Indian Journal of Agricultural Sciences **95** (9): 1048–1056, September 2025/Article
https://doi.org/10.56093/ijas.v95i9.167699

Phylogenetic relationship among potential citrus rootstock species based on seed characteristics and germination metrics

HEIPLANMI RYMBAI[1], HAMMYLLIENDE TALANG[1]*, ANJANI KUMAR JHA[2],
VEERENDRA KUMAR VERMA[1], JOIEDEVIVRESON MAWLEIN[1] and
MAYANGLAMBAM BILASHINI DEVI[1]

ICAR-Research Complex for North Eastern Hill Region, Umiam, Meghalaya 793 103, India

Received: 10 June 2025; Accepted: 29 August 2025

ABSTRACT

Wild citrus species could be utilised as potential rootstocks in the citriculture industry. Seed germination, seedling characteristics, and their metrics are important traits of rootstocks and their evolutionary relationships. However, the limited information available affects the propagation and field performance of citrus. The experiment was conducted during winter (*rabi*) season of 2022 to 2024 at ICAR-Research Complex for NEH Region, Umiam, Meghalaya to understand the germination, growth behaviours and phylogenetic relationship of citrus rootstock species. Fifteen potential citrus rootstocks selected for the study were *Citrus maxima, C. jambhiri, C. karna, C. latipes, C. limonia, C. aurantifolia, C. limon, C. macroptera, C. medica, C. paradisi, C. reshni, C. trifoliata, C. taiwanica, C. volkameriana*, and *C. indica*. Results showed that the maximum germination traits, seedling growth and their metrics were obtained in *C. jambhiri, C. latipes, C. limonia, C. maxima,* and *C. volkameriana*. The highest chlorophyll index was recorded in *C. limon* (80.8 ± 3.7) and *C. medica*. Germination was strongly correlated with the germination speed index (r = 0.746**), mean daily germination (r = 0.845**), peak (r = 0.512**), and germination value (r = 0.596**). Principal component analysis revealed the presence of a wider variability for germination and seedling traits, with the first five components (eigen value>1) contributing 78.69% of the total variation. Phylogenetic analysis revealed that cluster I was monogenotypic (*C. trifoliata*) and cluster II comprised commonly used rootstocks, indicating a close relationship between them. Therefore, C. *jambhiri, C. limonia*, *C. maxima*, and *C. latipes* exhibited higher performance in germination behaviours, growth, and vigour of seedlings. Germination percentage and germination metrics could be important selection criteria for the improvement and utilisation of these species in propagation.

Keywords: Citrus rootstocks, Citrus species, Propagation, Phylogenetic relationship, Seed germination

Citrus spp. are a significant component contributing to the rich agro-biodiversity of the NEH Region, India. Indigenous citrus species hold considerable potential as sources of genes that could contribute to several adaptability factors, including biotic and abiotic stress, as well as for broader horticultural applications. These species are valuable raw material for industries (food and pharmaceutical), and have been effectively utilised as rootstocks for various commercial scion cultivars. Rootstocks such as Rough lemon, Rangpur lime, and Karna Khatta are significant to the citriculture sector in India. These rootstocks have significantly contributed to increasing productivity, quality, and tolerance to insect pests, diseases, and salinity. The vegetative approach is the most commonly adopted propagation method for commercial scion cultivars (Rymbai *et al.* 2024). However, seed propagation remains the widely adopted multiplication technique for raising seedling rootstocks, as well as in hybridisation programmes.

The measurement of germination is significant due to the varying germination rates and percentages within individual and population levels of a species, as well as their stages and dormancy behaviours (Baski and Baskin 2014). The germination and growth parameters can be studied through mathematical expressions (Shiade and Boelt 2020). The study of germination and associated metrics can provide precise insights on the rate, peak, uniformity, percentage, and other germination-related traits in different citrus species. Therefore, seed germination metrics are a critical parameter to facilitate breeding programmes, establishment, and evolution for any crop, influencing both the distribution and abundance of plant species in communities.

The phylogeny of citrus is extremely complex, contested, and obscure (Nicolosi *et al.* 2000) due to factors such as sexual compatibility, extensive cultivation history, apomixis,

---

[1]ICAR-Research Complex for North Eastern Hill Region, Umiam, Meghalaya; [2]ICAR-Directorate of Knowledge Management in Agriculture, Krishi Anusandhan Bhawan-I, New Delhi. *Corresponding author email: rymbaihort@gmail.com

polyembryony, and somatic bud mutation. Phylogenetic studies have been primarily based on morphological and molecular markers (Penjor *et al.* 2013, Wali *et al*. 2013, Luro *et al*. 2017). However, no attempt was made to determine the phylogenetic relationship in citrus-based seed germination, growth and their metrics. Therefore, this study on germination behaviour and seedling growth will provide comprehensive information on the extent of genetic variability and their evolutionary relationship to facilitate breeding programmes, propagation, and conservation.

MATERIALS AND METHODS

Experimental plant and location: The experiment was conducted during winter (*rabi*) season of 2022 to 2024 at ICAR-Research Complex for NEH Region, Umiam (250°41'N, 91°55'E, and 1010 m amsl), Meghalaya. Fifteen potential citrus rootstocks, viz. Acid lime [*Citrus aurantifolia* (Christm. & Panz.) Swingle], Pummelo [*Citrus maxima* (Burm.) Merr.], Rough lemon (*Citrus jambhiri* Lush.), Karna khatta (*Citrus karna* Raf.), Khasi papeda [*Citrus latipes* (Swingle) Yu.Tanaka], Rangpur lime (*Citrus limonia* Osbeck), Lemon [*Citrus limon* (L.) Burm.], Soh kwit / Satkara (*Citrus macroptera* Montr.), Citron (*Citrus medica* L.)*,* Grapefruit (*Citrus paradisi* Mcfad.), Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.)*,* Trifoliate orange (*Citrus trifoliata* L.)*,* Sour orange (*Citrus × taiwanica* Tanaka & Shimada), Volkamer lemon (*Citrus volkameriana*), Indian wild orange or Memang Narang (*Citrus indica* Yu. Tanaka) were used for the experiment. The mother plants of the species are maintained in the Germplasm Block of the Institute under the standard package of practices.

The soil of the experiment site consisted of sandy loam soil with a *p*H of 4.85, 20.8 g/kg organic carbon, 203.6 kg/ha soil available N, 19.4 kg/ha soil available P, and 191.4 kg/ha soil available K. The recorded weather parameters included average temperature (minimum 7.4 °C and maximum 20.6 °C), precipitation (7.2 mm), relative humidity (minimum 56.1% and maximum 82.5%), and wind speed (1.8 km/h).

Seed collection, extraction and treatment: Well-ripened fruits for seed extraction were collected from a healthy, uniform, and known pedigree mother plant maintained in the germplasm block of the Institute. The fruits were manually cleaned, sliced in half, and then the seeds were separated from the fruit. Following extraction, the seeds were carefully rubbed and washed with clean water to remove the mucilaginous coating. After that, the seeds were spread in the shade for three to four hours. Following this, the seeds were treated with *Trichoderma viride* and *Pseudomonas fluorescens* (@7 g/kg of seed).

Seed sowing: The seeds were sown in nursery pro-trays filled with solar-sterilised media (soil: cocopeat: FYM, 1:1:1 v/v/v) at a depth of 1.5–2.0 cm. Each cavity of the pro-tray contained one seed. The sowing was carried out on the first day of December under a low-cost polyhouse having a temperature range (min 7.4°C and max 20.6°C) and relative humidity (max. 56.1% and max 82.5%). Light irrigation was applied immediately after sowing. Plant protective measures were taken, including the application of Nimbecidine (5 ml/L) after two weeks of planting to protect against pests. A prophylactic drenching of Bordeaux mixture (1.0%) was also applied twice to prevent any incidence of disease, particularly damping off, during the period of investigation.

Observations: The seed weight of mature and uniform seeds was measured using an electronic balance (LA 214i). The 10-seed weight was expressed in grams. The seed length was measured along the longest axis of the seed; seed width was taken along the second longest axis, perpendicular to the length axis; and seed thickness was measured along the third longest axis of the seed using a digital vernier calliper (INSIZE 1108-300).

The parameters for germination metrics were recorded; days to germination were counted from the day of seed sowing to the first emergence of the radicle. The protrusion of a seed radicle about 2 mm long indicates that a seed has germinated. The germination percentage was recorded daily until completion of germination and calculated as a percentage using the formula below:

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The germination speed index (GSI) was calculated by counting the germinated seeds within a specific time frame, as per the formula of Czabator (1962):

$$\text{GSI}=\sum \frac{\text{Pi}}{\text{Di}}$$

Where Pi, Number of germinated seeds on the ith day; Di, Number of days from the start of sowing to the ith day.

The mean germination time (MGT) was calculated using the formula given by Czabator (1962):

$$\text{MGT}=\sum \frac{(\text{Pi} \times \text{Di})}{\text{N}}$$

Where Pi, Number of germinated seeds on the ith day; Di, Number of days on the ith day; N, Total number of days.

The mean daily germination (MDG) was calculated as given Czabator (1962):

$$\text{Mean daily germination (MDG)} = \frac{\text{P}}{\text{N}}$$

Where, P is the total number of germinated seeds and N is the total number of days.

Peak value (PV) was calculated using the formula derived by Czabator (1962):

$$\text{Peak value (PV)} = \frac{\text{Maximum seed germination}}{\text{Number of days}}$$

The germination value (GV) was calculated as given by Czabator (1962):

$$\text{Germination value (GC)} = \text{PV} \times \text{MDG}$$

Seedling growth measurement: Seedling height, number of leaves, plant fresh weight and dry weight were recorded at 60 and 90 days after sowing (DAS). The seedling height was measured from the soil surface to the tip of the seedlings using a ruler. The number of leaves was counted, including young and fully opened ones. The fresh weight of the plant, including roots, stem, and leaves, was weighed on an electronic balance (LA 214i). These plants were dried in an oven (UNICO) at 60°C for 24 h until a constant weight was obtained, and then weighed on an electronic balance. Chlorophyll index of fully opened leaves was measured at 90 DAS using a chlorophyll meter (SPAD 502 Plus).

The absolute growth rate (AGR) and relative growth rate (RGR) were calculated as:

$$\text{AGR (Plant height)} = \frac{\text{H1} - \text{H1}}{\text{t2} - \text{t1}}$$

Where H1 and H2, Plant height (cm) during the time t1 to t2.

$$\text{RGR} = \frac{\text{logeW2} - \text{loge W1}}{\text{t2} - \text{t1}}$$

Where W1 and W2 denote plant dry weight (g) at times t1 and t2, $\log_e$ is the natural logarithm.

Statistical analysis: The experiment was laid out in a complete randomized block design (CRBD) with three replications, each replication consisting of 50 seeds. The statistical analysis was carried out using IBM SPSS (version 26.0) for the development of ANOVA and R Studio (version R 3.6.0+). The principal component analysis was performed using IBM SPSS (version 26.0). The phylogenetic analysis was conducted using the Neighbour-Joining method (DARwin version 6.0.021) to construct the phylogenetic tree.

RESULTS AND DISCUSSION

Seed characteristics: The results showed significant variations for seed characteristics among different potential citrus rootstocks (Table 1). The highest seed weight was recorded in *C. maxima* (5.18 ± 0.32 g), which was followed by *C. limon* (4.32 ± 0.19 g), while the lowest seed weight was recorded in *C. jambhiri*. Similarly, the seed length was maximum in *C. maxima* (16.92 ± 0.53 mm), which was followed by *C. taiwanica* (16.83 ± 0.87 mm), *C. macroptera* (15.35 ± 0.62 mm), and *C. latipes* (13.42 ± 0.52 mm). A similar pattern was observed for seed width and thickness, with the highest values recorded in *C. maxima* and *C. karna*. The result of the study indicated that the seed weight in *C. maxima* was 19.9% higher than *C. limon*, which is the second highest seed weight. Similarly, the seed length in *C. maxima* was 0.53% higher than in *C. taiwanica* and 17.2% higher than in *C. latipes*. Previous research has also been published on the variation in seed weight and dimensions within and across plant species (Eriksson 1999, Pelabon *et al*. 2021). These variations could be due to several reasons, such as microhabitat, the position of an ovule, or genetic control (Shaukat *et al*. 1999). In our study, the variations in weight and dimensions of the seeds among the different citrus species might be due to unique genotypic effects of the species.

Germination characteristics: The results showed significant variation among citrus spp. in terms of the number of days required for seed germination and germination percentage (Fig. 1A and B). The longest duration taken to seed germination was recorded in *C. macroptera* (58.6 ± 8.7 days), followed by *C. maxima* (55.7 ± 8.3 days). However, the shortest duration to seed germination was obtained in *C. limon* (42.4 ± 8.5 days), followed by *C. karna* (44.2 ± 9.6 days) and *C. jambhiri* (46.3 ± 9.9 days). The

Table 1 Seed characteristics of different potential citrus rootstocks

Species	Seed weight (g)	Seed length (mm)	Seed width (mm)	Seed thickness (mm)
C. aurantifolia	1.58 ± 0.1^{fgh}	8.47 ± 0.45^{gh}	4.18 ± 0.12^{h}	2.64 ± 0.11^{h}
C. maxima	5.18 ± 0.32^{a}	16.92 ± 0.53^{a}	9.67 ± 0.13^{a}	4.93 ± 0.24^{ab}
C. jambhiri	1.23 ± 0.08^{h}	8.97 ± 0.29^{g}	4.9 ± 0.53^{fgh}	3.58 ± 0.32^{g}
C. karna	3.11 ± 0.12^{c}	12.48 ± 0.35^{cd}	6.04 ± 0.39^{de}	5.19 ± 0.27^{a}
C. latipes	2.48 ± 0.13^{d}	13.42 ± 0.52^{c}	8.25 ± 0.29^{b}	4.38 ± 0.14^{cde}
C. limonia	1.28 ± 0.06^{gh}	10.44 ± 0.55^{ef}	5.09 ± 0.37^{fg}	3.62 ± 0.3^{fg}
C. limon	4.32 ± 0.19^{b}	12.48 ± 0.37^{cd}	5.26 ± 0.47^{fg}	4.06 ± 0.12^{efg}
C. macroptera	2.21 ± 0.17^{de}	15.35 ± 0.62^{b}	6.63 ± 0.27^{cd}	5.23 ± 0.15^{a}
C. medica	1.37 ± 0.07^{gh}	7.54 ± 0.68^{h}	4.51 ± 0.43^{gh}	2.91 ± 0.28^{h}
C. paradisi	2.48 ± 0.19^{d}	11.43 ± 0.45^{de}	4.88 ± 0.17^{fgh}	4.15 ± 0.09^{def}
C. reshni	1.81 ± 0.22^{ef}	10.67 ± 0.39^{ef}	7.42 ± 0.66^{bc}	4.85 ± 0.14^{abc}
C trifoliata	1.67 ± 0.11^{fg}	11.07 ± 0.86^{ef}	7.38 ± 0.22^{bc}	4.87 ± 0.12^{abc}
C. taiwanica	1.31 ± 0.15^{gh}	16.83 ± 0.87^{a}	7.11 ± 0.29^{c}	4.34 ± 0.31^{cde}
C. volkameriana	1.96 ± 0.19^{ef}	11.79 ± 0.65^{de}	5.67 ± 0.13^{ef}	3.95 ± 0.26^{efg}
C. indica	1.82 ± 0.18^{ef}	9.84 ± 0.16^{fg}	6.94 ± 0.13^{c}	4.61 ± 0.12^{bcd}

Values given are mean (n = 150) with ± SE. One-way analysis of variance (ANOVA) plus *post hoc* Tukey test was done to compare means. Superscript lowercase number on each column designated statistical significance (p<0.05).

percentage of seed germination was found to be the highest in *C. limonia* (88.8 ± 6.2%), which was at par with *C. jambhiri* (86.5 ± 4.7%), *C. volkameriana*, *C. indica*, and *C. latipes*. In contrast, the minimum seed germination was found in *C. medica* (67.2 ± 7.6%). Our study also indicates that germination of *C. limon* was 32.2% faster than *C. macroptera*, which took the longest days to germinate. The germination in *C. limonia* was 2.7% higher than that of *C. jambhiri*. The variation in germination behaviours among species in the present study might be due to the genetic effect of the species. Furthermore, our studies showed that the days to germination were twice longer than those previously studied, 10–25 days in *C. latipes* and *C. macroptera* and 7–20 days in *C. indica* (Upadhaya *et al*. 2019). This was probably because the previous studies were conducted under controlled optimum temperatures (28°C), which are a congenial environmental condition for seed germination. In our case, the studies were conducted under low-cost polyhouses at temperatures of 7.4°C/20.6°C (night/day). Citrus seeds have been shown to germinate in a wide range of temperatures, from as low as 6°C to as high as 39°C. However, the ideal temperature for seed germination of several citrus spp. is between 26°C and 30°C (Upadhaya *et al*. 2019, de Mello 2020). Therefore, the delay in seed germination observed in our studies may be due to the seeds having undergone brief dormancy at low temperatures. Several researchers (Geneve 2003, Shiade and Boelt 2020) have also established that citrus seeds undergo brief dormancy at low temperatures; for instance, trifoliate orange enters dormancy at temperatures of 13.9–15.6°C, Sour orange (8.9–10°C), and Rough lemon (below 8.9°C). Among different species, our studies also showed that species such as *C. limon, C. karna, C. jhamberi, C. latipes, C. taiwanica*, and *C. trifoliata* exhibit earlier germination, which may indicate that these species require lower minimum temperatures for seed germination than other species. Additionally, the integument that envelops the seeds varies from species to species; it may create a physical barrier to gas diffusion and water imbibition, or it may possess an inhibitor for embryo development, which is another reason for dormancy, causing non-uniform germination (Madruga *et al*. 2022). Therefore, germination response in citrus rootstock depends on the microclimate, seed macro-structures and the genetic influences of each species.

Mathematical expression of seed germination: The results showed significant differences among various citrus species for germination metrics (Table 2). The germination speed index was recorded as the highest in *C. jambhiri* (2.11 ± 0.01) and followed by *C. latipes, C. limonia, C. trifoliata,* and *C. reshni*. In comparison, the minimum was observed in *C. maxima* (1.39 ± 0.02), followed by *C. macroptera and C. medica*. The MGT was recorded the highest in *C. maxima* (54.92 ± 0.03), followed by *C. reshni*, *C. volkamerian*, and *C. macroptera*, while the minimum MGT was observed in *C. jambhiri* (39.7 ± 2.06), followed by *C.vtrifoliata*. MDG obtained the highest value in *C. jambhiri* (1.48 ± 0.02), which was at par with *C. trifoliata*, followed by *C. latipes, C. limonia, C. volkameriana,* and *C. indica*. However, the minimum MDG was noted in *C. medica* and *C. limon*. Similarly, *C. trifoliata* had the highest PV (1.12 ± 0.02), followed by *C. latipes* (0.98 ± 0.02) and *C. jambhiri* (0.88 ± 0.02). Furthermore, the GV was also found to be the maximum in *C. trifoliata* (1.64 ± 0.01),

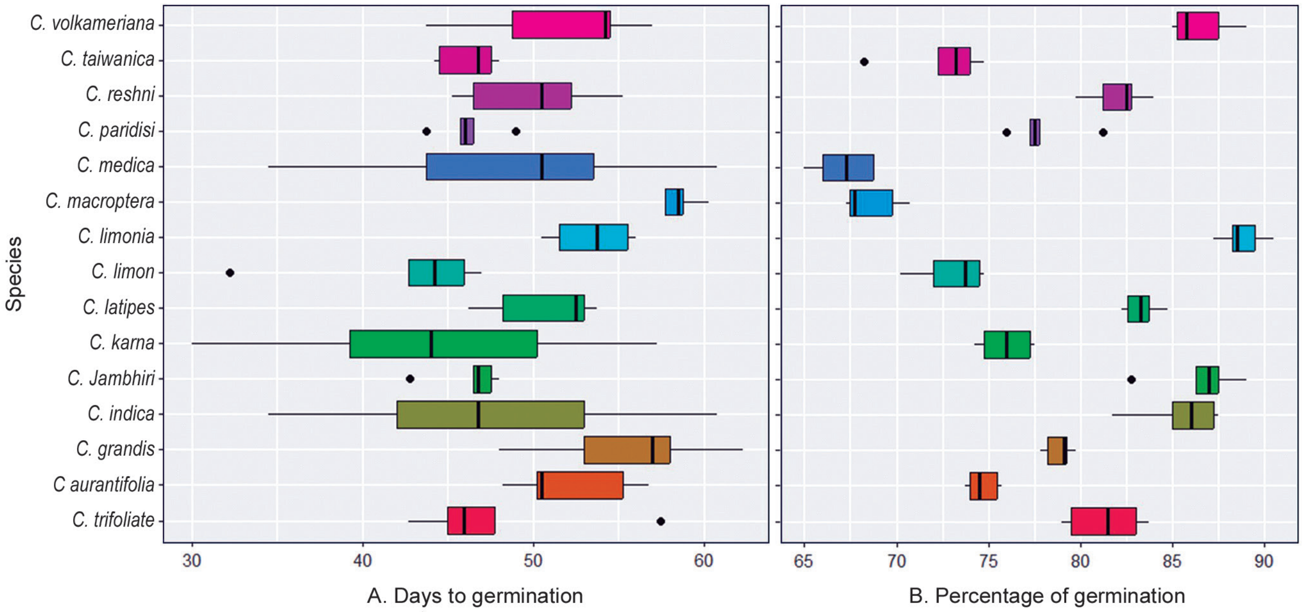

Fig. 1 Germination behaviours of different potential citrus rootstocks. A, Days taken to seed germination; and B, Seed germination percentage (%).
The boxplots representing the distribution of germination bahaviours in citrus species. Each boxplot illustrates the median, interquartile range, and outliers, providing a concise summary of the data distribution for germination bahaviours in citrus species.

followed by *C. latipes* (1.33 ± 0.04) and *C. jambhiri* (1.29 ± 0.1). This indicated that the differences among species in various germination metrics may be due to genotypic influences of the species. It is well known that a higher GSI value indicates faster and higher uniform germination. Our results also showed that the rate of germination was faster in *C. jambhiri, C. trifoliata*, *C. limonia, C. latipes*, and *C. volkameriana*. These species also exhibited higher daily germination rates, suggesting that these species had high seed vigour. This was further supported by a low value of MGT in these species, indicating their quick germination in a population. *C. jambhiri, C. trifoliata*, *C. limonia, C. latipes*, and *C. volkameriana*. *C. jambhiri* and *C. latipes* also had higher germination values and peak values. Therefore, it indicated that such species had high seed vigour for germination as well as the quality of germination. The germination value is known to have a direct association with the survival of seedlings, serving as an indicator of the rate and efficiency of seed germination (Ali and Elozeiri 2017, Shiade and Boelt 2020). Therefore, seeds of citrus species such as *C. jambhiri, C. trifoliata*, *C. limonia, C. latipes*, and *C. volkameriana*. *C. jambhiri, C. maxima*, and *C. latipes* with higher GSI, MDG, GV and PV, are endowed with higher germination vigour and survival and thus can be important in selection criteria for improvement and utilisation of this species in propagation and production.

Seedling growth behaviours and their metrics: A significant difference was observed among citrus species in terms of different seedling growth characteristics (Table 3). The highest seedling height at 60 and 90 DAS was recorded in *C. maxima* (5.08 cm and 10.47 cm, respectively), which was followed by *C. jambhiri* and *C. latipes*. Whereas *C. jambhiri* recorded the maximum number of leaves at 60 DAS (4.34/plant) and 90 DAS (6.13 DAS), which was followed by *C. maxima* and *C. latipes*. The highest plant fresh weight was also recorded in *C. maxima* at 60 DAS (0.328 g) and 90 DAS (2.81 g), followed by *C. jambhiri* and *C. latipes*. The plant dry weight was also recorded in a similar trend. Furthermore, *C. jambhiri* had the highest absolute growth rate (0.1913) and relative growth rate (0.0799), which was followed by *C. maxima* and *C. latipes*. Chlorophyll index was recorded as the highest in *C. limon* (80.8 ± 3.7), which was followed by *C. medica* (59.6 ± 2.1). The highest AGR was recorded in *C. jambhiri* (0.191 ± 0.02), which was followed by *C. limon* (0.099 ± 0.02) and *C. maxima* (0.178 ± 0.01). The maximum RGR was found in *C. reshni* and *C. taiwanica*. The variation among species might be due to the genetic effect of the species. It was found that *C. maxima*, *C. jambhiri,* and *C. latipes* had higher growth behaviours as compared to other citrus species. *C. maxima* growth was higher than 5.2–7.5% in plant height, 2.2–13.9% in plant fresh weight, and 13.1–15.5% in plant dry weight over *C. jambhiri* and *C. latipes*. The number of leaves per plant was 1.2–16.0% higher in *C. jambhiri* than in the next species (*C. maxima*). This indicated that *C. jambhiri* has vigourous growth habits, followed by *C. maxima* and *C. latipes*. It has been reported earlier that *C. jambhiri* exhibits a vigourous growth habit (Hayat *et al*. 2022). Similarly, this study observed that *C. latipes* is a vigourous plant, growing up to 7.5–9.5 m in height due to its higher growth traits. The assessment of plant growth is vital because it represents the

Table 2 Germination metrics of different potential citrus rootstocks

Species	Germination speed index (GSI)	Mean germination time (MGT)	Mean daily germination (MDG)	Peak value (PV)	Germination value (GV)
C. aurantifolia	1.63 ± 0.02^{f}	47.13 ± 1.45^{def}	1.19 ± 0.02^{de}	0.634 ± 0.03^{f}	0.753 ± 0.02^{fg}
C. maxima	1.39 ± 0.02^{i}	54.88 ± 1.97^{a}	1.17 ± 0.02^{de}	0.607 ± 0.02^{f}	0.723 ± 0.01^{g}
C. jambhiri	2.11 ± 0.02^{a}	39.7 ± 2.06^{g}	1.46 ± 0.02^{a}	0.881 ± 0.02^{c}	1.287 ± 0.1^{b}
C. karna	1.75 ± 0.03^{cd}	45.41 ± 1.31^{ef}	1.21 ± 0.01^{de}	0.501 ± 0.02^{g}	0.603 ± 0.01^{h}
C. latipes	1.95 ± 0.03^{b}	44.77 ± 1.29^{ef}	1.36 ± 0.04^{b}	0.976 ± 0.02^{b}	1.333 ± 0.04^{b}
C. limonia	1.89 ± 0.02^{b}	46.64 ± 1.47^{def}	1.37 ± 0.04^{b}	0.653 ± 0.02^{ef}	0.891 ± 0.02^{d}
C. limon	1.50 ± 0.02^{gh}	46.97 ± 1.02^{def}	1.11 ± 0.02^{f}	0.114 ± 0.01^{h}	0.125 ± 0.01^{i}
C. macroptera	1.52 ± 0.01^{h}	49.58 ± 1.26^{bc}	1.16 ± 0.02^{e}	0.464 ± 0.02^{g}	0.538 ± 0.02^{h}
C. medica	1.47 ± 0.03^{h}	47.35 ± 1.05^{cde}	1.08 ± 0.01^{f}	0.491 ± 0.04^{g}	0.529 ± 0.01^{h}
C. paradisi	1.67 ± 0.03^{ef}	47.47 ± 1.04^{cde}	1.24 ± 0.04^{cd}	0.653 ± 0.02^{ef}	0.809 ± 0.01^{ef}
C. reshni	1.79 ± 0.03^{c}	51.33 ± 1.37^{b}	1.29 ± 0.01^{c}	0.633 ± 0.01^{f}	0.813 ± 0.01^{ef}
C trifoliata	1.89 ± 0.02^{b}	43.79 ± 1.4^{f}	1.46 ± 0.02^{a}	1.119 ± 0.02^{a}	1.639 ± 0.01^{a}
C. taiwanica	1.56 ± 0.04^{g}	47.47 ± 0.82^{cde}	1.16 ± 0.01^{e}	0.694 ± 0.02^{de}	0.804 ± 0.01^{ef}
C. volkameriana	1.73 ± 0.03^{de}	51.94 ± 1.2^{ab}	1.40 ± 0.01^{b}	0.607 ± 0.01^{f}	0.848 ± 0.01^{de}
C. indica	1.79 ± 0.02^{cd}	48.51 ± 1.43^{bcd}	1.35 ± 0.01^{b}	0.735 ± 0.03^{d}	0.991 ± 0.01^{c}

Values given are mean (n = 150) with ± SEM. One-way analysis of variance (ANOVA) plus *post hoc* Tukey test was done to compare means. Superscript lowercase number on each column designated statistical significance ($p<0.05$).

Table 3 Seedling growth and their metrics of different potential citrus rootstocks

Species	Seedling height (cm)		Leaves number/ plant		Plant fresh weight (g/plant)		Plant dry weight (g/plant)		Chlorophyll content (SPAD value)	Annual growth rate (AGR)	Relative growth rate (RGR)
	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS			
C. aurantifolia	3.39 ± 0.48^{e}	3.44 ± 0.63^{b}	2.73 ± 0.9^{cd}	3.44 ± 0.63^{b}	0.231 ± 0.01^{de}	2.22 ± 0.07^{de}	0.064 ± 0.002^{efg}	0.837 ± 0.03^{d}	35.06 ± 1.9^{fg}	0.151 ± 0.02^{bc}	0.085 ± 0.001^{abc}
C. maxima	5.08 ± 0.16^{a}	6.06 ± 0.12^{a}	3.74 ± 0.44^{ab}	6.06 ± 0.12^{a}	0.328 ± 0.02^{a}	2.81 ± 0.16^{a}	0.112 ± 0.004^{a}	1.192 ± 0.08^{a}	46.13 ± 2.5^{d}	0.178 ± 0.0^{1a}b	0.079 ± 0.002^{bc}
C. jambhiri	4.21 ± 0.35^{cde}	6.13 ± 0.19^{a}	4.34 ± 0.38^{a}	6.13 ± 0.19^{a}	0.288 ± 0.02^{b}	2.54 ± 0.06^{b}	0.096 ± 0.012^{b}	1.054 ± 0.05^{b}	55.6 ± 2.7^{bc}	0.191 ± 0.02^{a}	0.080 ± 0.003^{bc}
C. karna	4.34 ± 0.19^{bcd}	3.82 ± 0.35^{b}	3.56 ± 0.97^{bc}	3.82 ± 0.35^{b}	0.256 ± 0.01^{cd}	2.41 ± 0.07^{bc}	0.082 ± 0.006^{bcd}	0.874 ± 0.04^{cd}	43 ± 2.2de	0.151 ± 0.01^{bc}	0.078 ± 0.002^{bc}
C. latipes	4.67 ± 0.65^{bcd}	5.46 ± 0.45^{a}	3.64 ± 0.45^{ab}	5.46 ± 0.45^{a}	0.272 ± 0.01^{bc}	2.75 ± 0.06^{a}	0.094 ± 0.005^{b}	0.992 ± 0.05^{bc}	29.04 ± 1.5^{g}	0.176 ± 0.02^{ab}	0.079 ± 0.002^{bc}
C. limonia	3.86 ± 0.42^{cde}	4.08 ± 0.5^{b}	2.82 ± 0.27^{cd}	4.08 ± 0.5^{b}	0.248 ± 0.01^{de}	2.42 ± 0.07^{bc}	0.088 ± 0.005^{bc}	0.876 ± 0.04^{cd}	48.6 ± 5.7^{cd}	0.165 ± 0.01^{bc}	0.077 ± 0.002^{bc}
C. limon	4.73 ± 0.43^{ab}	2.84 ± 0.76^{b}	2.35 ± 0.44^{cd}	2.84 ± 0.76^{b}	0.248 ± 0.01^{cd}	2.37 ± 0.05^{bcd}	0.074 ± 0.004^{cde}	0.852 ± 0.02^{d}	80.8 ± 3.7^{a}	0.099 ± 0.02^{d}	0.081 ± 0.001^{bc}
C. macroptera	3.37 ± 0.1^{cde}	3.41 ± 0.37^{b}	2.18 ± 0.36^{cd}	3.41 ± 0.37^{b}	0.192 ± 0.02^{f}	2.12 ± 0.03^{e}	0.064 ± 0.004^{efg}	0.818 ± 0.02^{d}	41 ± 2.4^{def}	0.172 ± 0.02^{bc}	0.086 ± 0.003^{ab}
C. medica	3.39 ± 0.48^{de}	3.44 ± 0.63^{b}	2.73 ± 0.9^{cd}	3.44 ± 0.63^{b}	0.23 ± 0.01^{de}	2.22 ± 0.07^{de}	0.064 ± 0.002^{efg}	0.827 ± 0.03^{d}	59.6 ± 2.1^{b}	0.151 ± 0.02^{bc}	0.084 ± 0.001^{abc}
C. paradisi	4.13 ± 0.14^{cde}	3.68 ± 0.85^{b}	2.84 ± 0.32^{bcd}	3.68 ± 0.85^{b}	0.254 ± 0.01^{cd}	2.25 ± 0.05^{cde}	0.07 ± 0.004^{def}	0.854 ± 0.05^{d}	54.6 ± 1.5^{bc}	0.165 ± 0.02^{bc}	0.082 ± 0.002^{abc}
C. reshni	3.26 ± 0.49^{cde}	3.31 ± 0.33^{b}	2.35 ± 0.75^{cd}	3.31 ± 0.33^{b}	0.178 ± 0.01^{f}	1.71 ± 0.04^{f}	0.052 ± 0.008^{g}	0.814 ± 0.05^{d}	37.58 ± 3.8^{ef}	0.159 ± 0.02^{bc}	0.090 ± 0.006^{a}
C trifoliata	3.05 ± 0.34^{e}	3.05 ± 0.34^{b}	2.68 ± 0.4^{cd}	3.05 ± 0.34^{b}	0.248 ± 0.01^{cd}	1.23 ± 0.02^{g}	0.06 ± 0.001^{efg}	0.666 ± 0.05^{e}	61.82 ± 2.5^{b}	0.141 ± 0.02^{cd}	0.082 ± 0.003^{abc}
C. taiwanica	3.31 ± 0.42^{de}	3.73 ± 0.38^{b}	2.46 ± 0.45^{cd}	3.73 ± 0.38^{b}	0.198 ± 0.01^{ef}	2.37 ± 0.08^{bcd}	0.056 ± 0.004^{fg}	0.85 ± 0.03^{d}	57.5 ± 4.8^{b}	0.153 ± 0.01^{bc}	0.090 ± 0.002^{a}
C. volkameriana	3.86 ± 0.38^{cde}	4.02 ± 0.89b	2.87 ± 0.68^{bcd}	4.02 ± 0.89^{b}	0.257 ± 0.02^{cd}	2.54 ± 0.05^{b}	0.091 ± 0.005^{b}	0.888 ± 0.06^{cd}	43.4 ± 2.9d^{e}	0.123 ± 0.02^{cd}	0.076 ± 0.003^{c}
C. indica	3.18 ± 0.14^{de}	3.12 ± 0.65b	1.87 ± 0.11^{d}	3.12 ± 0.65^{b}	0.180 ± 0.02^{f}	1.21 ± 0.05^{g}	0.034 ± 0.003^{h}	0.426 ± 0.07^{f}	29.28 ± 3.6g	0.142 ± 0.01^{cd}	0.083 ± 0.007^{bc}

Values given are mean (n=150) with ± SEM. One-way analysis of variance (ANOVA) plus *post hoc* Tukey test was done to compare means. Superscript lowercase number on each column designated statistical significance (p<0.05).

vigour of seedlings and predicts the potential for seedling emergence and establishment in the field (Nakagawa and Oak 2000, Franco *et al*. 2020). This was further supported by a higher AGR value in *C. jambhiri*, *C. limon*, *C. maxima,* and *C. latipes,* which can be categorised as indicating higher seedling growth and vigour.

Correlation among different seed and germination characteristics: A significant positive correlation was noted among seed characteristics and germination metrics of various potential citrus rootstocks (Fig. 2). Seed weight showed a positive correlation with seed length (r = 0.519**), width (r = 0.427**), thickness (r = 0.383**), and plant dry weight (r = 0.391** at 90 DAS). Although seed weight had no significant relationship with germination percentage (r = - 0.171) and other germination metrics, it showed a significant negative correlation with germination metrics, except for MGT (r = 0.408**). Similarly, seed weight had a significant positive correlation with fresh weight and dry weight. Earlier researchers have also demonstrated that larger seed weight is associated with higher germination performance (Fandohan *et al.* 2010, Franco *et al.* 2020). Our findings do not corroborate the assumption that large seeds germinate more quickly than small seeds among citrus species, which could be due to variations among different citrus species. Our results align with the findings of Moles *et al*. (2005a, b) and Norden *et al*. (2009), who reported faster germination in smaller seeds. Germination is a complex mechanism that may also be influenced by several factors; however, in our study, it may be attributed to the genetics of the species. Furthermore, it could be due to variations in dormancy among the species, desiccation sensitivity, and seed coat thickness (Daws *et al*. 2007). Furthermore, the seed germination was strongly correlated with germination metrics, GSI (r = 0.746**), MDG (r =

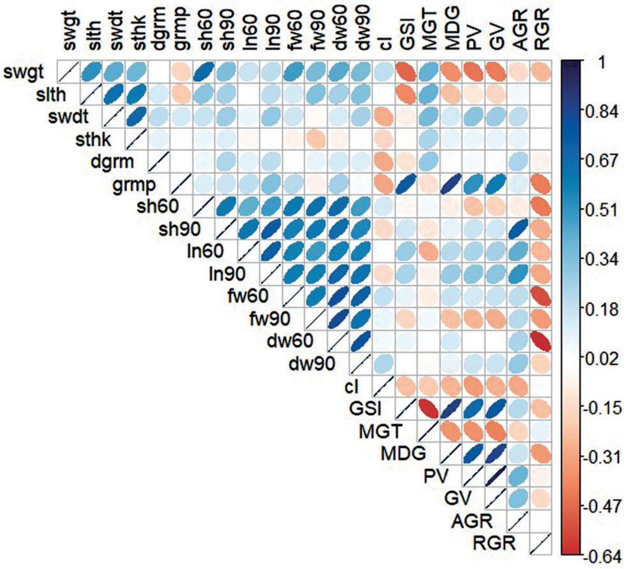

Fig. 2 Correlation among germination and growth behaviours and their metrics in different potential citrus rootstocks. swgt, Seed weight; slth, Seed length; Swdt, Seed width; sthk, Seed thickness; dgrm, Days to germination; grmp, Germination percentage; sh60, Seedling height at 60 DAS; sh90, Seedling height at 90 DAS; ln60, Leaf number at 60 DAS; ln90, Leaf number at 90 DAS; fw60, Plant fresh weight at 60 DAS; fw90, Plant fresh weight at 90 DAS; cl, Chlorophyll content; GSI, Germination speed index; MGT, Mean germination time; MDG, Mean daily germination; PV, Peak value; GV, Germination value; AGR, Absolute growth rate; RGR, Relative growth rate.

0.845**), PV (r = 0.512**), and GV (r = 0.596**). Therefore, this study suggests that GSI and other metrics can be effective indicators of high-performance seed germination behaviours in different citrus rootstocks.

Principal component analysis: The results of the PCA analysis also revealed the presence of variability for germination and seedling growth traits (Supplementary Table 1). The first five components had an extracted eigenvalue of >1 (Fig. 3), which contributed 78.69% of the total variation among the genotypes. Principal component 1 (PC1) contributed 28.18% of the total variability. PC2 contributed 23.33%, PC3 (12.60%), PC4 (9.1%), and PC5 (5.5%) to the total variability. PCA analysis reveals the presence of variability among genotypes for different traits. The principal component analysis revealed that the first component strongly correlated with plant dry weight, fresh weight, plant growth, and seed weight, indicating that these traits vary together in the same direction. Therefore, the variation in the first component can be attributed largely to plant growth and seed attributes. Thus, it can be inferred that the growth traits of citrus are linked to seed weight and dimension. In the second component, the variation was greatest in germination percentage and germination metrics (GSI, MDG, PV and GV), indicating a strong relationship between germination and germination metrics (Fig. 2). The third component exhibited variation due to seed characteristics, suggesting a strong association between seed weight and dimension. The fourth and fifth components varied as measures of growth metrics (AGR and RGR) and days to germination traits, respectively. *C. jambhiri, C. trifoliata*, *C. limonia, C. latipes*, and *C. volkameriana*. *C. jambhiri, C. maxima*, and *C. latipes* had very high values for the first component, and it is expected that this germplasm would have high values for the characters with which they are strongly correlated.

Phylogenetic tree: The phylogenetic analysis of different potential citrus rootstocks was reconstructed based on germination and seedling growth characteristics (Supplementary Fig. 1). Results formed three major groups of citrus based on germination and other metrics: Group I included only one species (*C. trifoliata*). The second cluster consisted of *C. indica, C. jambhiri, C. volkameriana, C. limonia, C. latipes,* and *C. reshni*. The third group comprised the remaining eight species. It was also previously reported that cluster analysis using the neighbour-joining method showed that *Poncirus*, Microcitrus, and Eremocitrus are very distant from *Citrus* (Pang *et al*. 2007). Our study also indicated that *C. trifoliata* (syn. *Poncirus trifoliata*) exhibited a wide dissimilarity in evolutionary relationship with other citrus species based on germination and seedling growth behaviours. The second cluster comprised the majority of commonly used rootstocks in commercial citrus production,

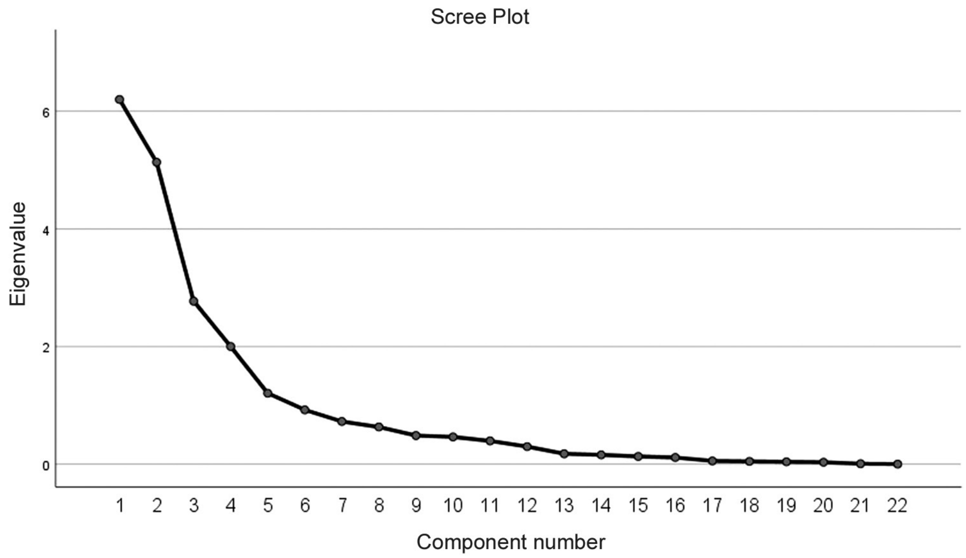

Fig. 3 Eingen value of different potential citrus rootstocks based on germination and seedling growth behaviours.

indicating their close genetic proximity.

Furthermore, cluster II comprised small-fruited species, except for *C. latipes*, which has a medium-sized fruit. Previously, *C. maxima, C. paradisi, C. sinensis, C. celebica, C. aurantium,* and *C. limon* were grouped in the identical cluster based on cpDNA data (Nicolosi *et al*. 2000). While *C. macroptera* and *C. aurantifolia* were grouped in a separate cluster (Nicolosi *et al*. 2000). Grapefruit is regarded as either an interspecific hybrid of pomelo and sweet orange or a hybrid or a mutant of pomelo (Wu *et al*. 2018). According to Hynniewta *et al*. (2014), *C. medica* and *C. limon* are closely related, and it is most probable that *C. limon* evolved from *C. medica* (Xie *et al*. 2008). Our study also indicated that *C. limon, C. karna, C. medica*, *C. macroptera, C. taiwanica, C. aurantifolia, C. maxima,* and *C. paradisi* were grouped in the same cluster, indicating a similar evolutionary relationship between them.

The study concluded that the potential citrus rootstocks exhibited significant variations in seed germination, physiology and metrics. The earliest seed germination was observed in *C. limon*, *C. karna,* and *C. jambhiri*. The highest percentages of seed germination and germination metrics (GSI, MDG, GV, and PV) were observed in *C. limonia*, *C. jambhiri*, *C. volkameriana*, *C. trifoliata*, *C. latipes*, and *C. maxima*. The highest seedling vigour was found in *C. jambhiri*, *C. maxima,* and *C. latipes.* Principal component analysis revealed the presence of variability for seed traits and germination. Based on phylogenetic analysis, *C. trifoliata* was found to be the most diverse form of other *Citrus* spp. Cluster II, comprising the majority of commonly used rootstocks in citrus (*C. indica, C. jambhiri, C. volkameriana, C. limonia, C. latipes,* and *C. reshni*), revealed their evolutionary relationships with each other. Therefore, this study suggests that seed germination, GSI, and other metrics can be reliable indicators of high-performance seed germination behaviours in different citrus rootstocks. A thorough investigation that combines molecular and multivariate methods can be employed to provide insight into the ecological and evolutionary factors influencing germination behaviours.

REFERENCES

Ali A S and eElozeiri A A. 2017. Metabolic processes during seed germination. *(In) Advances in Seed Biology*, 1st edn, Jimenez-Lopez J C (Ed). Intech Open. doi: 10.5772/intechopen.70653

Baskin C C and Baskin J M. 2014. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*, 2nd edn. Academic Press, San Diego, California.

Czabator F J. 1962. Germination value: An index combining speed and completeness of pine seed germination. *Forest Science* **8**(4): 386–96.

Daws M I, Ballard C, Mullins C E, Garwood N C, Murray B, Pearson T R H and Burslem D F R P. 2007. Allometric relationships between seed mass and seedling characteristics reveal trade-offs for neotropical gap-dependent species. *Oecologia* **154**: 445–54.

de Mello P A K X. 2020. Optimal germination temperature for 'swingle' citrumele seeds. ScientiaTec **7**: 03.

Eriksson O. 1999. Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*. *Acta Oecologica* **20**(1): 61–66.

Fandohan B, Achille E, Assogbadjo, Romain G K and Brice S. 2010. Variation in seed morphometric traits, germination and early seedling growth performances of *Tamarindus indica* L. *International Journal of Biological and Chemical Sciences* **4**(4): 1102–09.

Franco D, Cavalcante A I H L, Oliveira I V D M and Martins A B G. 2020. Evaluation of substrates in the initial development of six citrus rootstocks. *Citrus Research and Technology* **28**(1–2).

Geneve R L. 2003. Impact of temperature on seed dormancy. *HortScience* **38**(3): 336–41. doi: 10.21273/HORTSCI.38.3.336

Hayat F, Li J, Liu W, Li C, Song W, Iqbal S, Khan U, Umer J H, Ahsan A M, Tu P, Chen J and Liu J. 2022b. Influence of citrus rootstocks on scion growth, hormone levels, and metabolites profile of 'Shatangju' Mandarin (*Citrus reticulata* Blanco*). Horticulturae* **8**: 608. https://doi.org/10.3390/horticulturae8070608

Hynniewta M, Malik S and Rao S R. 2014. Genetic diversity and phylogenetic analysis of *Citrus* (L) from north-east India as revealed by meiosis, and molecular analysis of internal transcribed spacer region of rDNA. *Meta Gene* **2**(1): 237–51.

Lavagi-Craddock I, Campos R, Pagliaccia D, Kapaun T, Lovatt C and Vidalakis G. 2020. Citrus dwarfing viroid reduces canopy volume by affecting shoot apical growth of navel orange trees grown on trifoliate orange rootstock. *Journal of Citrus Pathology* **7**: 1–6.

Luro F, Curk F, Froelicher Y and Ollitrault P. 2017. Recent insights on citrus diversity and phylogeny. *(In) AGRUMED: Archaeology and History of Citrus Fruit in the Mediterranean: Acclimatization, Diversifications, Uses [online]*, pp. 1–13. Publications du Centre Jean, Bérard Naples. ISBN: 9782918887775. DOI: 10.4000/books.pcjb.2169

Madruga F, da Silva Almeida A, Rossetti C, Saraiva C R C, Figueiredo J C, Pagel G O, Klug E and Boeira K C. 2022. Overcoming dormancy in citrus rootstock seeds. *Colloquium Agrariae* **18**(3): 34–38.

Moles A T, Ackerly D D, Webb C O, Tweddle J C, Dickie J B, Pitman A J and Westoby M. 2005a. Factors that shape seed mass evolution. *(In) Proceedings of the National Academy of Sciences (PNAS) U S A* **102**: 10540–44.

Moles A T, Ackerly D D, Webb C O, Tweddle J C, Dickie J B and Westoby M. 2005b. A brief history of seed size. *Science* **307**: 576–80.

Nakagawa M and Oak N. 2000. *Seeds: Science, Technology and Production*, 4th edn, pp. 588. Jaboticabal FUNEP.

Nicolosi E, Deng Z, Gentile A, Malfa S L, Continella G and Tribulato E. 2000. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theoretical and Applied Genetics* **100**: 1155–66. doi:10.1007/s001220051419

Norden N, Daws M I, Antoine C, Gonzalez M A, Garwood N C and Chave J. 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. *Functional ecology* **23**: 203–10.

Pang X M, Hu C G and Deng X X. 2007. Phylogenetic relationships within *Citrus* and its related genera as inferred from AFLP markers. *Genetic Resources and Crop Evolution* **54**: 429–36.

Pélabon C, De Giorgi F, Opedal O H, Bolstad G H, Raunsgard A and Armbruster W S. 2021. Is there more to within-plant variation in seed size than developmental noise? *Evolutionary Biology* **48**: 366–77. doi:10.1007/s11692-021-09544-y

Penjor T, Yamamoto M, Uehara M, Ide M, Matsumoto N, Matsumoto R and Nagano Y. 2013. Phylogenetic relationships of citrus and its relatives based on matK gene sequences. *PLoS One* **8**(4): e62574. doi:10.1371/journal.pone.0062574

Rymbai H, Talang H D, Verma V K, Devi M B, Vanlaruati and Hazarika S. 2024. Innovative approaches for the production of certified quality planting materials in Khasi Mandarin (*Citrus reticulata* Blanco). *Indian Journal of Hill Farming* **37**(1): 205–13. doi:10.56678/iahf-2024.37.01.27

Shaukat S S, Siddique Z S and Aziz S. 1999. Seed size variation and its effects on germination, growth and seedling survival in *Acacia nilotica* subsp. Indica (Benth.) Brenan. *Pakistan Journal of Botany* **31**: 253–63.

Shiade S R G and Boelt B. 2020. Seed germination and seedling growth parameters in nine tall fescue varieties under salinity stress. *Acta Agric Scand, Section B- Soil and Plant Science* **70**(6): 485–94. doi:10.1080/09064710.2020.1779338

Upadhaya A, Chaturvedi S S, Tiwari B K and Paul D. 2019. Effect of temperature on germination of *Citrus macroptera, Citrus latipes* and *Citrus indica* seeds. *NEHU Journal* **17**(1): 12–20.

Wali S, Munir F and Mahmood T. 2013. Phylogenetic studies of selected Citrus species based on chloroplast gene, rps14. *International Journal of Agriculture and Biology* **15**(2): 357–61.

Wu G A, Terol J, Ibanez V, Lopez-García A, Perez-Roman E, Borreda C, Domingo C, Tadeo FR, Carbonell-caballero J, Alonso R, Curk F, Du D, Ollitrault P, Roose M L, Dopazo J, Gmitter Jr F G, Rokhsar D S and Talon M. 2018. Genomics of the origin and evolution of Citrus. *Nature* **554**: 311–16.

Xie R J, Zhou Z Q and Deng L. 2008. Taxonomic and phylogenetic relationships among the genera of the true citrus fruit trees group (*Aurantioideae, Rutaceae*) based on AFLP markers. *Journal of Systematics and Evolution* **46**(5): 682.