Analysis of phenotypic variation and selection of superior genotypes of *Balanites roxburghii* Planch. from South India

Guggalada Govardhana Yadav · Hosakatte Niranjan Murthy

Received: 10 August 2021 / Accepted: 20 February 2022 / Published online: 10 March 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract *Balanites roxburghii* Planch. is an important medicinal plant of India and used in the treatment of various diseases and disorders. Seeds possess a high amount of oil and could be used in the biodiesel production. This plant is also a source of diosgenin, a therapeutically useful compound. Domestication of this plant provides opportunities for the commercial production of those valuable products. Hence the present study was planned to assess the variability of *B. roxburghii* in Southern India and identifying the superior genotypes based on the morphological characterization. A total of 45 accessions from 35 populations were collected and variation in 9 quantitative and 3 qualitative morphological traits related to fruits was analyzed. Fruit weight showed the highest variation, ranging from 10.23 g in the accession KA-04C to 37.69 g in TN-03. Pulp weight ranged from 1.71 g in KA-04C to 10.24 g in TN-03. Seed kernel weight was highest in TE-04 (3.87 g) followed by KA-04A (3.21 g) and TE-05A (2.98 g). PCA analysis showed that fruit weight, fruit length, fruit width, pulp weight, rind weight and endocarp weight were the major traits contributing to the diversity. Cluster analysis resulted in the formation of 4 groups and among them, 2 accessions of cluster 3 and 6 accessions of cluster 4 showed superior fruit characters by having highest fruit weight, pulp weight and seed kernel weight. Accessions of these groups could be considered in the future domestication programs.

Keywords *Balanites roxburghii* · Diosgenin · Fruit morphology · South India

Introduction

Plants are being used as a major source of nutrition and medicine. They synthesize a wide array of chemicals, which can provide various health benefits. They are also utilized in garments, fuel-wood and feedstock for many industrial products such as timber, paper, chemicals and biodiesel. However, a large part of the plant species has not been recognized for their full potential. Such marginalized species are either semi-domesticated or wild and called as ‘underutilized species’. These underutilized species provide tremendous opportunities to fight against malnutrition and poverty by generating income for local people (Padulosi et al. 2013; Murthy and Bapat 2020).

Genus *Balanites* Delile (Zygophyllaceae) consists of several underutilized species, traditionally used for curing various diseases and disorders and also as a food source. The majority of the species of *Balanites* are distributed in the African continent. *Balanites*
aegyptiaca (L.) Delile is the most popular in the genus and have enormous traditional uses (Sands 2001). The fruits are eaten raw or processed to provide a variety of recipes and considered as nutritionally important due to the presence of 42.57% of carbohydrates and 9.57% of proteins with a good amount of minerals (Sands 2001; Sagna et al. 2014). The genus is characterized by a high amount of seed oil as in B. aegyptiaca (46.7%; Chapagain et al. 2009), B. rotundifolia (Tiegh.) Blatt. (31.0%; Radunz et al. 1985) and B. wilsoniana Dawe & Sprague (30.0%; Burkill 1985), which could be used in the biodiesel production (Chapagain et al. 2009).

In India, genus Balanites is represented by a single species, Balanites roxburghii Planch., and is distributed throughout the dry and arid lands including peninsular region (Sands 2001). Different parts of B. roxburghii are used in traditional medicine system to cure various ailments. The fruit is used to treat snake-bite and to deworm in children. Seeds are given in coughs and colic, and seed oil to cure burns, excoriation and freckles (Kirtikar and Basu 1918; Nadakarni 1954). Bark is used as anti-helminthic, spasmylic and also in cough. Paste of the bark is used to cure the skin diseases (Virendra et al. 2009).

Apart from the traditional uses, Balanites roxburghii is a good source of several valuable products. Similar to its allied species, B. roxburghii possesses a high amount of seed oil i.e. 40–42% (Arora and Tak 2013), that could be effectively utilized for the production of biodiesel (Khanvilkar et al. 2016). The different parts of B. roxburghii contain a good amount of diosgenin, a steroidal sapogenin used mainly as a precursor in the synthesis of most of the therapeutically useful steroidal drugs, including sex hormones and corticosteroids (Jesus et al. 2016). Dioscorea deltoidea Wall. ex Griseb. is the major source for the production of diosgenin in India, reported to contain 1.20% of diosgenin in its dried tuber (Nazir et al. 2021). D. deltoidea is recognized as critically endangered species due to overexploitation and trading for the production of diosgenin (Molur and Walker 1998). Various reports proved that B. roxburghii possess a good amount of diosgenin content in different parts such as root (0.97%), stem (0.36%), seed kernel (0.62%), fruit pulp (0.32%) and rind (0.15%); fluctuating up to 5.6% in fruits (Varshney and Vyas 1982; Amalraj and Shankarnarayan 1987). Hence, this species could be a good alternative for the production of diosgenin and reduce the pressure over D. deltoidea.

Systematic cultivation of wild plants could offer their full potential utilization (FAO 1999). This type of cultivation has the advantages of providing controlled and regular supply of valuable products and protects species from the risk of extinction due to overexploitation of wild populations (Schippmann et al. 2002). To bring a species into cultivation, it is very essential to assess the genetic diversity of the species, which enables the breeders to select genotypes with desirable characters (Govindaraj et al. 2015). Assessment of genetic variability at intraspecific level is performed by various techniques such as morphological, biochemical and DNA marker analysis (Govindaraj et al. 2015). However, comprehensive morphological diversity analysis provides a strong base to select the promising genotypes for the cultivation programs (Schlautman et al. 2020). Thus, it will be beneficial to assess the genetic diversity of B. roxburghii to bring the species into cultivation for the sustainable exploitation to produce valuable products such as seed oil and diosgenin, to meet the global demand. Amalraj and Shankarnarayan (1987) studied the variability of B. roxburghii in desert region of Rajasthan and found considerable diversity between the different genotypes. However, data on morphological diversity is lacking in the South Indian populations of B. roxburghii. Peninsular region of Southern India exhibits rich biodiversity and offer favourable conditions for the growth of B. roxburghii and probability of obtaining intraspecific variability will be more in this region. Therefore, main objectives of this paper were: (i) to evaluate the phenotypic diversity of B. roxburghii in Southern India; (ii) to assess diversity patterns of studied B. roxburghii populations; (iii) to identify morphologically superior genotypes which will be included in cultivation and production.

**Materials and methods**

Field survey

The present study was undertaken in the Southern region of India, including the states of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana, during the year 2019–2021 (Fig. 1; Table 1).
Extensive field studies were conducted in various forest areas that cover the major geographical regions of the above-mentioned states. Natural populations of *Balanites roxburghii* were located based on the floristic reports, herbarium reference and critical observation. Populations which were present in the forests or protected areas were only considered in the present study. Often, more than one type of fruit morphotypes that had considerable differences among them were identified within a single population and each morphotype was considered as an accession and materials were collected separately for further study.

**Plant material**

20 Matured plants were randomly selected from each accession and 10–15 fruits were collected from each plant and pooled together for further study. A minimum distance of 20 m was maintained between the selected plants. Fruits were collected in their ripened stage, indicated by their complete yellow colour, during the months of January-March.

**Morphological characterization**

A total of 12 morphological traits including qualitative and quantitative, were recorded for 45 accessions of *B. roxburghii* (Table 2). Characterization of fruits was done by randomly picking 30 fruits from each accession. Pulp and rind were separated and endocarp was broken mechanically to obtain the seed kernel. Quantitative characterization was carried out by taking measurements individually for all the selected fruits. The weight of fruit, pulp, rind, seed kernel and endocarp were recorded with the...
help of electronic balance and expressed in grams, whereas fruit length and fruit width were recorded using Vernier caliper and expressed in centimetres. Qualitative traits such as shape of fruit apex and ridges on fruits were recorded by their appearances and fruit shape was assigned according to Beentje (2016). Qualitative traits were assigned with numerical values (phenotypic scores) ranging from 1 to k (k equal to number of classes), to indicate different degrees of expression (Table 2). These values were further used to compare the qualitative and quantitative data together.

### Table 1 Geographical locations of populations of *Balanites roxburghii* distributed in the Southern India

| State          | Population | Location       | District     | Latitude          | Longitude         | Altitude (m) |
|----------------|------------|----------------|--------------|-------------------|-------------------|--------------|
| Andhra Pradesh | AP-01      | Giddangivaripalli | Kadapa       | 14° 18’ 14.07” N | 78° 19’ 28.46” E | 362          |
|                | AP-02      | Jaladurgam      | Kurnool      | 15° 15’ 06.25” N | 77° 53’ 42.56” E | 412          |
|                | AP-03      | Kallampilli     | Anantapuram  | 13° 55’ 15.82” N | 77° 38’ 48.29” E | 697          |
|                | AP-04      | Omkar Temple    | Kurnool      | 15° 36’ 08.45” N | 78° 36’ 37.95” E | 236          |
|                | AP-05      | Pacherlal       | Kurnool      | 15° 25’ 36.85” N | 78° 43’ 42.76” E | 320          |
|                | AP-06      | Rachepalli      | Kadapa       | 14° 12’ 48.74” N | 78° 49’ 30.19” E | 324          |
|                | AP-07      | Siddapuram      | Kurnool      | 15° 49’ 46.15” N | 78° 40’ 07.99” E | 285          |
|                | AP-08      | Thummalabailu   | Prakasam     | 15° 58’ 36.93” N | 78° 54’ 09.13” E | 656          |
|                | AP-09      | Venkatagaripalli| Anantapuram  | 14° 06’ 13.65” N | 77° 44’ 15.52” E | 508          |
| Karnataka      | KA-01      | Amareshwara     | Raichur      | 16° 15’ 50.96” N | 76° 32’ 35.95” E | 444          |
|                | KA-02      | Bannerghatta    | Bengaluru rural | 12° 42’ 22.54” N | 77° 34’ 55.56” E | 806          |
|                | KA-03      | Chennagiri      | Davanagere   | 13° 58’ 04.89” N | 76° 01’ 46.17” E | 732          |
|                | KA-04      | Chowdlapura     | Tumakuru     | 13° 19’ 21.80” N | 76° 43’ 50.49” E | 870          |
|                | KA-05      | Gudibande       | Chikballapurapura | 13° 41’ 58.25” N | 77° 41’ 50.87” E | 844          |
|                | KA-06      | Jenukallupalya  | Ramanagara   | 12° 52’ 22.15” N | 77° 14’ 03.19” E | 837          |
|                | KA-07      | Kalinganahalli  | Tumakuru     | 13° 34’ 14.44” N | 76° 48’ 28.43” E | 756          |
|                | KA-08      | Kappathagudda   | Gadaga       | 15° 13’ 14.34” N | 75° 42’ 31.29” E | 674          |
|                | KA-09      | Malebennuru     | Davanagere   | 14° 19’ 02.64” N | 75° 42’ 34.64” E | 637          |
|                | KA-10      | Moka            | Ballari      | 15° 15’ 18.55” N | 77° 04’ 16.36” E | 406          |
|                | KA-11      | Ranebennuru     | Haveri       | 14° 38’ 50.06” N | 75° 41’ 01.61” E | 585          |
|                | KA-12      | Ratnapura       | Raichur      | 15° 51’ 18.95” N | 76° 29’ 27.22” E | 495          |
|                | KA-13      | Shiruguppi      | Bagalakote   | 16° 14’ 58.74” N | 75° 47’ 16.88” E | 545          |
|                | KA-14      | Thondala        | Kolara       | 13° 10’ 57.82” N | 78° 14’ 49.33” E | 814          |
| Tamil Nadu     | TN-01      | Chinna Sakkanavaram | Krishnagiri | 12° 41’ 23.83” N | 78° 14’ 29.53” E | 620          |
|                | TN-02      | Coimbatore      | Coimbatore   | 11° 01’ 06.61” N | 76° 56’ 37.91” E | 436          |
|                | TN-03      | Madhanakupparam | Krishnagiri | 12° 35’ 34.08” N | 78° 19’ 21.72” E | 527          |
| Telangana      | TE-01      | Bornapalli      | Jagtial      | 19° 03’ 33.41” N | 78° 49’ 51.36” E | 202          |
|                | TE-02      | Buddharam       | Mancherial   | 18° 56’ 19.61” N | 79° 42’ 39.07” E | 167          |
|                | TE-03      | Burugpet        | Bhupalapalli | 18° 20’ 10.90” N | 80° 04’ 14.94” E | 198          |
|                | TE-04      | DoravariVempalli| Mahabubabad  | 17° 55’ 06.68” N | 80° 10’ 17.38” E | 321          |
|                | TE-05      | Katapuram       | Mulugu       | 18° 08’ 13.44” N | 80° 21’ 53.86” E | 209          |
|                | TE-06      | Pegadapalli     | Mulugu       | 18° 06’ 33.94” N | 80° 03’ 26.28” E | 204          |
|                | TE-07      | Pocharam        | Medak        | 18° 06’ 42.18” N | 78° 13’ 01.50” E | 464          |
|                | TE-08      | Talamalla       | Mancherial   | 18° 59’ 32.34” N | 79° 15’ 42.11” E | 239          |
|                | TE-09      | Udumpur         | Adilabad     | 19° 11’ 55.28” N | 78° 54’ 13.97” E | 247          |

Statistical analysis

Descriptive statistics such as mean, standard deviation, standard error and coefficient of variation were calculated for each quantitative trait for each
accession using Microsoft Excel 2016 and all other analyses were carried out by using IBM SPSS Statistics software version 20. Mean values and phenotypic scores were used for quantitative and qualitative traits respectively, for further analysis. Pearson correlation analysis has been carried out to find the strength of linear association between each morphological trait. Original values were standardized and Z-scores were used in the principal component analysis, to identify the prominent characteristics representing the accessions. Finally, accessions were grouped by k-means clustering method and scores of first two principal components were used to draw the scatter plot.

Table 2 Morphological traits used for the variability analysis of *Balanites roxburghii* accessions

| Trait Description                  | Phenotype score |
|-----------------------------------|-----------------|
| **Qualitative traits**            |                 |
| Fruit shape                       | 1. Round        |
| The shape of matured fruit        | 2. Elliptic     |
| 1. Round                          | 3. Oval         |
| 2. Elliptic                       | 4. Obovate      |
| 3. Oval                           | 5. Oblong       |
| 4. Obovate                        | 6. Pyriform     |
| 5. Oblong                         |                 |
| 6. Pyriform                       |                 |
| Fruit apex                        | 1. Flattened    |
| The appearance of apex of fruit   | 2. Round        |
| 1. Flattened                      | 3. Acute        |
| 2. Round                          |                 |
| 3. Acute                          |                 |
| Ridges on fruit                   | 1. Absent       |
| Ridges/lines on the surface of the fruit | 2. Pale     |
| 1. Absent                         | 3. Prominent    |
| 2. Pale                           |                 |
| 3. Prominent                      |                 |
| **Quantitative traits**           |                 |
| Fruit weight                      | Measured in gram|
| Measured in gram                  |                 |
| Fruit length                      | Measured in cm  |
| Measured in cm                    |                 |
| Fruit width                       | Measured in cm  |
| Measured in cm                    |                 |
| Pulp weight                       | Measured in gram|
| Measured in gram                  |                 |
| Rind weight                       | Measured in gram|
| Measured in gram                  |                 |
| Seed Kernel weight                | Measured in gram|
| Measured in gram                  |                 |
| Endocarp weight                   | Measured in gram|
| Measured in gram                  |                 |
| % Composition of pulp             | Per 100 g of fruit|
| Per 100 g of fruit                |                 |
| % Composition of seed kernel      | Per 100 g of fruit|
| Per 100 g of fruit                |                 |

**Results and discussion**

**Distribution**

The Southern India, including the states of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana possess considerable topographic, edaphic and climatic heterogeneity and resulted in the rich biodiversity. In all the states except Kerala, we identified the natural populations of *B. roxburghii*. A total of 45 accessions from 35 natural populations were identified and the details of localities of populations and list of accessions are presented in Tables 1 and 3 respectively. *B. roxburghii* is distributed throughout the dry and arid regions of Southern India, majorly concentrated in Central, Southern and Northern interiors of Karnataka, and Rayalaseema and Eastern Ghats regions of Andhra Pradesh. Northern Telangana and North-Western Tamil Nadu regions are also major centres of distribution (Fig. 1). The distributional altitude ranges from 167 to 870 m above mean sea level (Table 1).

Genus *Balanites* consists of nine species, distributed in the dry and arid lands of Africa, part of Western Asia and Indian Subcontinent. Among them, one species is from India, one from Myanmar and remaining are from African continent. They are usually found in low rainfall areas, dry and open regions, and prefer comparatively high-water available places such as river banks and seasonal water-logged flood plains where ground water compensates for low rain fall. But only *B. wilsoniana* is reported to be distributed in humid, semi-evergreen
| Accession number | Accession | Fruit weight (g) | Fruit length (cm) | Pulp weight (g) | Seed kernel weight (g) | Endocarp weight (g) | % Composition of seed kernel (%) | Cluster group |
|------------------|-----------|------------------|------------------|----------------|-----------------------|------------------|-------------------------------|--------------|
| AP-01A           | AP-01     | 23.12 ± 1.13     | 4.73 ± 0.13      | 3.50 ± 0.19    | 5.98 ± 0.50           | 3.11 ± 0.09      | 1.30 ± 0.09                    | 1            |
| AP-01B           | AP-01     | 15.23 ± 0.89     | 5.04 ± 0.19      | 3.21 ± 0.07    | 6.05 ± 0.38           | 3.50 ± 0.19      | 1.24 ± 0.02                    | 2            |
| AP-02A           | AP-02     | 21.80 ± 0.70     | 4.97 ± 0.07      | 3.59 ± 0.07    | 6.05 ± 0.38           | 3.50 ± 0.19      | 1.24 ± 0.02                    | 3            |
| AP-02B           | AP-02     | 18.66 ± 0.93     | 4.68 ± 0.06      | 3.59 ± 0.07    | 6.05 ± 0.38           | 3.50 ± 0.19      | 1.24 ± 0.02                    | 4            |
| AP-03            | AP-03     | 17.25 ± 0.62     | 4.86 ± 0.06      | 3.45 ± 0.05    | 4.32 ± 0.22           | 3.18 ± 0.15      | 1.57 ± 0.10                    | 2            |
| AP-04            | AP-04     | 16.52 ± 0.65     | 3.75 ± 0.10      | 3.16 ± 0.05    | 2.99 ± 0.12           | 3.25 ± 0.20      | 1.03 ± 0.10                    | 4            |
| AP-05            | AP-05     | 15.80 ± 0.12     | 3.80 ± 0.11      | 3.20 ± 0.12    | 2.84 ± 0.20           | 3.18 ± 0.20      | 1.03 ± 0.10                    | 7            |
| AP-06            | AP-06     | 22.66 ± 0.89     | 5.28 ± 0.14      | 3.32 ± 0.05    | 4.29 ± 0.16           | 2.44 ± 0.21      | 1.21 ± 0.09                    | 8            |
| AP-07            | AP-07     | 17.21 ± 0.47     | 5.36 ± 0.04      | 3.69 ± 0.03    | 5.94 ± 0.15           | 3.09 ± 0.15      | 2.47 ± 0.10                    | 9            |
| AP-08            | AP-08     | 15.60 ± 0.73     | 5.08 ± 0.15      | 3.35 ± 0.04    | 3.89 ± 0.25           | 2.68 ± 0.23      | 2.24 ± 0.13                    | 10           |
| AP-09            | AP-09     | 16.52 ± 0.65     | 3.75 ± 0.10      | 3.16 ± 0.05    | 2.99 ± 0.12           | 3.25 ± 0.20      | 1.03 ± 0.10                    | 11           |
| AP-10            | AP-10     | 17.10 ± 0.60     | 3.80 ± 0.11      | 3.20 ± 0.12    | 2.84 ± 0.20           | 3.18 ± 0.20      | 1.03 ± 0.10                    | 12           |
| AP-11            | AP-11     | 19.73 ± 0.28     | 4.56 ± 0.12      | 3.47 ± 0.12    | 4.28 ± 0.50           | 4.76 ± 0.44      | 2.34 ± 0.12                    | 13           |
| KA-01            | KA-01     | 19.73 ± 0.28     | 4.56 ± 0.12      | 3.47 ± 0.12    | 4.28 ± 0.50           | 4.76 ± 0.44      | 2.34 ± 0.12                    | 14           |
| KA-02            | KA-02     | 22.04 ± 0.92     | 5.37 ± 0.11      | 3.59 ± 0.08    | 5.94 ± 0.15           | 3.09 ± 0.15      | 2.47 ± 0.10                    | 15           |
| KA-03            | KA-03     | 29.57 ± 0.14     | 7.33 ± 0.29      | 5.26 ± 0.12    | 7.11 ± 0.14           | 5.26 ± 0.12      | 2.51 ± 0.10                    | 22           |
| KA-04A           | KA-04     | 29.57 ± 0.14     | 7.33 ± 0.29      | 5.26 ± 0.12    | 7.11 ± 0.14           | 5.26 ± 0.12      | 2.51 ± 0.10                    | 23           |
| KA-04B           | KA-04     | 31.49 ± 0.50     | 14.40 ± 0.94     | 14.00 ± 0.64   | 16.90 ± 0.17          | 16.90 ± 0.17     | 2.51 ± 0.10                    | 24           |
| KA-05            | KA-05     | 30.98 ± 0.58     | 5.17 ± 0.26      | 6.58 ± 0.67    | 4.13 ± 0.21           | 4.13 ± 0.21      | 2.51 ± 0.10                    | 25           |
| KA-06            | KA-06     | 31.48 ± 0.58     | 5.17 ± 0.26      | 6.58 ± 0.67    | 4.13 ± 0.21           | 4.13 ± 0.21      | 2.51 ± 0.10                    | 26           |
| KA-07            | KA-07     | 32.49 ± 0.68     | 6.04 ± 0.26      | 6.79 ± 0.67    | 4.13 ± 0.21           | 4.13 ± 0.21      | 2.51 ± 0.10                    | 27           |
| KA-08            | KA-08     | 31.92 ± 0.65     | 5.48 ± 0.20      | 7.24 ± 0.37    | 3.04 ± 0.10           | 3.04 ± 0.10      | 2.51 ± 0.10                    | 28           |
| KA-09            | KA-09     | 20.55 ± 0.69     | 4.47 ± 0.08      | 5.89 ± 0.67    | 4.13 ± 0.21           | 4.13 ± 0.21      | 2.51 ± 0.10                    | 29           |
| KA-10A           | KA-10A    | 22.04 ± 0.90     | 4.37 ± 0.08      | 5.89 ± 0.67    | 4.13 ± 0.21           | 4.13 ± 0.21      | 2.51 ± 0.10                    | 30           |
| KA-11B           | KA-11B    | 20.55 ± 0.69     | 4.47 ± 0.08      | 5.89 ± 0.67    | 4.13 ± 0.21           | 4.13 ± 0.21      | 2.51 ± 0.10                    | 31           |
| KA-12            | KA-12     | 16.50 ± 0.65     | 4.86 ± 0.07      | 5.90 ± 0.28    | 4.25 ± 0.12           | 4.25 ± 0.12      | 2.51 ± 0.10                    | 32           |
Table 3 (continued)

| Accession number | Accession | Fruit weight (g) | Fruit length (cm) | Fruit width (cm) | Pulp weight (g) | Rind weight (g) | Seed kernel weight (g) | Endocarp weight (g) | % Composition of pulp (%) | % Composition of seed kernel (%) | Cluster group |
|------------------|-----------|------------------|-------------------|------------------|----------------|----------------|-----------------------|--------------------|--------------------------|-----------------------------|--------------|
| 33               | TN-03     | 37.69 ± 0.94     | 5.21 ± 0.05       | 4.26 ± 0.05      | 10.24 ± 0.52   | 6.83 ± 0.18    | 2.47 ± 0.13           | 18.15 ± 0.41       | 27.18 ± 1.30               | 6.55 ± 0.36                  | 4            |
| 34               | TE-01     | 26.14 ± 1.25     | 5.16 ± 0.07       | 3.44 ± 0.06      | 5.66 ± 0.42    | 3.51 ± 0.16    | 1.96 ± 0.11           | 15.00 ± 0.76       | 21.67 ± 0.82               | 7.52 ± 0.76                  | 1            |
| 35               | TE-02     | 28.50 ± 1.10     | 5.70 ± 0.30       | 3.80 ± 0.22      | 4.99 ± 0.32    | 4.18 ± 0.21    | 2.37 ± 0.16           | 16.82 ± 0.60       | 17.50 ± 0.55               | 8.30 ± 0.36                  | 1            |
| 39               | TE-03     | 28.11 ± 0.92     | 5.35 ± 0.40       | 3.46 ± 0.19      | 4.72 ± 0.48    | 4.17 ± 0.45    | 2.34 ± 0.29           | 16.90 ± 0.87       | 16.79 ± 0.87               | 8.32 ± 0.92                  | 1            |
| 37               | TE-04     | 33.41 ± 0.92     | 7.66 ± 0.19       | 3.43 ± 0.05      | 5.05 ± 0.25    | 4.16 ± 0.13    | 3.87 ± 0.15           | 20.33 ± 0.51       | 15.10 ± 0.47               | 11.59 ± 0.40                 | 3            |
| 38               | TE-05A    | 36.38 ± 0.91     | 6.63 ± 0.05       | 3.72 ± 0.06      | 8.58 ± 0.24    | 6.48 ± 0.25    | 2.98 ± 0.19           | 18.33 ± 0.44       | 23.57 ± 0.36               | 8.20 ± 0.46                  | 4            |
| 39               | TE-05B    | 19.69 ± 1.50     | 4.63 ± 0.15       | 3.26 ± 0.09      | 4.45 ± 0.30    | 3.17 ± 0.20    | 1.89 ± 0.22           | 10.17 ± 0.50       | 22.61 ± 0.88               | 9.60 ± 0.98                  | 2            |
| 40               | TE-06A    | 22.01 ± 1.08     | 4.98 ± 0.06       | 3.52 ± 0.06      | 5.51 ± 0.27    | 3.46 ± 0.15    | 2.03 ± 0.18           | 11.00 ± 0.69       | 25.03 ± 1.03               | 9.22 ± 0.59                  | 1            |
| 41               | TE-06B    | 18.07 ± 1.01     | 5.34 ± 0.12       | 3.04 ± 0.08      | 4.15 ± 0.15    | 3.45 ± 0.17    | 1.63 ± 0.13           | 8.84 ± 0.70        | 22.97 ± 1.08               | 9.02 ± 0.24                  | 2            |
| 42               | TE-07A    | 25.48 ± 0.47     | 4.32 ± 0.07       | 3.77 ± 0.04      | 5.61 ± 0.20    | 4.54 ± 0.13    | 1.52 ± 0.15           | 13.72 ± 0.34       | 22.02 ± 0.70               | 5.97 ± 0.50                  | 1            |
| 43               | TE-07B    | 21.19 ± 0.69     | 3.87 ± 0.03       | 3.42 ± 0.04      | 6.48 ± 0.34    | 2.77 ± 0.10    | 2.36 ± 0.18           | 9.59 ± 0.31        | 30.56 ± 0.89               | 11.12 ± 0.62                 | 1            |
| 44               | TE-08     | 23.99 ± 1.09     | 5.07 ± 0.17       | 3.47 ± 0.06      | 5.65 ± 0.38    | 3.50 ± 0.15    | 2.62 ± 0.10           | 12.21 ± 0.60       | 23.56 ± 0.58               | 10.94 ± 0.49                 | 1            |
| 45               | TE-09     | 17.93 ± 0.61     | 4.80 ± 0.08       | 3.17 ± 0.06      | 5.18 ± 0.26    | 2.94 ± 0.12    | 1.06 ± 0.05           | 8.73 ± 0.34        | 28.91 ± 0.95               | 5.91 ± 0.17                  | 2            |
| Min              | 10.23     | 3.56             | 2.72              | 1.71             | 2.15           | 0.77           | 4.92                  | 15.05              | 4.15                     |                           |              |
| Max              | 37.69     | 7.66             | 4.37              | 10.24            | 7.08           | 3.87           | 20.33                 | 35.62              | 14.37                    |                           |              |
| Average          | 22.51     | 4.72             | 3.49              | 5.09             | 3.91           | 1.98           | 11.52                 | 22.49              | 8.98                     |                           |              |
| SD               | 6.44      | 0.87             | 0.35              | 1.81             | 1.20           | 0.62           | 3.66                  | 4.71               | 2.28                     |                           |              |
| CV%              | 28.59     | 18.49            | 10.05             | 35.46            | 30.63          | 31.16          | 31.78                 | 20.93              | 25.34                    |                           |              |

Each value represents the mean ± standard error of 30 fruits of respective accession. Min – minimum value; Max – maximum value; SD—Standard deviation; CV– Coefficient of variation.
or rain forests. They grow on variety of soil types including deep sands, sandy-loam, clay-loam and alluvium soils and are rarely found in stony soils. Generally, they are associated with spiny species of *Acacia* Mill., *Ziziphus* Mill., *Capparis* Tourn. ex L. and *Albizia* Durazz. (Sands 2001).

Similar to its allied species, *B. roxburghii* is also a highly drought-resistant tree species, occurring mainly in scruffy and deciduous forests. It is mostly found along the roadsides, banks of streams and base of the hills where water availability is relatively high and disappears on slopes and top of the hills. Plants which grow in thick forests prefer open areas. *B. roxburghii* grow rich in black cotton soil, sandy soil and red soil, but rarely found in rocky areas. Sands (2001) also reported the similar ecological conditions for *B. roxburghii*. Eastern part of the Karnataka and Western part of the Andhra Pradesh are the major distribution centres for *B. roxburghii* in Southern India and those regions receives less rainfall compared to the average rainfall of the respective states (Guhathakurta et al. 2020a, 2020b). The other major centres, Northern Telangana and North-Western Tamil Nadu regions receive comparatively more rainfall than the districts of Karnataka and Andhra Pradesh (Guhathakurta et al. 2020c, 2020d). Ouedraogo et al. (2020) reported the geographical effect on the production of fruits in *B. aegyptiaca*. They found that, the average fruit biomass produced in Sahelian and Sudano-Sahelian regions of Africa was 2.99 and 6.32 kg per tree respectively. They also found that the number of fruits and fruit biomass produced per tree had significant positive correlation with stem diameter and tree height. In another study, Weber et al. (2019) reported that, plants grown from the seeds of 12 geographical regions of Niger, where the annual rainfall ranges between 300 to 550 mm, shown significant variability in the trait of root weight to shoot weight ratio. These findings suggest that, environmental conditions combined with genetic variation resulted in the creation of a great morphological diversity of fruits among the different accessions of *B. roxburghii* in Southern India.

Morphological characterization

Morphological variability is an adaptive strategy expressed by plants which allows them to be better fitted to the environment. Analysis of 12 morphological characters including the quantitative and qualitative traits (Table 2) among the 45 accessions has shown significant variability between the accessions and they are presented in Tables 3 and 4 respectively. Fruit weight is the prominent trait and it was influenced by weight of individual constituents such as pulp, rind, seed kernel and endocarp. Fruit weight ranged from 10.23 g in the accession KA-04C to 37.69 g in the accession TN-03 (Table 3). More than half of the accessions (51%) have the fruit weight in the range of 17 to 24 g and seven accessions have more than 30 g (KA-04A, KA-04B, KA-13A, KA-13B, TN-03, TE-04 and TE-05A; Table 3). Fruit weight is directly affected by fruit length and fruit width. Fruit length ranged from 3.56 cm (AP-07) to 7.66 cm (TE-04) and fruit width ranged from 2.72 cm (AP-01B) to 4.37 cm (KA-13B). Similarly, previous reports showed a high variability in *B. roxburghii* with respect to fruit weight (6 to 80 g), fruit length (2.6 to 8.2 cm) and fruit width (2.2 to 6.3 cm) among the 50 types of fruits collected in 10 locations in the desert region of Rajasthan (Amalraj and Shankarnarayan 1987).

Pulp is the edible portion of the fruit, used for its medicinal and nutritional properties. It showed higher variation between the accessions, ranging from 1.71 g (KA-04C) to 10.24 g (TN-03). In some cases, percentage composition of pulp had considerable impact on the weight of the pulp. For instance, accession TE-04 had the fruit weight of 33.41 g and pulp weight of 5.05 g whereas accession TE-09 has only...
17.93 g of fruit weight, nearly half of the former one, and the pulp weight of 5.18 g. This is because, despite of smaller size and less weight, fruits of TE-09 have higher pulp composition (28.91%). On the other hand, though fruits of TE-04 are large sized and have more weight, contribution of pulp to the total fruit weight is very less (15.10%) when compared to that of TE-09. The maximum percentage composition of pulp was observed in KA-10B (35.62%). Rind is the outer covering of the fruit, which showed less variation compared to the pulp, ranging from 2.15 g (KA-04C) to 7.08 g (KA-04B). Seed kernel is the innermost portion of the fruit that contains high amount of oil. Larger seed kernel could be considered as a desired character to select an accession for the domestication programs. Seed kernel weight is highest in the accession TE-04 (3.87 g) followed by KA-04A (3.21 g) and TE-05A (2.98 g). Usually, larger fruit comprise of larger seed kernel but similar to the pulp weight, seed kernel weight is also affected by percentage composition of seed kernel and it ranged from 4.15% (AP-02B) to 14.37% (AP-07). Endocarp constitutes about half of the fruit weight and it ranged between 4.92 g in accession KA-04C to 20.33 g in TE-04. Abasse et al. (2011) reported variation in seed kernel weight among the different populations of B. aegyptiaca, ranging from 0.53 g to 0.63 g. Seed kernels of B. roxburghii are larger than the seed kernels of B. aegyptiaca and its weight ranged from 0.77 g to 3.87 g in different accessions.

Coefficient of variation is the measure of relative dispersion and variability extent of a character in relation to its average value (Brown 1998). In the present study, pulp weight showed greater variation among the accessions than other traits by having a coefficient of variation of 35.46% followed by endocarp weight (CV = 31.78%), seed kernel weight (CV = 31.15%), rind weight (CV = 30.63%) and fruit weight (CV = 28.59%). Fruit width and fruit length were least variable compared to all other characters with the CV of 10.05% and 18.49% respectively. Variability of fruit weight among the populations of B. roxburghii in desert region of Rajasthan is very high with a coefficient of variation of 44.56% (Amalraj and Shankarnarayan 1987). Fruit length and fruit width were less diverse as compared to fruit weight with coefficient of variation of 21.48% and 18.03% respectively, in that region (Amalraj and Shankarnarayan 1987). Similar type of variability pattern has been reported among the different populations of B. aegyptiaca grown in Maradi region of Eastern Niger (Abasse et al. 2011). In that study, seed kernel weight (CV = 38.89%), endocarp weight (CV = 29.15%) and fruit weight (CV = 26.23%) had the higher variation compared to fruit length (CV = 13.81%) and fruit width (CV = 11.45%). The high degree of variability of fruit morphological traits among the accessions of B. roxburghii reflects the genotypic effects, environmental effects and interaction between the both.

Among the qualitative traits studied, fruit shape shown the highest variability among the accessions (Fig. 2). Elliptic shape is the prominent one, represented by 46.67% of the accessions followed by obovate (17.78%) and oblong (13.33%) shapes. Pyriform fruit shape presents only in TE-04 accession (Table 4). Flattened type of fruit apex is the predominant character represented by 55.56% of the accessions and pointed type was present in few accessions (13.33%) (Table 4). Majority of the accessions (68.89%) have ridges as pale lines and very few (8.89%) have no ridges or lines on the fruits (Table 4).

In their report on the diversity of B. aegyptiaca in Maradi region of Eastern Niger, Abasse et al. (2011) hypothesize that, trees present in drier regions tends to produce longer and narrower seeds and heavier seed kernels and show less variability in fruit and seed width. Longer and thinner seeds provide more surface area for water absorption and larger seed kernels can store more energy for rapid seedling development, which are very essential in water scarce regions. However, the present study revealed an abundant diversity in the fruit morphological traits among the 45 accessions distributed in different geographical locations of Southern India. Often, there was a considerable difference between the accessions of a same population. This variation within a population where the environmental conditions are same for all the plants is perhaps due to the existence of genetic variability and interaction between genotypic and environmental conditions.

Correlation between the traits

Understanding the relationship between the morphological traits could facilitate the selection of genotypes for cultivation programs. Pearson’s correlation coefficients have been used to measure the strength of linear association between the traits, where the ‘r’
Fig. 2  Morphological variability of fruits of *Balanites roxburghii* accessions collected from Southern India; Scale: Bar = 1 cm
Table 5  Pearson’s correlation matrix for the morphological traits used to assess the diversity of *Balanites roxburghii*

|            | Fruit weight | Fruit length | Fruit width | Pulp weight | Rind weight | Seed kernel weight | Endocarp weight | % Composition of pulp | % Composition of seed kernel | Fruit shape | Fruit apex | Ridges on fruit |
|------------|--------------|--------------|-------------|-------------|-------------|-------------------|-----------------|-----------------------|-------------------------------|-------------|------------|-----------------|
| Fruit weight | 1.000        |              |             |             |             |                   |                 |                       |                               |             |            |                 |
| Fruit length | 0.736**      | 1.000        |             |             |             |                   |                 |                       |                               |             |            |                 |
| Fruit width  | 0.794**      | 0.287        | 1.000       |             |             |                   |                 |                       |                               |             |            |                 |
| Pulp weight  | 0.825**      | 0.484**      | 0.808**     | 1.000       |             |                   |                 |                       |                               |             |            |                 |
| Rind weight  | 0.863**      | 0.525**      | 0.815**     | 0.745**     | 1.000       |                   |                 |                       |                               |             |            |                 |
| Seed kernel weight | 0.664**     | 0.564**      | 0.457**     | 0.437**     | 0.394**     | 1.000             |                 |                       |                               |             |            |                 |
| Endocarp weight | 0.950**     | 0.783**      | 0.646**     | 0.633**     | 0.750**     | 0.651**           | 1.000           |                       |                               |             |            |                 |
| % Composition of pulp | 0.105       | -0.081       | 0.336*      | 0.632**     | 0.095       | -0.101            | -0.146          | 1.000                 |                               |             |            |                 |
| % Composition of seed kernel | -0.318*     | -0.199       | -0.325*     | -0.395**    | -0.435**    | 0.457**           | -0.295*         | -0.292                | 1.000            |             |            |                 |
| Fruit shape  | 0.184        | 0.494**      | -0.168      | 0.005       | -0.040      | 0.196             | 0.300*          | -0.174                | -0.066           | 1.000       |            |                 |
| Fruit apex   | 0.146        | 0.564**      | -0.221      | -0.029      | -0.047      | 0.102             | 0.267           | -0.205                | -0.021           | 0.270       | 1.000      |                 |
| Ridges on fruit | 0.071        | 0.143        | -0.075      | -0.054      | 0.025       | -0.016            | 0.143           | -0.181                | -0.106           | 0.080       | 0.203     | 1.000           |

**Correlation is significant at the 0.01 level (2-tailed)**

*Correlation is significant at the 0.05 level (2-tailed)
value of ‘1’ indicates a complete positive correlation and ‘-1’ indicates a complete negative correlation (Table 5). The majority of the analyzed traits have a positive correlation with each other. Fruit weight is an important trait, which is directly influenced by individual constituents and has shown high correlation with the pulp weight, rind weight, seed kernel weight and endocarp weight (Table 5). Among them, endocarp weight showed very high positive correlation with fruit weight (r = 0.95) and had a very dominant effect on it by accounting for 37.65% (KA-10B) to 62.65% (AP-04) of the total weight. Fruit weight also had a high correlation with pulp weight (r = 0.83), rind weight (r = 0.86), fruit length (r = 0.74) and fruit width (r = 0.79). Though fruit length and fruit width have no significant correlation between each other, they seem to influence the other characters. Fruits with more length tend to have high endocarp (r = 0.78) and seed kernel content (r = 0.56) whereas those with more width tend to have a high amount of pulp (r = 0.81) and rind content (r = 0.82). Percentage composition of pulp also had a significant positive correlation (r = 0.34) with fruit width. Seed kernel weight is another important trait, which has great commercial value because of high seed oil content. It has a good correlation with fruit weight (r = 0.66), fruit length (r = 0.56) and fruit width (r = 0.46) and thus fruit weight and its dimensions could be the good indicators of large seed kernels. Correlation pattern of seed kernel with fruit weight (r = 0.80), fruit length (r = 0.79) and fruit width (r = 0.82) was reported in Prunus armeniaca L. (Zargar et al. 2021). Abasse et al. (2011) reported the similar pattern of correlation between the different traits in B. aegyptiaca. In that study, fruit weight had significant correlation with fruit length (r = 0.46) and fruit width (r = 0.61). However, they reported a negative correlation of the seed kernel weight with fruit weight. Ahmed et al. (2020) reported a significant negative correlation between percentage composition of seed kernel and fruit weight (r = -0.45) in B. aegyptiaca. In the present study, seed kernel weight has significant positive correlation with fruit weight (r = 0.66) but percentage composition of seed kernel is negatively correlated with fruit weight (r = -0.32), indicating that the percentage composition of seed kernel will be more in small fruits.

Quantitative and qualitative traits have very weak correlation between them, except for the fruit length. Fruit length had a significant positive correlation with fruit shape and fruit apex (Table 5). As the fruit length increases, fruits tend to acquire round to elliptic to oblong shape and acute apex. Even though ridges/lines on the surface of the fruit won’t have any correlation with other characters, fruit with no ridges is an appreciative character, because the fruits with prominent ridges have their rind deeply inserted into the pulp which makes the rind very difficult to separate from the pulp. In contrast to that, rind with no or pale ridges is easily separable. Zargar et al. (2021) tested the correlation among the 28 morphological characters in Prunus armeniaca and they found that fruit shape and fruit apex traits didn’t had any significant correlation with other traits. The present results suggest that we can select particular genotypes for both fruit and seed kernel traits. Along with that, we can also consider selecting and improving distinct genotypes for fruit and seed kernel traits.

PCA analysis

PCA analysis was carried out to know the significant variables in the analyzed morphological traits. First two principal components explained 62.74% and the first 5 principal components explained 90.50% of the total variance (Table 6). The degrees of association of analyzed traits with these axes were obtained and are presented as their Eigenvectors (Table 6). The first principal component corresponds to 43.24% of the total variance, represented by six dominant traits, fruit weight, fruit length, fruit width, pulp weight, rind weight and endocarp weight. The second PC corresponds to 19.49% of the total variance and was represented by percentage composition of pulp, fruit shape and fruit apex. The third PC is represented by seed kernel characteristics, seed kernel weight and percentage composition of seed kernel and accounts for 12.36% of the total variance. The fourth PC had the highest coefficient for ridges on fruit trait and corresponds to 8.63% of the total variance. PCA analysis in the morphological diversity of B. aegyptiaca also revealed the similar type of results (Abasse et al. 2011). In that study, first principal component explained the 45% of the total variance and it was represented by the traits of fruit weight, fruit length and fruit width. Second principal component explained about 21% of the total variance and it was represented by seed kernel weight. In the present study, the results...
indicated that the first and second principal components explained more than half of the variance and hence characters representing these PCs are more significant in assessing the diversity of *B. roxburghii*.

### Clustering of accessions

Clustering of the accessions provides details about the distribution pattern of the variables. In the present study, the resulting data were grouped using k-means clustering method and a scatter plot was created using the scores of first two principal components which represents the 62.74% of the total variance among the accessions (Table 7; Fig. 3). Grouping of 45 accessions resulted in the formation of 4 clusters that were closely associated with their fruit characteristics. The minimum value, maximum value, mean value, standard deviation and coefficients of variation of analyzed quantitative traits of each cluster are presented in Table 7. Cluster 1 included 19 accessions that had medium-sized fruits with average fruit weight of 23.48 g and have the highest value of percentage composition of pulp (24.20%) than clusters 2, 3 and 4, with the average pulp weight of 5.59 g (Fig. 3; Table 7). Cluster 2 included 18 accessions and it is characterized by smallest fruits (average fruit weight of 16.68 g) among all the clusters (Fig. 3; Table 7). Clusters 3 and 4 were characterized by their large fruits with the average fruit weight of 32.29 g and 33.67 g respectively (Fig. 3; Table 7). Even though clusters 3 and 4 have more or less equal average fruit weight, they are distinguished by fruit length, fruit width, pulp weight, seed kernel weight, percentage composition of pulp and percentage composition of seed kernel. Cluster 3 is formed by two accessions i.e. KA-04A and TE-04, had larger seed kernel (average weight of 3.54 g) and moderate amount of pulp weight (average weight of 5.70 g) (Fig. 3; Table 7). Seed kernel has high commercial importance because of the presence of good amount of diosgenin (0.62%) as well as oil (40–42%) (Varshney and Vyas 1982; Arora and Tak 2013). The present study suggests that accessions of cluster 3 could be effectively utilized for seed kernel properties. Cluster 4 included six accessions viz. KA-03, KA-04B, KA-13A, KA-13B, TN-03 and TE-05A, and had a high pulp weight (average weight of 8.10 g) and comparatively low seed kernel weight (average weight of 2.37 g) (Fig. 3; Table 7). Pulp also has economic importance because of the presence of considerable amount of diosgenin (0.32%; Varshney and Vyas 1982). Hence accessions of cluster 4 could be effectively utilized in future domestication programs for their pulp characteristics. In the similar way, Manohar and Murthy (2011) reported the phenotypic diversity among the 30 collections of *Cucumis sativus* var. *sativus* L. in the

### Table 6 Principal component analysis of the morphological traits used to assess the diversity of *Balanites roxburghii*

| Traits                        | PC 1  | PC 2  | PC 3  | PC 4  | PC 5  |
|-------------------------------|-------|-------|-------|-------|-------|
| Fruit weight                  | 0.992 | 0.036 | 0.051 | −0.057| −0.030|
| Fruit length                  | 0.756 | 0.520 | −0.149| 0.202 | 0.038 |
| Fruit width                   | 0.811 | −0.446| 0.166 | −0.154| −0.029|
| Pulp weight                   | 0.859 | −0.392| −0.032| 0.207 | 0.210 |
| Rind weight                   | 0.872 | −0.209| −0.025| −0.250| −0.176|
| Seed kernel weight            | 0.631 | 0.331 | 0.661 | 0.058 | 0.166 |
| Endocarp weight               | 0.921 | 0.272 | 0.005 | −0.131| −0.130|
| % Composition of pulp         | 0.184 | −0.692| −0.159| 0.507 | 0.429 |
| % Composition of seed kernel  | −0.360| 0.345 | 0.812 | 0.057 | 0.258 |
| Fruit shape                   | 0.198 | 0.592 | −0.224| 0.432 | −0.284|
| Fruit apex                    | 0.161 | 0.660 | −0.340| 0.210 | 0.312 |
| Ridges on fruit               | 0.064 | 0.321 | −0.378| −0.592| 0.511 |
Table 7  Descriptive statistics for the quantitative traits of clusters identified among the accessions of *Balanites roxburghii*

|                      | Cluster 1 |         |         |         |          |          |          |          |          |          |          |          |          |          |          |          |          |
|----------------------|-----------|---------|---------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|                      | Min       | Max     | Average | SD      | CV (%)   | Min       | Max     | Average | SD      | CV (%)   | Min       | Max     | Average | SD      | CV (%)   | Min       | Max     | Average | SD      | CV (%)   |
| Fruit weight (g)     | 20.30     | 28.50   | 23.48   | 2.56    | 10.89    | 10.23     | 19.73   | 16.68   | 2.22    | 13.31    | 31.17     | 33.41   | 32.29   | 1.58    | 4.91     | 29.57     | 37.69   | 33.67   | 3.20    | 9.49     |
| Fruit length (cm)    | 3.72      | 5.70    | 4.84    | 0.59    | 12.10    | 3.56      | 5.34    | 4.12    | 0.53    | 12.86    | 5.28      | 7.66    | 6.47    | 1.68    | 26.01    | 4.80      | 6.63    | 5.55    | 0.73    | 13.10    |
| Fruit width (cm)     | 3.32      | 3.90    | 3.57    | 0.18    | 4.94     | 2.72      | 3.56    | 3.21    | 0.23    | 7.10     | 3.43      | 3.86    | 3.65    | 0.30    | 8.34     | 3.67      | 4.37    | 4.04    | 0.31    | 7.70     |
| Pulp weight (g)      | 3.89      | 7.24    | 5.59    | 0.83    | 14.83    | 1.71      | 5.18    | 3.50    | 0.99    | 28.46    | 5.05      | 6.34    | 5.70    | 0.91    | 16.02    | 6.98      | 10.24   | 8.10    | 1.25    | 15.43    |
| Rind weight (g)      | 2.77      | 5.20    | 3.80    | 0.59    | 15.55    | 2.15      | 4.76    | 3.14    | 0.59    | 18.65    | 4.16      | 4.67    | 4.42    | 0.36    | 8.17     | 5.26      | 7.08    | 6.42    | 0.64    | 9.92     |
| Seed kernel weight (g)| 1.30     | 2.62    | 2.11    | 0.37    | 17.49    | 0.77      | 2.47    | 1.53    | 0.42    | 27.37    | 3.21      | 3.87    | 3.54    | 0.47    | 13.18    | 1.60      | 2.98    | 2.37    | 0.49    | 20.54    |
| Endocarp weight (g)  | 7.64      | 16.90   | 11.95   | 2.53    | 21.17    | 4.92      | 10.35   | 8.54    | 1.30    | 15.19    | 16.90     | 20.33   | 18.62   | 2.43    | 13.03    | 14.46     | 18.33   | 16.77   | 1.51    | 9.03     |
| % Composition of pulp (%) | 16.79   | 35.62   | 24.20   | 5.09    | 21.05    | 15.05     | 28.91   | 20.71   | 4.23    | 20.43    | 15.10     | 20.34   | 17.72   | 3.70    | 20.90    | 21.50     | 27.18   | 23.99   | 1.92    | 8.00     |
| % Composition of seed kernel (%) | 5.62   | 11.77   | 9.07    | 1.71    | 18.88    | 4.15      | 14.37   | 9.32    | 2.80    | 30.07    | 10.31     | 11.59   | 10.95   | 0.91    | 8.32     | 4.91      | 8.49    | 7.05    | 1.32    | 18.74    |

Min – minimum value; Max – maximum value; SD – Standard deviation; CV – Coefficient of variation
Karnataka state of Southern India, and they grouped the different collections into 7 clusters. The clusters were formed based on the fruits, seeds and vegetative characters and percentage of pollen viability. The results have shown that collections of clusters 1 and 4 were superior with respect to larger fruits, larger seeds, good vegetative characters and high pollen viability. They suggested the accessions of those clusters should be given importance in future breeding programs. In the present study, the clustering results proves the correlation pattern (Table 5) that high fruit length favours the larger seed kernels and high fruit width favours the more amount of pulp content (Table 5). Thus, the accessions of clusters 3 and 4 could be utilized for the future cultivation programs in general and accessions of cluster 3 for seed kernel traits and accessions of cluster 4 for pulp traits in particular.

Conclusion

*Balanites roxburghii* is an underutilized medicinal tree in India, used to cure various diseases and disorders. This species is also a source of several valuable products such as diosgenin and seed oil. It is advantageous to use this species sustainably for the production of those valuable products. The present study was undertaken to assess the diversity of *B. roxburghii* in Southern India and it revealed the existence of large amount of variability in South Indian populations. Among the 45 accessions studied, accessions of clusters 3 (KA-04A and TE-04) and cluster 4 (KA-03, KA-04B, KA-13A, KA-13B, TN-03 and TE-05A) have superior fruit qualities with respect to pulp and seed kernel content and could be used in the future cultivation programs. Majority of the areas where the *B. roxburghii* is distributed, receive very less rainfall, and have low irrigation facility and suitable soil conditions. It could be grown commercially in those areas, to get the valuable products and generate income for local people. Diversity within the population is common in *B. roxburghii*, where the environmental conditions are same for all plants, is perhaps due to the genetic variability and interaction between the genetic and environmental factors. However, further analysis is needed on the variability of fruit yield, tree height, canopy area and other morphological traits.

Fig. 3  Cluster diagram constructed using the k-means clustering method and accessions were scattered using the scores of first two principal component axes. Each numerical number represents an accession as given in Table 3.
traits along with the effect of geographic, environmental and genetic factors on the phenotypic variability. That could assist the geneticists and breeders in identifying, selecting and improving the genotypes for various cultivation programs.

**Declarations**

**Conflict of interest** The authors declare no conflict of interest.

**Human and animals rights** This article does not contain any studies involving animals performed by any of the authors.

**References**

Abasse T, Weber JC, Katokore B, Boureima M, Larwanou M, Kalinganine A (2011) Morphological variation in *Balanites aegyptiaca* fruits and seeds within and among parkland agroforests in eastern Niger. Agroforest Syst 81:57–66

Ahmed AAO, Kita A, Nems A, Miedzianka J, Foligni R, Abdalla AMA, Mozzon M (2020) Tree-to-tree variability in fruits and kernels of a *Balanites aegyptiaca* (L.) Del. population grown in Sudan. Trees 34:111–119

Amalraj VA, Shankarnarayan KA (1987) Variability in *Balanites roxburghii* Pl. in the Indian desert. J Bombay Nat Hist Soc 84:476–478

Arora A, Tak L (2013) *Balanites roxburghii*: Physico-chemical properties and composition of fatty acid from arid zone of Rajasthan. Int J Basic Appl Chem Sci 3(2):1–5

Beentje H (2016) The Kew plant glossary: An illustrated dictionary of plant terms. 2nd edn. Royal Botanic Gardens, Kew, UK

Brown CE (1998) Applied multivariate statistics in geohydrology and related sciences. Springer-Verlag, Berlin, Germany

Burkill HM (1985) The useful plants of West Tropical Africa, Vol.1, Families A-D, Royal Botanic Gardens, Kew, UK

Chapagain BP, Yehoshua Y, Wiesman Z (2009) Desert date *Balanites aegyptiaca* (L.) Delile in tropical areas (Burkina Faso)? Int J Fruit Sci 20:282–299

FAO (1999) Use and potential of wild plants in farm households. Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome, Italy

Govindaraj M, Vetrienthvan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Genet Res Int. https://doi.org/10.1155/2015/431487

Guhathakurta P, Sanap S, Menon P, Prasad AK, Sable ST, Advani SC (2020a) Observed rainfall variability and changes over Karnataka state. India Meteorological Department, Pune, India

Guhathakurta P, Sanap S, Menon P, Prasad AK, Sangwan N, Advani SC (2020b) Observed rainfall variability and changes over Andhra Pradesh state. India Meteorological Department, Pune, India

Guhathakurta P, Sanap S, Menon P, Prasad AK, Sable ST, Advani SC (2020c) Observed rainfall variability and changes over Telangana state. India Meteorological Department, Pune, India

Guhathakurta P, Krishnan U, Menon P, Prasad AK, Sable ST, Advani SC (2020d) Observed rainfall variability and changes over Tamil Nadu state. India Meteorological Department, Pune, India

Jesus M, Martins APJ, Gallardo E, Silvestre S (2016) Diosgenin: recent highlights on pharmacology and analytical methodology. J Anal Methods Chem. https://doi.org/10.1155/2016/4156293

Khanvilkar P, Patel G, Nagar PS, Shah SN (2016) *Balaniates roxburghii* plant oil as potential non-edible feedstock for biodiesel production. Energy Power 6:21–27

Kirtikar KR, Basu BD (1918) Indian medicinal plants vol.1, part I, Suduindranath Basu, Panini office, Bhuwaneswari asrama, Bahadurganj, India

Manohar SH, Murthy HN (2011) Estimation of phenotypic divergence and powdery mildew resistance in a collection of *Cucumis sativus* L. Afr J Biotechnol 10:1978–1987

Molur S, Walker S (1998) Report of the workshop on “Conservation assessment and management plan for selected medicinal plant species of Northern, Northeastern and Central India (BCPP- Endangered Species Project). Zoo Outreach Organisation, Conservation Breeding Specialists Group, India, Coimbatore, India

Murthy HN, Bapat VA (2020) Importance of underutilized fruits and nuts. In: Murthy HN, Bapat VA (eds) Bioactive compounds in underutilized fruits and nuts, Reference series in phytochemistry. Springer, Cham, Switzerland, pp 3–19

Nadakarni KM (1954) Indian materia medica, 3rd edn. Bombay Popular Prakashan, Mumbai, India

Nazir R, Pandey DK, Pandey B, Kumar V, Dwivedi P, Kham-pariya A, Dey A, Malik T (2021) Optimization of diosgenin extraction from Dioscorea deltoidea tubers using response surface methodology and artificial neural network modelling. PLoS ONE 16:e0253617. https://doi.org/10.1371/journal.pone.0253617

Ouedraogo S, Bonde L, Ouedraogo O, Ouedraogo A, Thom-biano A, Boussim IJ (2020) To what extent do tree size, climate and land use influence the fruit production of *Balanites aegyptiaca* (L) Delile in tropical areas (Burkina Faso)? Int J Fruit Sci 20:282–299

Padulosi S, Thompson J, Rudebjørn P (2013) Fighting poverty, hunger and malnutrition with neglected and underutilized species (NUS): Needs, challenges and the way forward. Biodiversity International, Rome, Italy

Radunz A, Grosse W, Mewi-Sutz J (1985) Seeds of the East African savannah bush *Balaniates orbiculavis* as a possible new source of lipids for commercial use. J Am Oil Chem Soc 62:1251–1252

Sagna MB, Diallo A, Sarr NS, Ndiaye O, Goffner D, Guisse A (2014) Biochemical composition and nutritional value of *Balaniates aegyptiaca* (L.) Del fruit pulps from Northern Ferlo in Senegal. Afr J of Biotechnol 13:336–342
Sands MJS (2001) The Desert date and its relatives: A revision of the genus *Balanites*. Kew Bull 56:1–128
Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues. Food and Agriculture Organization of the United Nations, Rome, Italy
Schlautman B, Diaz-Garcia L, Barriball S (2020) Morphometric approaches to promote the use of exotic germplasm for improved food security and resilience to climate change: a kura clover example. Plant Sci. https://doi.org/10.1016/j.plantsci.2019.110319
Varshney IP, Vyas P (1982) Saponin and sapogenin contents of *Balanites roxburghii*. Int J Crude Drug Res 20:3–7
Virendra S, Priyanka T, Patel JR, Kori ML, Dixit VK (2009) Preliminary phytochemical and antiasthmatic studies on stem bark of *Balanites roxburghii* Planch. Int J Pharm Clin Res 1:40–42
Weber JC, Montes CS, Soumana I, Diallo BO, Abasse T, Larwanou M, Bationo AB (2019) Genetic and geographic variation in growth of *Balanites aegyptiaca* in Niger: comparing results from provenance/progeny tests in the nursery and field. New Forest 50:643–661
Zargar SA, Wani AA, Saggo MIS (2021) Analysis of phenotypic diversity of apricot (*Prunus armeniaca* L.) accessions from Jammu and Kashmir, India. Plant Genet Resour. https://doi.org/10.1017/S1479262121000241

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.