Identification of potential accessions of *Asparagus racemosus* for root yield and shatavarin IV content

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ABSTRACT

Successful restoration of over exploited species (*Asparagus racemosus*) depends upon variability, conservation and cultivation. Twelve elite accessions were characterized for fifteen quantitative and qualitative traits for sustainable cultivation and industrial uses. The evaluated accessions varied in morphology, herbage, root yield and shatavarin IV content. The accession DAR-7 was showing maximum herbage yield (1860 and 1850 g plant\(^{-1}\)), fresh root weight (36.33 and 37.33 g plant\(^{-1}\)), root girth (18.25 and 18.45 cm) and root yield (14.26 and 12.79 kg plant\(^{-1}\)) in both the harvesting years. Shatavarin IV content in roots was maximum in DAR-14 (152.06 and 151.72 μg g\(^{-1}\)), followed by DAR-28 (81.16 and 83.16 μg g\(^{-1}\)). For economic yield accessions DAR-7, DAR-19, DAR-14, DAR-28 were found superior therefore, they may be further used in crop improvement program as valuable seed sources.

1. Introduction

Lesser known horticultural plants as raw material has been used by people since time immemorial throughout the world for food, edible products, culinary ingredients besides medicinal, ornamental and aesthetic purposes. They are genetically very diverse group and play a major role in modern society and economy as well (Hayes et al., 2008).

The centre of origin for Shatavari (*Asparagus racemosus*) belongs to Liliaceae family originated from eastern parts of the world wherein Indian peninsular is also included. *A. racemosus* is commonly known as Satavar, Satamuli and Shatavari in Indian subcontinent. The genus *Asparagus* comprised about 350 species and in India, around 22 species of asparagus, *A. racemosus* (AR) are frequently and commonly used in traditional or indigenous medicine system (Gaur, 1999; Goyal et al., 2003). Prevalence of Asparagus in India and its habitat is found in tropics, sub-tropics and in Himalayan ranges up to 1000–1500 m. The species considered as endangered in its natural habitat which warrants sustainable conservation and cultivation as a top priority. It is required to understand the existing genetic variability for the successful retrieval of these species (Bopana and Saxena, 2007). Satavari grown as wild in Himachal Pradesh, Punjab, Jammu and Kashmir, Uttar Pradesh, Bihar, West Bengal, Orissa, Madhya Pradesh, Gujarat, lower and foothills of Himalayas. Usually, this plant prefers light to medium soils. Cultivation in sandy, sandy loam and silt type soils are in vogue but black, deep, loose and well-drained fertile sandy loam soils are considered to be suitable for cultivation. A soil with a pH of 6–7 neutral to slightly alkaline is optimum for higher yield but sustainable yield can easily be cultivated up to pH 8, electrical conductivity 0.15, organic carbon 0.79 % and phosphorus 7.3 kg acre\(^{-1}\). Even under gravelly and rock soils there is formation of roots with minimum soil depth 30 cm (Goyal et al., 2003; Anupam et al., 2012). The roots are ready for harvesting after six months of transplanting and life cycle is likely to continue up to 15 years (Goyal et al., 2003; Joshi, 2016). On turning of plants to yellow after 18 months, is indicative of the time of harvesting. Winter season (November–December) considered to be best time for harvesting tuberous roots of crop.

Roots are rich source of steroidal saponins showing different properties and thus find numerous pharmacological uses (Joshi, 2016; Saran et al., 2019b). It is known to having a wide range of chemical constituents viz. saponins, alkaloids, proteins and tannins (Zhang et al., 2018). Other important phytochemical ingredients in asparagus are arginine, tyrosine, asparagine, flavonoids, essential oils, resin and tannins (Negi et al., 2010; Joshi, 2016). Shatavar are low in calories, sodium and also a good source

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Figure 1. Optimization of chromatographic separation of shatavarin IV on UHPLC-MS/MS. A) Specificity of shatavarin IV. B) Calibration curve of shatavarin IV.
Evaluation of *A. racemosus* accessions for growth and carpological parameters.

| Accession | Fresh herbage yield (g plant⁻¹) | Stem diameter (mm) | Spike length (mm) | Spike base girth (mm) | Internode length (cm) | Number of cladodes | Fruit girth (mm) | Fruit height (mm) |
|-----------|---------------------------------|-------------------|------------------|----------------------|----------------------|-------------------|-----------------|-----------------|
| DAR-10    | 1090                            | 790e              | 9.64b            | 9.57b                | 1.70b                | 4.35d            | 4.23d           | 2.57de          |
| DAR-11    | 1770                            | 1720ab            | 9.12bc           | 9.15bc               | 1.10e                | 3.59e            | 3.56e           | 3.17a           |
| DAR-19    | 1800                            | 1720ab            | 9.02bc           | 9.03c                | 2.27a                | 5.22b            | 5.26b           | 2.23fg          |
| DAR-26    | 1010                            | 1010d             | 9.16bc           | 9.26bc               | 1.27de               | 2.78f            | 2.82f           | 2.07g           |
| DAR-28    | 1250                            | 1170cd            | 9.70b            | 9.17bc               | 1.40cd               | 3.76e            | 3.73e           | 2.20fg          |
| DAR-40    | 1350                            | 1070d             | 9.81b            | 9.57b                | 1.80b                | 4.71c            | 4.75c           | 2.87bc          |
| DAR-51    | 1440                            | 1320bcd           | 9.25bc           | 9.05c                | 1.13e                | 2.85f            | 2.81f           | 3.03ab          |
| DAR-54    | 1250                            | 1060d             | 9.34b            | 9.31bc               | 1.40cd               | 4.74c            | 4.71c           | 2.63cd          |
| DF        | 1740                            | 1670abc           | 10.79a           | 10.78a               | 2.13ab               | 5.64a            | 5.52a           | 2.73a           |

Means with the same letter (superscript) in the columns do not showing significantly different (P ¼ 0.05) (Duncan Multiple Range Test).

2. Materials and methods

2.1. Experimental site

The experiments were carried out at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Borli, Anand, Gujarat India, during 2011-12 and 2015-16. Harvesting at four years interval was carried out during 2015-16 and 2019-20, respectively. The experimental site located at 22°35′ N and 72°55′ E at an altitude of about 45.1 m above MSL, falling in semiarid, subtropical climate with hot dry summers and mild winters.

2.2. Plant materials

Selected accessions were harvested and measured for root weight and yield contributing parameters. The suckers of accessions were transplanted in replicates of three at spacing of 100 × 200 cm (plant to plant and row to row) and each accession was replicated thrice. The crop was irrigated once in every month except rainy season. One hand weeding was also carried out to avoid the weed competition. All the standard agronomic practices under crop production were followed. The observations were recorded at the harvesting stage for fresh herbage yield, stem diameter, spike length, spike girth, internodal length, number of cladodes, fruit girth and fruit height. Parameters for observations were fresh root weight, root length, root girth, full root length, root yield and thickness of central root portion of all the selected accessions using standard methods. The mean value of three replications from each treatment served for analysis in both the harvests. Roots were separated from plants for measuring root parameters dried properly after peeling at room temperature so as to reach constant moisture content.

2.3. Determination of shatavarin IV

Certified Reference Material for Shatavarin IV (99.9 %, Sigma Aldrich, Germany), MS Grade methanol, MS Grade water, MS Grade acetonitrile (Merck, Darmstadt, Germany) and formic Acid (99.5 %, Optima™, Fisher Chemical, Fair lawn, NJ, USA) was used for the quantitative analysis on LC-MS/MS.

The peeled dried roots of *A. racemosus* were ground to a fine powder, kept in air tight container and stored in a deep freezer at -20 °C for further analysis of shatavarin-IV content. The shatavarin-IV content was determined using the protocol as standardized with some minor modifications (Saran et al., 2019b). For this approximately 50 mg dried root samples of *A. racemosus* were taken into screw-capped tubes having 10 ml methanol and heated at 50 °C for 5 min. Thereafter, tubes were centrifuged (Eppendorf 5804, Germany) at 4000 rpm for 5 min. For the Shatavarin IV quantification on LC-MS/MS, supernatant was collected and filtered through 0.2 μm PTFE membrane filter in a 2 ml capacity vial. A Thermo Scientific made TSQ Quantum Access MAX triple stage quadrupole mass spectrometer was used for quantitative analysis of shatavarin-IV. A Dionex made ultra-high-performance liquid chromatograph (UHPLC) system equipped with an autosampler, a quaternary pump system and a
Intermediate and working standards were prepared (4 Methanol: 1 Water v/v) from the stock solution (339.6 μg mL⁻¹) and stock solution was prepared in the MS grade methanol stored at -20 °C (Saran et al., 2019c). Linearity of Shatavarin IV at various concentration levels (1, 2.5, 5, 10, 25, 50, 100, 250, 500 ng mL⁻¹) on LC-MS/MS exhibited linear response with correlation coefficient (R²) of 0.998 (Fig. 1A and B). Sensitivity of the analytical method was done out in terms of limit of detection (LOD) and limit of quantification (LOQ) considering proper signal-to-noise ratio. Mathematically, values assumed for LOD and LOQ were 2.5 ng mL⁻¹ and 7.5 ng mL⁻¹, respectively as described (Saran et al., 2019). Fifteen characters were recorded using shrub cladode, fruit and root specimens from 12 accessions of this crop. Data of various characters were standardized by using the YBAR option using the NTSYS-pc 2.1 software (Rohlf, 2000). Identical measurements for each specimen were replaced by shatavari under high-density plantation (45 × 60 cm) with drip irrigation system. On an average, fresh and dry root yield (40.8 t ha⁻¹ and 4.13 t ha⁻¹, respectively) was obtained from harvests after 24 months of the plantation. The primary data were collected through personal interview using a pre-tested questionnaire. To examine the economics, simple cost accounting method was followed and the financial feasibility was worked out by comparing costs and returns. The prices used in the analysis were averages for the period 2018-19.

2.4. Economics under high density plantation

Front-line demonstrations (FLD) in 2.5 ha area during 2016-17 were laid out in farmers field located at Botad, Bhavnagar, Gujarat. Earlier farmers are doing cultivation of cotton, groundnut, jeera, gram and getting approximately Rs. 1.6 Lakhs ha⁻¹ year⁻¹. These crops were replaced by shatavari under high-density plantation (45 × 60 cm) with drip irrigation system. On an average, fresh and dry root yield (40.8 t ha⁻¹ and 4.13 t ha⁻¹, respectively) was obtained from harvests after 24 months of the plantation. The primary data were collected through personal interview using a pre-tested questionnaire. To examine the economics, simple cost accounting method was followed and the financial feasibility was worked out by comparing costs and returns. The prices used in the analysis were averages for the period 2018-19.

Table 2. Evaluation of A. racemosus accessions for root characters.

| Accession/Fresh root weight of rootlet (g)/Root length (cm) Root girth (cm) Fill root length (cm)/Root yield per plant (kg)/Thickness of central part of roots (mm)/Shatavarin IV (μg g⁻¹) | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 | 2015-16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DAR-7 36.33a 37.33a 29.50bcd 29.57abc 18.25a 18.45a 18.10fg 17.40de 14.26a 12.79a 2.48a 2.49a 19.81e 18.21e | | | | | | | | | | | | | | |
| DAR-10 26.67b 23.67b 27.52d 27.07e 13.24f 13.23f 19.43f 17.97de 15.22f 4.08f 1.58de 1.57de 30.73f 31.33d | | | | | | | | | | | | | | |
| DAR-11 28.33b 26.67b 37.36ab 38.93ab 13.81b 13.78b 34.65b 29.40ab 7.72f 7.31b 1.41f 1.39f 67.22f 72.07c | | | | | | | | | | | | | | |
| DAR-14 26.67b 25.67b 37.86b 39.50b 13.36bc 12.90bc 12.90bc 12.90bc 23.44cd 23.23cd 6.17ef 4.93de 1.55ef 1.56cde | | | | | | | | | | | | | | |
| DAR-19 24.33bc 24.33b 32.83abc 32.67abc 11.66de 11.59cde 24.89c 24.83bc 11.00b 10.98ab 1.69cd 1.70bcd 11.45f 10.88g | | | | | | | | | | | | | | |
| DAR-26 15.33d 15.33d 29.70bc 29.77b 12.46b 12.46b 16.07b 16.07b 5.50ef 6.17de 1.39f 1.39f 8.96f 8.92f | | | | | | | | | | | | | | |
| DAR-28 25.33b 26.33b 31.16abcd 31.00abc 13.70b 13.78b 19.04efg 18.80de 6.90ef 6.54df 1.73cd 1.72cd 81.16b 83.16b | | | | | | | | | | | | | | |
| DAR-40 25.33b 25.33b 37.33b 36.80b 12.85bc 12.34bcd 21.77b 22.43cd 5.95ef 5.90de 1.12f 1.11f 7.18b 7.4b | | | | | | | | | | | | | | |
| DAR-44 14.33d 13.33d 25.40d 23.13f 10.70d 10.78e 14.48f 14.53g 6.39ef 5.62de 1.48fg 1.47f 5.68f 5.54f | | | | | | | | | | | | | | |
| DAR-51 20.67b 19.67f 31.84f 31.93f 12.81f 12.93f 20.69f 19.83f 5.46g 5.10f 1.43f 1.39f 27.46f 31.40d | | | | | | | | | | | | | | |
| DAR-54 14.33d 14.67b 35.28gh 35.27gh 11.20d 11.02d 17.62e 17.23f 5.61i 6.43g 1.53de 1.53de 4.12f 3.15f | | | | | | | | | | | | | | |
| DF 25.33b 25.33b 37.23gh 37.23gh 12.97f 12.97f 30.67f 30.67f 9.08b 9.08b 1.84f 1.84f 10.65f 12.98f | | | | | | | | | | | | | | |

Means with the same letter (superscript) in the columns do not showing significantly different (P = 0.05) - (Duncan Multiple Range Test).

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2.5. Statistical analysis

The analysis of variance was done in randomized block design for various observations observed during the experiment by using statistical
software SAS 9.2. DMRT comparisons among the different shade-net intensities. The results were presented at 5% level of significance ($P = 0.05$). The critical difference (CD) values were calculated to compare the various treatment means.

3. Results

3.1. Quantitative and qualitative variations

Twelve accessions of shatavari were evaluated for morphological variations and yield attributing characters after four years of plantation at two harvesting years (Tables 1 and 2). The results revealed DAR-7 accession to have maximum herbage yield (1860 and 1855 g plant$^{-1}$), fresh root weight (36.33 and 37.33 g plant$^{-1}$), root girth (18.25 and 18.45 cm), root yield (14.26 and 12.79 kg plant$^{-1}$) and thickness of a central portion of the root (2.48 and 2.49 mm) during both the years of harvest (Figures 2 and 3).

Accession DF registered maximum stem diameter (10.79 and 10.78 mm) and spike base girth (5.64 and 5.52 mm) were observed for both the harvesting years, respectively. The highest spike length was observed in DAR-19 (2.27 and 2.33 mm), while minimum in DAR-11 (1.10 and 1.07 mm) (Figure 2). Maximum internode length (3.17 and 3.13 cm), number of cladodes per branchlets (115.67 and 110.67), fruit girth (8.90 mm and 8.87 mm), fruit height (5.31 and 5.28 mm) and fill root length (34.65 and 29.40 cm) were observed in DAR-11. Maximum root length (37.86 and 39.50 cm) was recorded in DAR-14 followed by DAR-11 (37.36 and 38.93 cm), while minimum in DAR-44 (25.40 and 23.13 cm) for both harvesting years (Table 2 and Figure 3). The Shatavarin IV contents in roots of different shatavari accessions were subjected to chemical characterization in order to determine medicinal property of raw drugs. The roots were harvested during last week of December for quality analysis. Highest recovery of Shatavarin IV was recorded in DAR-14 (152.06 and 151.72 μg g$^{-1}$) followed DAR-28 (81.16 and 83.16 μg g$^{-1}$), while minimum in DAR-54 (4.12 and 3.15 μg g$^{-1}$) in both the harvest (Table 2 and Figure 4).

The primary shoot characters like stem diameter, spike length, spike base girth, internodal length, number of cladodes, fruit size and root characters like root length, root girth, fill root length, thickness of central part of roots play major role in production, identification, cataloging and documentation of wild species for improvement. The morphological and chemical characterization provide the information on extent of variability and step towards identification of elites for root yield and shatavarin-IV content with distinct characters. Comparative analysis based on the fifteen morphological and biochemical characters was carried out and results revealed significant variation. A dendrogram generated from morphometric data grouped all 12 accessions into different clusters. In the 2-D plot, accessions DAR-14, DAR-11 have been

Figure 2. Shatavari accessions with distinct morphological characters (spine and central part of roots).
grouped in one cluster and DAR-7 and DAR-28 in another cluster (Figure 5). However, accession namely, DAR-7, DAR-54, DAR-51 and DAR-19 were distinct in 2-D plot. As important source of breeding program, the morphological and biochemical markers of shatavari can be successfully exploited.

3.2. Economics

This shatavari accession DAR-26 was selected decisively for cultivation at farmer’s field due to medium to small fill root and spine length suitable for the gravelly or culturable wasteland. It prefers moist, humid and arid conditions for cultivation. Roots harvesting of FLD at 24 months of transplanting were carried out when the plants above ground turned green to pale yellow. FLD at farmers field fetched Rs. 4.87 l ha$^{-1}$ year$^{-1}$ net returns and B:C ratio was 3.66 over net return from the sale of dry roots (4.13 t ha$^{-1}$) (Figure 6).

4. Discussion

Besides the genetic constituents, morphological traits also play crucial role in the yield and production. Morphological traits of A. curillus, A. lycopodineus, A. penicillatus and A. racemosus were studied and significant variation was reported in Nepal. The variants for distinct morphological characters such as root weight, root size with root length and biochemical characters for steroidal saponin content reported in A. racemosus (Shrestha et al., 2016). Average root yield was observed 13.53 kg–4.65 kg per plant in DAR-7 and DAR-10, respectively. Variation for the time of harvesting and tuberous root yield was also reported under the Himalayan region in A. racemosus (Goyal et al., 2003; Joshi, 2016). Yield parameters also influenced by harvesting duration where roots yield was less from one-year harvest as compare to the harvesting from subsequent years. Genetic variation among the germplasm also play a major role in quantity as well as quality of roots (Saran and Meena, 2020).

Simple economics worked out to attract farmers having poor soil (gravelly culturable wasteland) under high density plantation of satavari. For that, roots were harvested after 24 months of transplanting at colour turning stage of above ground parts from green to pale yellow. Crop prefers gravelly or rocky soils having at least 30 cm depth as the crop having the ability to store and capture maximum

Figure 3. Variation for root morphology and root yield in shatavari accessions.
moisture from dry soils as earlier reported that gravels reflects its potential for restocking balance to stress (Goyal et al., 2003; Anupam et al., 2012; Singh et al., 2018). The highest benefit-cost ratio (BCR) 5.29 was observed when plants were sown at spacing 30 × 45 cm, followed by 45 × 45 cm. This spacing gave a significant increase in biomass yield per unit area. It accommodated a greater number of plants in per hectare area (Thakur, 2016). It might be due to a greater number of plants per unit area under high density plantation. Therefore, it could be considered as a suitable crop for field having stony soil for high returns per rupee investment as compared to traditional crops such as cumin, gram, cotton, groundnut, etc. Overall, it could be a suitable crop for degraded soils for high returns per rupee investment as compared to traditional cropping system (Saran et al., 2019a).

5. Conclusion

Among the evaluated accessions, DAR-7, exhibited maximum herbage and root yield whereas DAR-14 was containing maximum Shatavarin IV in both the harvesting years. Accessions DAR-7, DAR-19, DAR-14,
DAR-28 were found at par for root yield and shatavarin IV content therefore, may be recommended in crop improvement programs and commercial cultivation as a new selection. The accession DAR-26 was found suitable for shallow, steep and stony soil under high-density plantation instead of traditional crops. Farmers can get approximately Rs. 4.87/ha year net return with 3.66 B:C ratio over net return. DAR-26 as promising accession is being recommended as viable substitute for farmers under stony soils.

Declarations

Author contribution statement

Parmeshwar Lal Saran: Conceptualized and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Figure 5. Genotype by trait biplots and trait relationship analysis (2-D).

Figure 6. Average yearly based economic parameters of shatavari cultivation under high density plantation crop harvested after two years at farmer's field.
S. Singh, V. H. Solanki, R. V. Kansara: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

G. Devi: Performed the experiments.

P. Manivel: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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