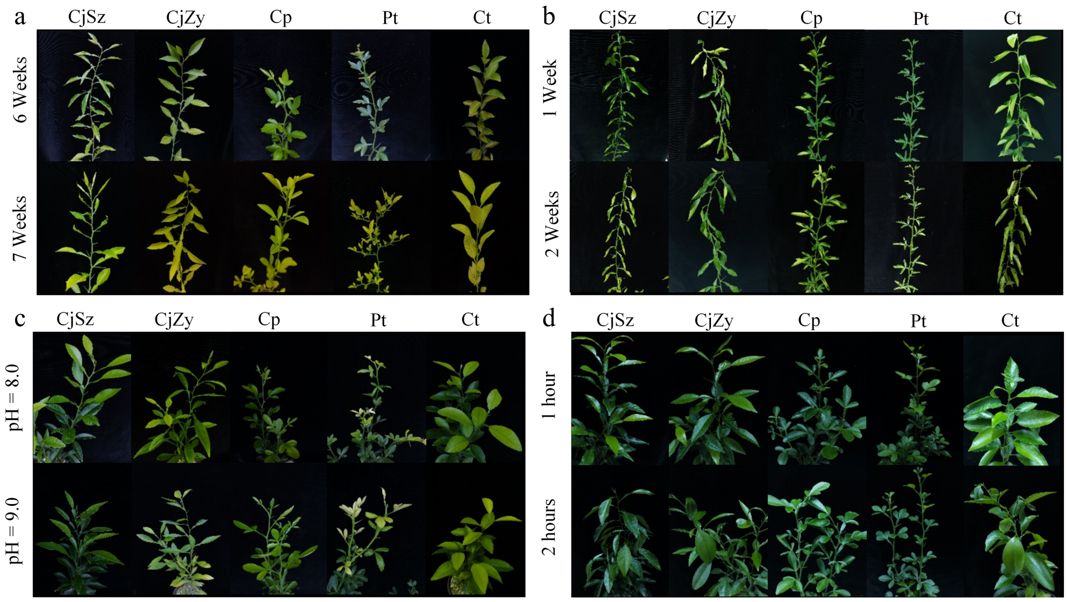

Fig. 2 Growth state of five rootstocks under abiotic stresses. (a) Flooding stress; (b) drought stress; (c) alkaline stress (pH = 8.0 and pH = 9.0); (d) freezing stress.

Table 1. Statistics for chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid compared with controls.

	Species	Flooding stress		Drought stress		Alkaline stress		Freezing stress	
		6 weeks	7 weeks	1 week	2 weeks	pH = 8.0	pH = 9.0	1 h	2 h
Chl a	CjSz	0.72 ± 0.12a	0.60 ± 0.04a	0.53 ± 0.08a	0.48 ± 0.15a	0.86 ± 0.26a	0.74 ± 0.35ab	0.96 ± 0.24a	0.83 ± 0.11a
	CjZy	0.67 ± 0.22a	0.68 ± 0.09a	0.59 ± 0.16a	0.45 ± 0.22a	0.79 ± 0.14a	0.69 ± 0.19ab	0.85 ± 0.17a	0.79 ± 0.19a
	Cp	0.77 ± 0.26a	0.69 ± 0.26a	0.66 ± 0.28a	0.61 ± 0.16a	0.83 ± 0.26a	0.77 ± 0.20a	0.88 ± 0.12a	0.85 ± 0.18a
	Pt	0.63 ± 0.06ab	0.63 ± 0.19a	0.63 ± 0.03a	0.60 ± 0.08a	0.72 ± 0.03ab	0.68 ± 0.07ab	0.91 ± 0.20a	0.88 ± 0.05a
	Ct	0.44 ± 0.10b	0.38 ± 0.09b	0.28 ± 0.05b	0.23 ± 0.07b	0.48 ± 0.04b	0.47 ± 0.07b	0.92 ± 0.06a	0.69 ± 0.15a
Chl b	CjSz	0.78 ± 0.12a	0.70 ± 0.08a	0.67 ± 0.14a	0.64 ± 0.15a	0.87 ± 0.26a	0.77 ± 0.31a	1.05 ± 0.22a	1.00 ± 0.12a
	CjZy	0.70 ± 0.20a	0.65 ± 0.09a	0.72 ± 0.09a	0.52 ± 0.20a	0.76 ± 0.11a	0.74 ± 0.13a	0.86 ± 0.11ab	0.92 ± 0.17ab
	Cp	0.76 ± 0.24a	0.68 ± 0.25a	0.71 ± 0.25a	0.68 ± 0.15a	0.85 ± 0.18a	0.78 ± 0.16a	0.82 ± 0.09b	0.83 ± 0.11b
	Pt	0.71 ± 0.13a	0.66 ± 0.20a	0.66 ± 0.05a	0.68 ± 0.11a	0.69 ± 0.09ab	0.66 ± 0.08ab	0.86 ± 0.19ab	0.87 ± 0.11ab
	Ct	0.47 ± 0.09b	0.50 ± 0.12a	0.39 ± 0.04b	0.33 ± 0.06b	0.51 ± 0.04b	0.47 ± 0.05b	1.00 ± 0.10ab	0.64 ± 0.12c
Total Chl	CjSz	0.74 ± 0.12a	0.63 ± 0.05ab	0.57 ± 0.10a	0.53 ± 0.15a	0.86 ± 0.26a	0.75 ± 0.33a	0.99 ± 0.23a	0.89 ± 0.12a
	CjZy	0.68 ± 0.21a	0.67 ± 0.09a	0.63 ± 0.14a	0.47 ± 0.21a	0.78 ± 0.13a	0.71 ± 0.17ab	0.85 ± 0.15a	0.84 ± 0.19ab
	Cp	0.76 ± 0.25a	0.68 ± 0.26a	0.67 ± 0.27a	0.63 ± 0.16a	0.84 ± 0.23a	0.77 ± 0.19a	0.86 ± 0.11a	0.84 ± 0.15ab
	Pt	0.65 ± 0.08ab	0.64 ± 0.19a	0.63 ± 0.03a	0.63 ± 0.09a	0.71 ± 0.05ab	0.67 ± 0.07ab	0.89 ± 0.19a	0.87 ± 0.07a
	Ct	0.45 ± 0.10b	0.42 ± 0.10b	0.32 ± 0.04b	0.27 ± 0.07b	0.49 ± 0.03b	0.47 ± 0.06b	0.95 ± 0.08a	0.67 ± 0.14b
Total Car	CjSz	0.83 ± 0.12a	0.65 ± 0.10a	0.68 ± 0.11a	0.55 ± 0.18ab	0.96 ± 0.30a	0.71 ± 0.27a	0.96 ± 0.20a	0.95 ± 0.13a
	CjZy	0.62 ± 0.15bc	0.65 ± 0.08a	0.56 ± 0.14a	0.51 ± 0.25ab	0.68 ± 0.11bc	0.69 ± 0.12ab	0.77 ± 0.14a	0.76 ± 0.12bc
	Cp	0.76 ± 0.24ab	0.67 ± 0.23a	0.63 ± 0.19a	0.69 ± 0.13a	0.79 ± 0.15ab	0.75 ± 0.17a	0.92 ± 0.06a	0.86 ± 0.14ab
	Pt	0.69 ± 0.08ab	0.62 ± 0.16ab	0.64 ± 0.04a	0.65 ± 0.09a	0.73 ± 0.04b	0.63 ± 0.05ab	0.91 ± 0.23a	0.95 ± 0.05a
	Ct	0.45 ± 0.12c	0.43 ± 0.13b	0.38 ± 0.05b	0.37 ± 0.10b	0.47 ± 0.03c	0.49 ± 0.05b	0.89 ± 0.14a	0.67 ± 0.14c

Note: Chl: chlorophyll, Car: carotenoids. Data shown in the table are expressed as ratios relative to the values obtained on control seedlings. Three biological replicates (five seedlings per replicate) were set randomly for each treatment. Significance was tested for indicators of different rootstocks in the same treatment, and different lowercase letters indicate significant differences at $p < 0.05$.

weeks of flooding stress. Additionally, CjSz exhibited the highest POD ratio value under alkaline (1.37 and 1.46) and freezing stresses (1.62). Under drought stress, CjSz had significantly lower MDA and POD ratio values than the other four rootstocks.

The regulation of soluble protein and sugar contents in response to abiotic stressors varied among the citrus rootstock genotypes (Table 3). Compared with other rootstocks, CjSz had the lowest ratio of soluble proteins during 7 weeks of flooding stress, but the highest ratio under drought, alkaline, and freezing stresses. CjSz exhibited the greatest increase in soluble sugars under 7 weeks of flooding stress and 1 week of drought stress, whereas other rootstocks showed moderate increases. Specifically, the overall ratio of soluble proteins in CjSz was the highest (2.16) under 2 h of freezing stress and the lowest (1.10) during 7 weeks of flooding stress. However, the ratio of soluble sugars displayed opposite trends.

Comprehensive evaluation of rootstocks under abiotic stresses

To provide a comprehensive assessment of the tolerance of different rootstocks, we conducted a principal component analysis to calculate various physiological and biochemical

Table 2. Mean comparison of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD).

	Species	Flooding stress		Drought stress		Alkaline stress		Freezing stress	
		6 weeks	7 weeks	1 week	2 weeks	pH = 8.0	pH = 9.0	1 h	2 h
MDA	CjSz	1.42 ± 0.30bc	2.02 ± 0.16ab	1.07 ± 0.11d	1.90 ± 0.26b	1.11 ± 0.11b	1.50 ± 0.13c	1.02 ± 0.22b	1.24 ± 0.39b
	CjZy	1.45 ± 0.17bc	1.81 ± 0.30b	1.79 ± 0.22b	2.10 ± 0.14a	1.47 ± 0.30ab	1.75 ± 0.28bc	1.02 ± 0.13b	1.15 ± 0.27b
	Cp	1.67 ± 0.20ab	1.84 ± 0.41b	1.31 ± 0.23cd	2.25 ± 0.61a	1.33 ± 0.39ab	1.36 ± 0.20c	1.49 ± 0.29a	1.92 ± 0.63a
	Pt	1.26 ± 0.16c	1.48 ± 0.34b	1.45 ± 0.31bc	2.03 ± 0.43a	1.84 ± 0.40a	2.41 ± 0.42a	0.91 ± 0.34b	1.01 ± 0.54b
	Ct	1.75 ± 0.25a	2.42 ± 0.66a	2.30 ± 0.36a	2.29 ± 0.37a	1.36 ± 0.10ab	1.92 ± 0.36b	1.00 ± 0.17b	1.46 ± 0.26ab
SOD	CjSz	1.60 ± 0.26a	1.71 ± 0.03a	1.53 ± 0.02ab	1.63 ± 0.11a	1.25 ± 0.08a	1.31 ± 0.15ab	1.31 ± 0.06ab	1.35 ± 0.16a
	CjZy	1.65 ± 0.10a	1.70 ± 0.07a	1.62 ± 0.07a	1.71 ± 0.06a	1.31 ± 0.29a	1.36 ± 0.29a	1.12 ± 0.27b	1.54 ± 0.21a
	Cp	1.25 ± 0.18c	1.19 ± 0.18b	1.18 ± 0.16c	1.15 ± 0.15b	1.12 ± 0.05ab	1.26 ± 0.02ab	1.25 ± 0.17ab	1.05 ± 0.23b
	Pt	1.56 ± 0.10ab	1.50 ± 0.46ab	1.69 ± 0.16a	1.51 ± 0.28a	0.94 ± 0.30b	0.94 ± 0.39c	1.39 ± 0.19a	1.56 ± 0.11a
	Ct	1.36 ± 0.10bc	1.61 ± 0.18a	1.35 ± 0.17bc	1.23 ± 0.18b	1.08 ± 0.07ab	1.04 ± 0.09bc	1.18 ± 0.24ab	1.07 ± 0.08b
POD	CjSz	1.40 ± 0.31a	1.50 ± 0.10ab	1.25 ± 0.38c	1.45 ± 0.61b	1.37 ± 0.49a	1.46 ± 0.22a	1.62 ± 0.32ab	1.62 ± 0.37a
	CjZy	1.61 ± 0.33a	1.62 ± 0.38ab	2.23 ± 0.68ab	2.69 ± 0.72a	1.29 ± 0.24a	1.38 ± 0.54a	1.25 ± 0.66b	1.55 ± 0.59a
	Cp	1.45 ± 0.16a	2.16 ± 0.86a	2.59 ± 1.09a	2.90 ± 0.70a	1.27 ± 0.28a	1.31 ± 0.32a	1.28 ± 0.44b	1.46 ± 0.49a
	Pt	0.85 ± 0.17b	1.25 ± 0.21b	1.40 ± 0.41bc	1.57 ± 0.50b	0.83 ± 0.22b	1.17 ± 0.32a	1.85 ± 0.28a	1.83 ± 0.23a
	Ct	1.00 ± 0.26b	1.99 ± 0.65a	2.64 ± 0.67a	2.25 ± 1.20ab	0.80 ± 0.24b	1.06 ± 0.32a	1.21 ± 0.21b	1.25 ± 0.48a
CAT	CjSz	0.98 ± 0.20ab	0.77 ± 0.10a	1.14 ± 0.14ab	1.00 ± 0.10ab	0.91 ± 0.30a	0.67 ± 0.09c	0.87 ± 0.14a	0.67 ± 0.10c
	CjZy	0.74 ± 0.03b	0.91 ± 0.09a	1.52 ± 0.28a	0.93 ± 0.08b	0.57 ± 0.17a	1.08 ± 0.17b	0.71 ± 0.09ab	1.56 ± 0.24a
	Cp	0.90 ± 0.06ab	0.79 ± 0.18a	0.91 ± 0.04b	1.08 ± 0.14ab	0.70 ± 0.14a	1.59 ± 0.10a	0.90 ± 0.08a	0.61 ± 0.03c
	Pt	1.08 ± 0.11a	0.72 ± 0.02a	0.47 ± 0.06c	1.28 ± 0.08a	1.04 ± 0.14a	0.84 ± 0.15bc	0.53 ± 0.14b	0.85 ± 0.10bc
	Ct	0.77 ± 0.10 ab	0.66 ± 0.12 a	1.33 ± 0.44 a	1.15 ± 0.15 ab	0.79 ± 0.11 a	0.88 ± 0.11 bc	0.70 ± 0.13 ab	1.09 ± 0.19 b

Note: Data showed in the table are expressed as ratios relative to the values obtained on control seedling. Different lowercase letters indicate significant differences at $p < 0.05$.

Table 3. Mean comparison of soluble proteins and soluble sugars.

	Species	Flooding stress		Drought stress		Alkaline stress		Freezing stress	
		6 weeks	7 weeks	1 week	2 weeks	pH = 8.0	pH = 9.0	1 h	2 h
Soluble proteins	CjSz	1.11 ± 0.17bc	1.10 ± 0.26d	1.35 ± 0.36a	1.52 ± 0.08ab	1.86 ± 0.18a	2.04 ± 0.17a	2.15 ± 0.18a	2.16 ± 0.17a
	CjZy	1.01 ± 0.17c	1.46 ± 0.28bc	1.19 ± 0.19a	1.17 ± 0.28b	1.81 ± 0.06a	1.82 ± 0.31a	1.58 ± 0.09c	1.77 ± 0.32b
	Cp	1.59 ± 0.19a	1.88 ± 0.19a	1.29 ± 0.32a	1.67 ± 0.28a	1.62 ± 0.10b	1.83 ± 0.18a	1.74 ± 0.17bc	1.77 ± 0.17b
	Pt	1.09 ± 0.07bc	1.24 ± 0.17cd	1.13 ± 0.19a	1.11 ± 0.17b	1.13 ± 0.13c	1.19 ± 0.26b	1.84 ± 0.09b	1.93 ± 0.10ab
	Ct	1.26 ± 0.02b	1.54 ± 0.12b	1.21 ± 0.55a	1.35 ± 0.51ab	1.23 ± 0.15c	1.30 ± 0.15b	1.28 ± 0.23d	1.36 ± 0.17c
Soluble sugars	CjSz	2.78 ± 0.48a	3.24 ± 0.40a	2.50 ± 0.62a	1.87 ± 0.71bc	1.77 ± 0.23ab	2.08 ± 0.51b	1.37 ± 0.20a	1.25 ± 0.21b
	CjZy	1.52 ± 0.45c	1.79 ± 0.10b	1.36 ± 0.37b	1.62 ± 0.13bc	1.42 ± 0.37bc	2.32 ± 0.44ab	1.29 ± 0.13a	1.53 ± 0.23ab
	Cp	2.80 ± 0.15a	2.83 ± 0.53a	1.96 ± 0.30ab	2.76 ± 0.92a	2.22 ± 0.63a	2.77 ± 0.37a	1.14 ± 0.41a	1.74 ± 0.22a
	Pt	2.12 ± 0.49b	2.13 ± 0.33b	1.86 ± 0.13ab	1.26 ± 0.43c	0.98 ± 0.16c	1.15 ± 0.16c	1.23 ± 0.28a	1.41 ± 0.32ab
	Ct	3.06 ± 0.26a	3.16 ± 0.64a	2.44 ± 0.93a	2.25 ± 0.17ab	1.01 ± 0.24c	1.18 ± 0.25c	1.17 ± 0.33a	1.63 ± 0.31a

Note: Data shown in the table were expressed as ratios relative to the values obtained on control seedling. Different lowercase letters indicate significant differences at $p < 0.05$.

parameters. By comparing the comprehensive evaluation values of all citrus rootstocks, we observed that the ranking of tolerance for each rootstock varied with treatment time. The comprehensive evaluation values were determined using a membership function and weight calculation, which allowed us to assess the performance of each rootstock under each different abiotic stress treatment (Table 4).

The results showed that CjSz had the highest comprehensive evaluation values during 6 and 7 weeks of flooding (0.710 and 0.966), at pH 8.0 (0.810), and after 2 h of freezing treatment (0.749). On the other hand, Ct exhibited the lowest comprehensive evaluation values during 6 weeks of flooding (0.141), and 2 weeks of drought (0.171), at pH 9.0 (0.272), and after 2 h of freezing treatment (0.018) (Table 4).

Performances of different graft combinations

Among the different graft combinations, the success rate of grafting 'Chunjian' and 'Mingrijian' onto Pt rootstocks wad below 80%, with success rates of 70.67% and 79.33%, respectively (Table 5). The highest survival rates of 100% were observed in 'Buzhihuo' was grafted onto CjSz and 'Dafen' grafted onto Ct. In terms of graft joint thickness (Ta), 'Dafen' grafted onto CjSz had the highest value of 20.13 mm, while 'Mingrijian' grafted onto Pt had the lowest value of 6.65 mm. The stem thickness below the graft joint (Tb) was the highest in 'Mingrijian' grafted onto CjSz (28.62 mm) and the lowest in 'Buzhihuo' grafted onto Pt (11.80 mm). The Ta/Tb ratio, which indicates the relative thickness above the blow the graft joint, was the highest in 'Tarocco' grafted onto CjZy and 'Tarocco' grafted onto Ct (0.82 for both), followed by 'Dafen' and 'Buzhihuo' grafted onto Ct (0.81 and 0.80, respectively). The lowest Ta/Tb ratio was observed in 'Mingrijian' and 'Chunjian' grafted onto Pt (0.52 and 0.58, respectively).

The tree growth was significantly affected by different rootstocks (Table 6). The citrus scions grafted onto CjSz rootstock exhibited strong tree vigor, followed by Ct, CjZy, and Pt (Table 6). The leaf greenness, as indicated by the SPAD value, was the highest in trees with 'Tarocco' grafted onto CjZy (86.46) and 'Buzhihuo' grafted onto CjSz (85.79), while it was lowest for 'Mingrijian', 'Chunjian', and 'Buzhihuo' grafted onto Pt (71.43,

Table 4. Comprehensive evaluation of five genotypes citrus rootstocks under different stresses.

Treatment	Variety	Comprehensive evaluation			Membership function			Comprehensive evaluation value	Order
		F1	F2	F3	U1	U2	U3		
Flooding 6 weeks	CjSz	2.778	−0.145	0.154	1.000	0.397	0.677	0.710	1
	CjZy	−0.703	−1.678	0.998	0.130	0.000	1.000	0.229	4
	Cp	−1.222	0.620	0.569	0.000	0.596	0.836	0.375	3
	Pt	−0.270	2.180	−0.108	0.238	1.000	0.577	0.592	2
	Ct	−0.582	−0.978	−1.614	0.160	0.181	0.000	0.141	5
	Weights				0.440	0.388	0.172		
Flooding 7 weeks	CjSz	2.261	1.481	0.119	1.000	1.000	0.621	0.966	1
	CjZy	0.985	−1.106	0.866	0.715	0.000	1.000	0.565	2
	Cp	−1.878	−0.464	0.269	0.075	0.248	0.698	0.174	5
	Pt	−2.215	1.047	−0.146	0.000	0.832	0.487	0.249	4
	Ct	0.847	−0.957	−1.108	0.684	0.058	0.000	0.468	3
	Weights				0.663	0.246	0.091		
Drought 1 week	CjSz	−0.468	1.581	0.303	0.239	1.000	0.504	0.516	2
	CjZy	−1.545	0.762	−0.946	0.000	0.755	0.000	0.228	4
	Cp	0.003	−1.758	−0.943	0.343	0.000	0.001	0.178	5
	Pt	2.964	0.349	0.050	1.000	0.631	0.401	0.781	1
	Ct	−0.954	−0.934	1.535	0.131	0.247	1.000	0.321	3
	Weights				0.519	0.302	0.179		
Drought 2 weeks	CjSz	−0.005	1.386	1.251	0.386	1.000	1.000	0.672	2
	CjZy	−1.787	0.898	−1.024	0.000	0.864	0.000	0.287	4
	Cp	0.005	0.146	−0.150	0.388	0.654	0.384	0.476	3
	Pt	2.826	−0.236	−0.492	1.000	0.547	0.234	0.747	1
	Ct	−1.039	−2.194	0.415	0.162	0.000	0.632	0.171	5
	Weights				0.535	0.332	0.133		
Alkaline pH = 8.0	CjSz	0.492	1.831	0.873	0.674	1.000	1.000	0.810	1
	CjZy	2.056	0.273	−0.941	1.000	0.551	0.000	0.761	2
	Cp	1.059	−1.087	0.226	0.792	0.160	0.643	0.574	3
	Pt	−2.749	0.626	−0.598	0.000	0.653	0.189	0.229	5
	Ct	−0.859	−1.643	0.440	0.393	0.000	0.761	0.301	4
	Weights				0.582	0.323	0.094		
Alkaline pH = 9.0	CjSz	0.178	1.765	−0.992	0.681	1.000	0.000	0.699	2
	CjZy	1.244	0.969	0.689	0.941	0.770	0.990	0.885	1
	Cp	1.484	−1.154	0.590	1.000	0.156	0.932	0.676	3
	Pt	−2.614	0.115	0.706	0.000	0.523	1.000	0.342	4
	Ct	−0.292	−1.695	−0.993	0.567	0.000	0.000	0.272	5
	Weights				0.481	0.372	0.148		
Freezing 1 h	CjSz	−0.127	0.600	1.842	0.330	0.644	1.000	0.568	2
	CjZy	−1.364	−1.396	−0.133	0.021	0.000	0.284	0.068	5
	Cp	2.549	0.457	−0.447	1.000	0.598	0.170	0.701	1
	Pt	−1.447	1.702	−0.916	0.000	1.000	0.000	0.324	3
	Ct	0.389	−1.364	−0.346	0.459	0.010	0.207	0.263	4
	Weights				0.473	0.324	0.203		
Freezing 2 h	CjSz	1.279	0.579	−0.031	0.907	0.650	0.416	0.749	1
	CjZy	−1.946	1.708	0.603	0.000	1.000	0.714	0.443	4
	Cp	0.900	−1.426	1.209	0.800	0.029	1.000	0.567	3
	Pt	1.609	0.661	−0.915	1.000	0.676	0.000	0.746	2
	Ct	−1.843	−1.521	−0.866	0.029	0.000	0.023	0.018	5
	Weights				0.516	0.340	0.144		

75.63, and 75.70, respectively). Overall, the results suggest that CjSz exhibited good graft compatibility with the test scions.

Discussion

Grafting is widely used in citrus propagation and provides many agronomical advantages to scion[32,33]. Rootstock is vital for the citrus industry as it provides resistance to multiple stresses[3,34]. The rootstocks used in citrus have certain issues with stress resistance, disease resistance, or grafting compatibility[14−16], rendering them inflexible to the varying soil environment and climatic conditions, and consequently, cannot be widely used in various cultivars[17]. Therefore, citrus rootstock cultivation and evaluation are critical for the industry's healthy, stable, and sustainable development[27].

In this study, abiotic stress altered the physiological, metabolic, and molecular processes[17,35]. Almost all rootstock leaf photosynthetic pigments (*Chl* a, *Chl* b, total *Chl*, and carotenoid) were decreased under abiotic stress treatments (Table 1), creating an imbalance in the photosynthetic machinery[36]. Under stress conditions, MDA, soluble protein, soluble sugar contents, and antioxidant enzyme activities were in an unbalanced equilibrium state[23, 24,37]. Alkaline stress

Table 5. Survival rate and graft union situation of different graft combinations.

Graft combination		Survival rate (%)	Diameter of scion (mm)	Diameter of rootstock (mm)	Ration of scion to rootstock
Rootstock	Scion				
CjSz	Chunjian	95.67	18.81 ± 0.35ab	26.52 ± 2.75b	0.70 ± 0.03ab
	Buzhihuo	100.00	18.31 ± 2.34ab	23.96 ± 2.49c	0.77 ± 0.02a
	Mingrijian	91.33	17.87 ± 1.95b	28.62 ± 2.88a	0.63 ± 0.06c
	Dafen	90.67	20.13 ± 2.52a	28.40 ± 3.42ab	0.70 ± 0.06ab
	Tarocco	95.50	20.10 ± 1.68a	30.12 ± 2.74a	0.67 ± 0.01bc
CjZy	Chunjian	86.33	11.92 ± 0.94b	16.00 ± 0.46b	0.75 ± 0.05ab
	Buzhihuo	95.50	12.59 ± 1.87b	16.05 ± 1.39b	0.78 ± 0.06ab
	Mingrijian	81.67	11.26 ± 1.28b	16.01 ± 1.52b	0.71 ± 0.01b
	Dafen	95.50	14.78 ± 1.99a	20.48 ± 2.29a	0.74 ± 0.12ab
	Tarocco	90.67	14.82 ± 2.01a	17.66 ± 2.85ab	0.82 ± 0.05a
Ct	Chunjian	91.33	15.94 ± 1.64ab	21.17 ± 1.71a	0.75 ± 0.03ab
	Buzhihuo	95.67	14.77 ± 2.50b	18.83 ± 2.51ac	0.80 ± 0.12ab
	Mingrijian	90.67	15.02 ± 2.48b	20.91 ± 2.27ab	0.72 ± 0.05b
	Dafen	100.00	16.44 ± 0.81ab	21.60 ± 3.82a	0.81 ± 0.09a
	Tarocco	87.33	17.37 ± 1.07a	21.28 ± 1.65a	0.82 ± 0.03a
Pt	Chunjian	70.67	8.19 ± 0.49bc	14.10 ± 2.02abc	0.58 ± 0.05ab
	Buzhihuo	95.67	6.66 ± 0.66c	11.80 ± 0.44c	0.57 ± 0.07ab
	Mingrijian	79.33	6.65 ± 0.76c	12.81 ± 1.13bc	0.52 ± 0.06b
	Dafen	91.67	10.94 ± 0.97a	17.01 ± 1.67a	0.65 ± 0.07a
	Tarocco	91.33	10.36 ± 1.84ab	16.55 ± 0.68ab	0.62 ± 0.18a

Table 6. The growth situation of different graft combinations.

Graft combination		Scion length (cm)	Crown breadth		Leaf greenness (SPAD)
Rootstock	Scion		Longitudinal (cm)	Horizontal (cm)	
CjSz	Chunjian	97.54 ± 9.53b	87.83 ± 14.42a	89.18 ± 16.32b	82.98 ± 0.74abc
	Buzhihuo	79.11 ± 10.63d	73.67 ± 5.78b	78.00 ± 17.04c	85.79 ±1.85a
	Mingrijian	115.78 ± 8.88a	91.33 ± 13.53a	110.38 ± 19.01a	84.97 ± 0.47a
	Dafen	88.11 ± 5.98c	97.33 ± 20.63a	98.00 ± 18.89ab	81.34 ± 0.14c
	Tarocco	81.11 ± 12.07cd	61.56 ± 11.59c	65.56 ± 9.19d	84.36 ± 1.55ab
CjZy	Chunjian	69.67 ± 11.15ab	66.99 ± 4.20a	66.88 ± 15.99a	82.16 ± 2.12b
	Buzhihuo	55.89 ± 9.71c	52.33 ± 3.71b	61.11 ± 3.20ab	82.93 ± 0.57b
	Mingrijian	69.67 ± 5.93ab	58.33 ± 14.25ab	71.00 ± 13.91a	79.54 ± 3.83c
	Dafen	73.56 ± 6.00a	72.17 ± 16.54a	71.33 ± 18.34a	84.93 ± 3.30ab
	Tarocco	57.56 ± 10.84bc	64.22 ± 11.52a	52.67 ± 20.54b	86.46 ± 0.80a
Ct	Chunjian	96.11 ± 10.24a	77.44 ± 12.30a	83.33 ± 14.88ab	81.37 ± 1.92ab
	Buzhihuo	82.44 ± 15.61b	62.56 ± 8.55bc	69.22 ± 14.34b	81.36 ± 1.68abc
	Mingrijian	97.89 ± 9.34a	76.89 ± 5.43ab	77.67 ± 31.56ab	78.81 ± 1.97bcd
	Dafen	87.33 ± 5.46ab	89.22 ± 1.26a	92.67 ± 20.85a	83.47 ± 1.70a
	Tarocco	73.22 ± 13.77c	55.22 ± 12.85c	53.33 ± 16.68c	83.43 ± 1.28a
Pt	Chunjian	41.18 ± 7.44ab	38.09 ± 12.96ab	42.13 ± 14.27a	75.63 ± 2.67b
	Buzhihuo	25.56 ± 3.17c	18.89 ± 1.92c	15.56 ± 1.50bc	75.70 ± 2.04b
	Mingrijian	32.11 ± 7.93bc	20.78 ± 5.23bc	15.11 ± 2.12c	71.43 ± 4.99c
	Dafen	51.44 ± 4.17a	50.56 ± 10.40a	48.00 ± 6.66a	80.92 ± 4.33a
	Tarocco	42.13 ± 16.27ab	27.89 ± 10.00bc	33.89 ± 15.79ab	81.71 ± 0.70a

inhibits plant growth far more than salt stress[38]. CjZy is widely used as an alkaline-tolerant citrus rootstock in calcareous soil areas in China[5]. Under alkaline stress, CjSz performed similarly to CjZy. Low temperatures cause the leaves to wilt and dehydrate, reducing the photosynthesis rate[39]. The disruption of photosynthetic mechanisms causes excessive production of ROS, leading to oxidative stress, one of the most damaging consequences of freezing stress. Pt has shown the highest resistance to cold stress[40−42]. CjSz and Pt undergo similar physiological and biochemical changes, indicating that CjSz is also highly resistant to freezing. Flooding is a seasonal stress factor affecting Chinese citrus production areas[2]. Under flooding stress, the MDA content in Ct was the highest, and that in Pt was the lowest. Comprehensive analysis revealed that Cp is the most flood-resistant genotype, correlating with previous research findings[43]. CjSz had reasonable flooding resistance. Cp has been considered drought-resistant citrus rootstocks owing to its higher chlorophyll content and POD activity under drought stress than CjSz. CjSz had less MDA content than Cp but more soluble sugar and soluble protein content. The comprehensive evaluation revealed that CjSz's drought tolerance was second only to Pt and superior to other rootstocks.

Rootstocks significantly affect tree performance in multiple aspects[9]. Several studies have indicated that rootstocks have a significant effect on shoot growth[3,18]. In this study, the effects of rootstocks on the horticultural performance of scion varieties were investigated. Rootstock genotypes influence compatibility[9]. In our study, the graft success rate of CjSz with five citrus cultivars ranged from 91.33% to 100%. Scions on CjSz developed faster than those on Pt, suggests that graft compatibility is related to the genetic relationship between scion and rootstock. The supply of root-derived nutrients, such as water and minerals, to the shoots may be limited due to incompatibility, leading to poor shoot growth and leaf function[44]. Scions on Pt exhibited a much smaller canopy size and lower SPAD value than those on other rootstocks, consistent with the findings in three late-ripening navel oranges[45] and Folha Murcha sweet oranges[46]. These results suggest that Pt can be used for dense planting and that two genotypes of *C. junos* are preferable for sparse planting, consistent with previous findings[45]. Therefore, scion-rootstock compatibility based on graft success and tree vigor supports that 'Shuzhen No.1' has a high potential for usage as a citrus rootstock.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization and supervision: Wang X; methodology: He W; investigation: Chai J, Wang Y, Wu Z, Li M, Lin Y, Luo Y, Yong Zhang, Yunting Zhang, Wang H; bioinformatic analyses: He W, Chai J; data curation: He W, Xie R, Chai J; manuscript preparation: He W; writing—review and editing: He W, Tang H, Wang X. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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