TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2021) 45: 475-483
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doi:10.3906/tar-2006-42

# Evaluation of *Asparagus adscendens* accessions for root yield and shatavarin IV content in India

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**Received:** 08.06.2020 • **Accepted/Published Online:** 03.06.2021 • **Final Version:** 18.08.2021

**Abstract**: The study was carried out to evaluate fifty-six accessions/species suitable for industrial purpose. Understanding the bioactive compound diversity among both the shatavari species, *Asparagus racemosus* (AR) and *A. adscendens* (AA) are an important species for crop development. Sufficient variation for bioactive compounds (shatavarin IV) were registered in both the species. AR and AA confined average 22.84 µg $g^{-1}$ and 117.74 µg $g^{-1}$ shatavarin IV, respectively. The *A. adscendens* produced about five-folds higher shatavarin IV content as compared to *A. racemosus*. Wide variation for shatavarin IV content were also observed within the AA accessions. Successful restoration of such species depends upon variability available within the species for crop improvement. Twelve elite accessions were evaluated for quantitive and qualitative traits. The morphological parameters such as cladode, spike, internodal space and root weight play an important role as a trade and industrial characters. The evaluated accessions varied in herbage, root yield and shatavarin IV content. The accession DAA-28 had the highest fresh weight of rootlet, root length and shatavarin IV content. Simultaneously, root girth and root yield were recorded maximum in accession DAA-41. In both the years, DAA-41, DAA-44, DAA-28 and DAA-32 were found superior, therefore, they may be used in diverse parental combination for enhancing genetic gain in varietal selection, advancement and release of varieties.

**Key words:** *Asparagus adscendens*, *Asparagus racemosus,* variability, root yield, shatavarin IV, species

## 1. Introduction

*Asparagus racemosus* and *A. adscendens* belonging to the family *Liliaceae* are originated in the eastern parts of the world, including India. *A. racemosus* is commonly known as *Satawar* or *Satamuli* and *A. adscendens* is known as Shweta musali or Satavari bhed in the country (Panda et al., 2011). The genus Asparagus comprised about 350 species and in India approximately 22 species are commonly used in traditional or indigenous medicine (Goyal et al., 2003).

Prevalence of *Asparagus racemosus* (AR) in India and its habitat is found in tropicas, subtropics and in Himalayan region up to 1000 m mean sea level (MSL), while *A. adscendens* (AA) is distributed in the western Himalayas up to 1800 m MSL. The AA plants are considered by suberect, prickly herb besieged habit having 10–100 cm long roots (Kawale et al., 2014). Both the species are considered as endangered in its natural habitat, which warrants conservation and cultivation as a top priority. These species are grown as wild in Himachal Pradesh, Punjab, Jammu and Kashmir, Uttar Pradesh, Bihar, West Bengal, Orissa, Madhya Pradesh, Gujarat, lower and foothills of Himalayas (Saran et al., 2020). This plant prefers light to medium soils but can be grown in sandy, sandy loam and silt type of soils. The black, deep, loose and well-drained fertile sandy loam soils are considered to be ideal for cultivation. Soil with a pH neutral to slightly alkaline (6–7.5) is optimum but it can be sustained under cultivated up to pH 8, electrical conductivity 0.15, organic carbon 0.79 % and phosphorus 7.3 (kg $acre^{-1}$). Even under gravely and rock soils formation of roots takes place with minimum soil depth of 100 cm (Goyal et al., 2003; Anupam et al., 2012). The roots may be harvested from six months of transplanting and this continues up to fifteen years (Joshi, 2016; Saran et al., 2021). After 18 months of plantation, the plant begins to turn yellowish in colour (maturity index). Winter season (from November to December) is the best time for tuberous roots harvesting. *A. racemosus* produced about 1.6-fold higher root yield as compared to *A. adscendens* (Ram et al., 2001).

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Root powder of *A. adscendens* is rich in carbohydrates (30.65 mg $g^{-1}$) and proteins (0.76 mg $g^{-1}$) on dry weight basis as reported by Kawale et al. (2014). It also contains dietary fibre and vitamins (A, C, $B_1$, $B_2$, E and folic acid), minerals (Ca, Mg and P) therefore, it is frequently used for preparation of health tonics. Primarily roots are containing steroidal saponins exhibiting variety of properties and thus find numerous pharmacological applications (Joshi, 2016; Saran et al., 2019). Out of several asparagus' species grown in India, *A. racemosus, A. gonaclades* and *A. adscendens* are most commonly used in indigenous medicines (Hayes et al., 2008).

Shatavari is known to possess a wide range of phytochemical constituents i.e. saponins, alkaloids, proteins and tannins (Kawale et al., 2014; Zhang et al., 2019). Consequently, it is well known for their multiple health benefits especially improving fertility, vitality in women and men. Entire plant contains saponins but roots are the richest source of steroidal saponins (Kamar et al., 2000; Thakur and Sharma, 2015). Wide variation in the bioactive compounds (steroidal sapogenins) were reported in different asparagus species (Sharma et al., 1982; Kawale et al., 2014; Negi et al., 2010; Saran et al., 2020). The morphological and chemotypic diversities are main measures for identification and selection of suitable species as well as accession. A wide dissimilarity between and within species is observed based on their morphological and chemical variability (Shrestha et al., 2016; Saran et al., 2020). Evaluation studies on AR and AA are negligible, hereafter the selection of potential accessions for commercial cultivation are in need of the hours.

## 2. Materials and methods

### 2.1. Experimental site

The experiments were carried out at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat India, during 2015–2016. Crop harvested at four years interval was carried out during 2019–2020. The experimental farm is located at 22°35′N and 72°55′E at an altitude of about 45.1 m above MSL. It has a semiarid, subtropical climate with hot dry summers and mild winters. The soil type of experimental site was sandy loam supplied with 5 t $ha^{-1}$ farmyard manure (FYM). The qualitative and quantitative analysis was performed at Food Quality Testing laboratory, N.M. College of Agriculture, Navsari Agricultural University, Navsari following the standard protocols.

### 2.2. Plant materials

Roots of fifty-six accessions of both the species (AR and AS) were harvested from germplasm block during the initial experimental period (2015–2016) and dried for comparative performance. Twelve selected elite accessions of AS rich in shatavarin IV were evaluated for root weight and yield contributing parameters during 2019–2020. The healthy and uniform suckers of selected accessions were transplanted during 2015–2016 with the spacing of 100 × 200 cm (plant to plant and row to row respectively) and each accession was replicated three times. The crop was irrigated once in every month except rainy season and one hand weeding was carried out. All the standard agronomic practices were followed for good crop production. The observations were recorded at harvesting stage for fresh herbage yield, stem diameter, spike length, spike base girth, internodal length and number of cladodes. Root parameters i.e. fresh root weight, root length, root girth, root yield, thickness of central root part and dry root recovery (without peeling) of all the selected accessions were recorded using the standard methodology (Saran et al., 2020). The mean value of three replications from each treatment served for analysis. Roots were separated from plants for measuring root parameters at room temperature so as to dry properly and reach constant moisture level.

### 2.3. Determination of shatavarin IV

The peeled and dried roots were grounded to make fine powder. It was stored in deep freezer (–20 ˚C) and served for further analysis of shatavarin IV content. For shatavarin IV estimation, 50 mg dried root samples of *A. racemosus* were taken into screw-capped tubes containing 10 mL methanol. The tubes were heated at 50 °C for 5 min and mixed. Thereafter, tubes were cooled down, centrifuged for 5 min at 4000 rpm and supernatant transferred to another vial. It was filtered through 0.2 µm PTFE membrane filter prior to quantification with LC-MS/MS system. LC-MS grade methanol, water and acetonitrile were procured from Merck (Darmstadt, Germany). The chemical formic acid (99.5+%, Optima) LC/MS grade was purchased from Fisher Chemical (Fair lawn, NJ, USA) and shatavarin IV with purity 99.9% was purchased from Sigma-Aldrich (Taufkirchen, Germany).

### 2.4. Quantitative analysis

The quantitative analysis of shatavarin IV was performed with Thermo Fisher Scientific made Quantum Access MAX triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with a heated electrospray ionization (HESI) source. A Dionex UltiMate 3000 Rapid Separation's made ultrahigh-performance liquid chromatograph (UHPLC) system equipped with an autosampler, a quaternary pump system and column compartment was used for separation of the shatavarin IV. The separation was achieved on Hypersil Gold $C_{18}$ column (150 × 4.6 mm, 5 µm particle size) with flow rate 0.3 mL/min and column oven temperature 35 °C. An elution gradient was used with solvents A (99.9% water: 0.1% formic acid) and B (99.9% acetonitrile: 0.1% formic acid): 0–10 min, 0%–65% B; 10–17 min, 65%–95 % B; 17 to 20 min, 95%–35% B and 20–30 min 35%–0% B. The retention time observed for shatavarin IV was 12.89 min. The MS parameters of shatavarin IV were optimized in negative ion mode with capillary voltage - 5000 V; vaporizer temperature 350 °C; sheath gas ($N_2$) 35 unit; aux gas ($N_2$) 10 unit and capillary temperature 270 °C. The parent ion (885.6 m/z) and product ion (739.3, 221.4 and 179.0 m/z) were obtained for shatavarin IV as reported (Saran et al., 2019; Saran et al., 2020).

**2.4.1. Method optimization parameters**

The external standard method was castoff for the quantification of shatavarin IV. A stock solution of shatavarin IV (339.6 μg $mL^{-1}$) in methanol was prepared and stored in deep freezer at –20.0 °C and was subjected to dilution to get intermediate and working standards. The fresh working standards (1 to 500 ng $mL^{-1}$ range) were prepared from intermediate standard by properly diluting with MeOH-Water (4:1 v/v) at the time of analysis. For shatavarin IV, the single reaction monitoring (SRM) chromatogram was integrated and the peak area was plotted against concentration and subjected to regression analysis as shown in Figure 1. The limit of detection of the shatavarin IV was defined as the lowest sample concentration (signal-to-noise ratio = 3). The limit of quantification was defined as the lowest sample concentration (signal-to-noise ratio = 10) that can be quantitatively determined with suitable precision and accuracy.

**2.5. Statistical analysis**

The data for variance analysed using randomized block design (RBD) for various observations were recorded during experimentation by using statistical software SAS 9.2. The DMRT was adopted to compare different accessions. The results were presented at 5% level of significance ($p = 0.05$) and critical difference values were calculated to compare the various treatment means.

## 3. Results and discussion

**3.1. Root yield contributing parameters**

Fifty-six accessions of both the species were screened for shatavarin IV content in harvested and dried root samples. On an average AR and AA accessions contained 22.84 and 117.74 μg $g^{-1}$ shatavarin IV, respectively as shown in Figure 2. AA accessions contained five times higher shatavarin IV as compared to AR accessions. Several asparagus species have a variety of biological properties due to variable chemical constituents in different plant parts including roots (Negi et al., 2010; Singh et al., 2018; Saran et al., 2020).

On the basis of initial screening of both the species for shatavarin IV content in root, twelve selected accessions of AA (rich species) were evaluated for morphological variations and yield contributing parameters after four years of plantation (Table 1). The result revealed DAA-16 and DAA-41 accessions to have highest fresh herbage yield (3795 and 3681 g $plant^{-1}$), stem diameter (12.47 and 14.70 mm), spike length (1.53 mm), spike base (3.12 and 3.20 mm), internodal length (3.40 and 3.53 cm) and number of cladodes (131 and 147) at maturity as shown in Table 1 and Figure 3.

The highest fresh weight of rootlet (31.33 g), root length (64.23 cm) and shatavarin IV content (570.80 μg $g^{-1}$) was observed in DAA-28. Accession DAA-41 registered highest root girth (9.37 mm) and root yield (9039 g). The highest thickness of central portion of root (0.82 mm) was recorded in DAA-32 and the highest dry root recovery (25.04%) was in DAA-44 as shown in Table 2 and Figure 4. Besides the genetic constituents, morphological traits also play a crucial role in crop production. The level of quantitative and qualitative variation among AA accessions collected from different parts of country and cultivated under same environment is due to diversity among the selected accessions. Morphological parameters are good indicators for plant growth that witnessed for considerable variability (Saran et al., 2020). Morphology of *A. curillus*, A. *lycopodineus*, *A. penicillatus* and *A. racemosus* from Nepal was studied and the significant variations for root weight, root size and steroidal saponin content were reported by Shrestha et al. (2016). The present finding is comparable to the reports from Gujarat for quantitative characters. It has been reported that the twelve accessions of *A. racemosus* were significant variations for yield and contributing parameters in both the harvesting years (Saran et al., 2020). These morphological parameters exert a strong influence on root yield of shatavari. High root yield is a desirable characteristic from an economic perspective.

**3.2. Shatavarin IV content in root**

Twelve elite accessions were evaluated for quality parameters and the variations for Shatavarin IV content in different accessions were observed under same environmental conditions as shown in Table 2 and Figure 5. Maximum shatavarin IV content in DAA-28 (570.80 μg $g^{-1}$) was followed by DAA-32 (347.27 μg $g^{-1}$) while minimum shatavarin IV content was in DAA-9 (100.45 μg $g^{-1}$). Entire plant parts contain saponins, however, tuberous roots are the richest source of steroidal saponins and used for medicinal purposes. Shatavari having a group of steroidal saponin including steroidal glycosides (shatavarins I–IV) and shatavarin IV are the principle bioactive constituents used in numerous pharmacological applications. The shatavarin IV contents in roots of different shatavari accessions were subjected to chemical characterization in order to determine their impact on medicinal properties of the raw drug. The significant difference for concentration of various steroidal saponins (shatavarin I–IV) was also

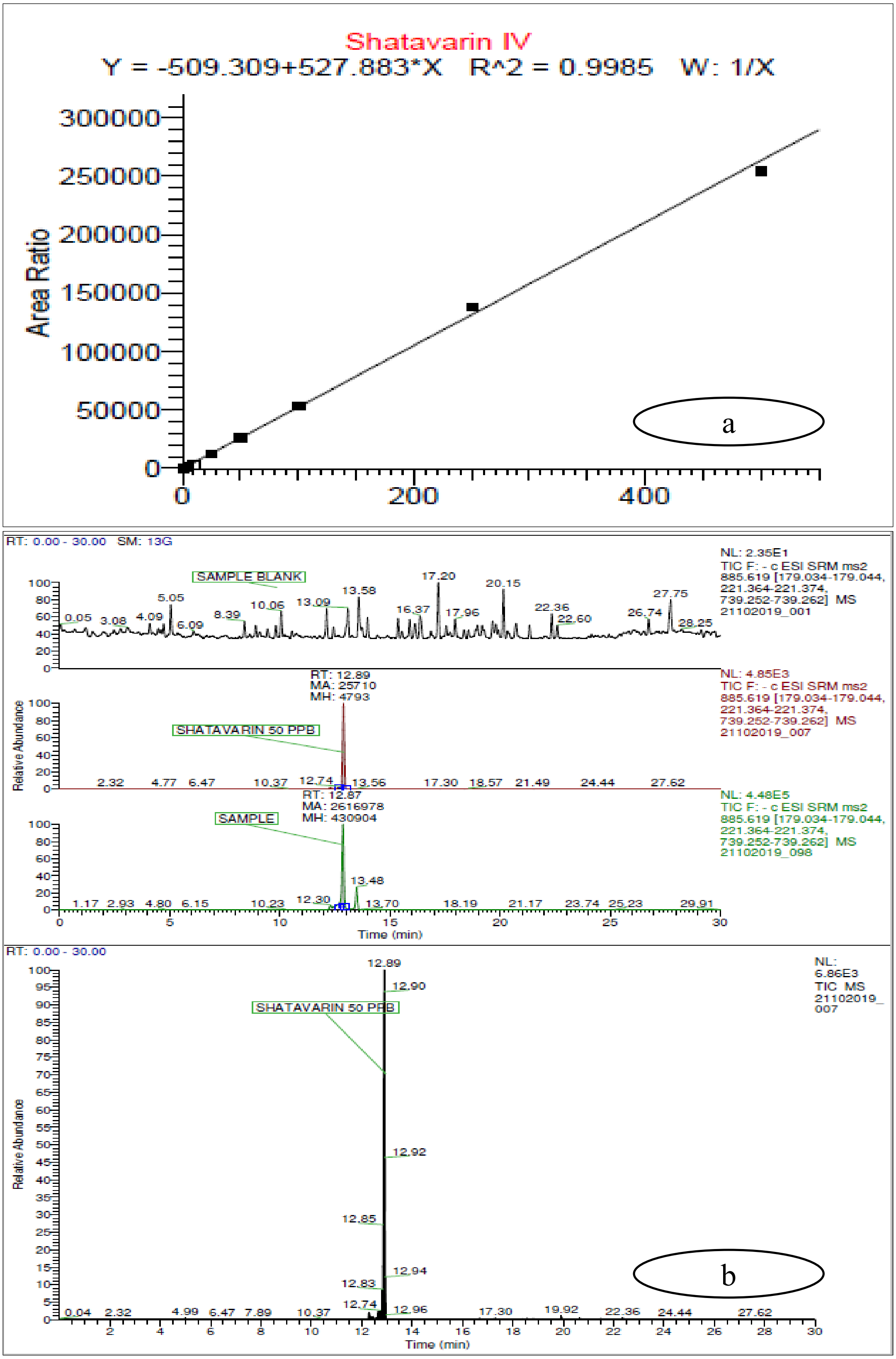


**Figure 1.** Optimization of chromatographic separation of shatavarin IV. 1a) Specificity of shatavarin IV. 1b) Calibration curve of shatavarin IV.

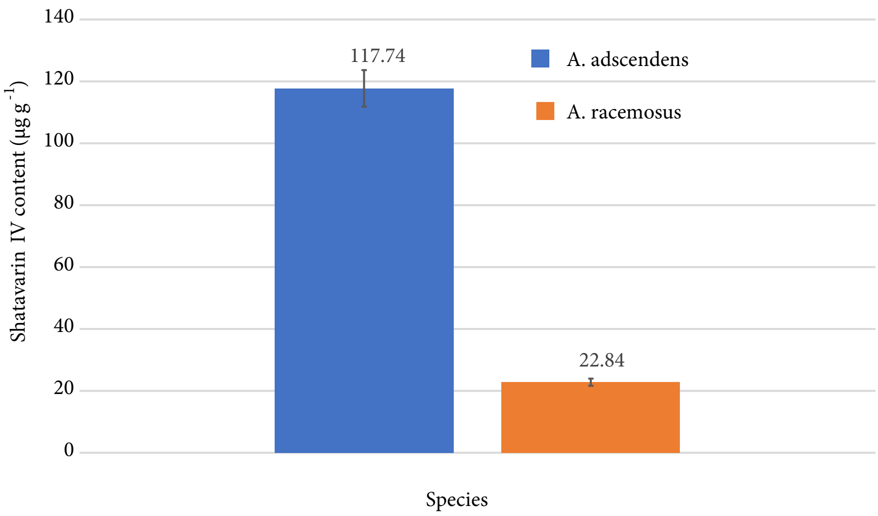


**Figure 2.** Average Shatavarin IV content in *Asparagus racemosus* and *A. adscendens* species.

**Table 1.** Evaluation of *A. adscendens* accessions for growth parameters.

| Accession | Fresh herbage yield per plant (g plant$^{-1}$) | Stem diameter (mm) | Spike length (mm) | Spike base girth (mm) | Internode length (cm) | Number of cladodes (branchlet$^{-1}$) |
|---|---|---|---|---|---|---|
| DAA-9 | 776c | 6.11e | 0.67f | 1.61ef | 2.80bc | 115bc |
| DAA-14 | 810c | 3.36f | 0.93e | 0.97g | 2.10e | 82def |
| DAA-16 | 3795a | 12.47b | 1.53a | 3.12a | 3.40a | 131ab |
| DAA-20 | 820c | 5.85ef | 0.57f | 1.37f | 1.77f | 62f |
| DAA-23 | 1171bc | 8.16d | 1.23bc | 2.12d | 2.97b | 71ef |
| DAA-26 | 3200ab | 6.80e | 1.47a | 2.84b | 2.67cd | 79def |
| DAA-28 | 1516abc | 10.55c | 1.27b | 2.49c | 3.63a | 148a |
| DAA-32 | 1286bc | 10.39c | 1.03de | 2.38cd | 2.50d | 103cd |
| DAA-36 | 1268bc | 7.76de | 1.13cd | 1.74e | 2.60cd | 147a |
| DAA-41 | 3681a | 14.70a | 1.47a | 3.20a | 3.53a | 94cde |
| DAA-43 | 2945abc | 12.65b | 1.53a | 3.24a | 2.20e | 74ef |
| DAA-44 | 2848abc | 9.92d | 0.97e | 2.43c | 2.83bc | 113bc |

Different letters within columns indicate significant differences ($p = 0.05$) – (Duncan multiple range test).

reported under semiarid conditions of India (Hayes et al., 2008; Kumar et al., 2016). The variation for root yield and chemical content as affected by shade net intensity was also reported in shatavari (AR) under western part of India (Saran et al., 2019). Genetic variation among the germplasm also plays a significant role in quantity as well as quality of roots. Sixty genotypes of shatavari have been grouped into three groups based on saponin contents (33.02 to 53.46 mg g$^{-1}$) showing variability between genotypes (Kumar et al., 2016). Significant variations for shatavarin IV content (151.89–3.64 µg g$^{-1}$) in roots were registered among twelve accessions of *A. racemosus* (Saran et al., 2020). The comparative analysis based on morphological and biochemical characters revealed the significant variations between the species and accessions were observed. The results shows that there is a potential for bioactive principal in this species as compared to other asparagus species. The accessions exhibited significant variation for economic parameters i.e. fresh herbage yield, root weight, root length, root width, root yield and shatavarin IV content. The quantification of such active compounds from different accession is valuable for standardization and preparation of formulations for improving fertility and vitality in women.


**Figure 3.** Variation for spine morphology in different AA accessions.

**Table 2.** Evaluation of *A. adscendens* accessions for root parameters and shatavarin IV content.

| Accession | Fresh weight of rootlet (g) | Root length (cm) | Root girth (mm) | Root yield per plant (g) | Thickness of central portion of roots (mm) | Dry root recovery (%) | Shatavarin IV ($\mu g\ g^{-1}$) |
|---|---|---|---|---|---|---|---|
| DAA-9 | 12.67ef | 48.73b | 7.61cd | 2376g | 0.82g | 23.71b | 100.45i |
| DAA-14 | 8.00f | 29.43e | 6.71d | 2723fg | 0.94fg | 21.79c | 113.31h |
| DAA-16 | 10.33f | 39.90cd | 8.29bc | 6596bcd | 1.31bc | 21.63c | 105.01i |
| DAA-20 | 8.33f | 28.30e | 8.63bc | 2273g | 1.43ab | 20.18d | 175.35d |
| DAA-23 | 11.33ef | 38.47d | 9.00ab | 6456cd | 1.15cde | 17.64e | 156.63ef |
| DAA-26 | 17.00cde | 48.67b | 7.87c | 5853d | 1.24cd | 25.36a | 207.03c |
| DAA-28 | 31.33a | 64.23a | 8.97ab | 7066bc | 1.29bc | 20.15d | 570.80a |
| DAA-32 | 26.33ab | 55.67b | 9.16ab | 4482e | 1.51a | 17.69e | 347.27b |
| DAA-36 | 20.67bc | 50.67b | 9.40a | 3330f | 1.12de | 20.00d | 176.13d |
| DAA-41 | 20.00cd | 49.63b | 9.37a | 9039a | 1.41ab | 23.90b | 125.57g |
| DAA-43 | 19.67cd | 47.67bc | 8.85abc | 7384b | 1.05ef | 22.86b | 164.14e |
| DAA-44 | 14.00def | 52.50b | 6.77d | 8316a | 1.49a | 25.04a | 152.22f |

Different letters within columns indicate significant differences (p = 0.05) – (Duncan multiple range test).

## 4. Conclusion

In conclusion, species *A. adscendens* exhibited five times higher shatavarin IV content in roots as compared to *A. racemosus*. Among the selected twelve accessions, DAA-28 registered maximum shatavarin IV content and DAA-41 for root yield. Overall, DAA-41, DAA-44, DAA-28 and DAA-32 accessions were found to be superior in root yield and shatavarin IV content. These accessions are diverse and

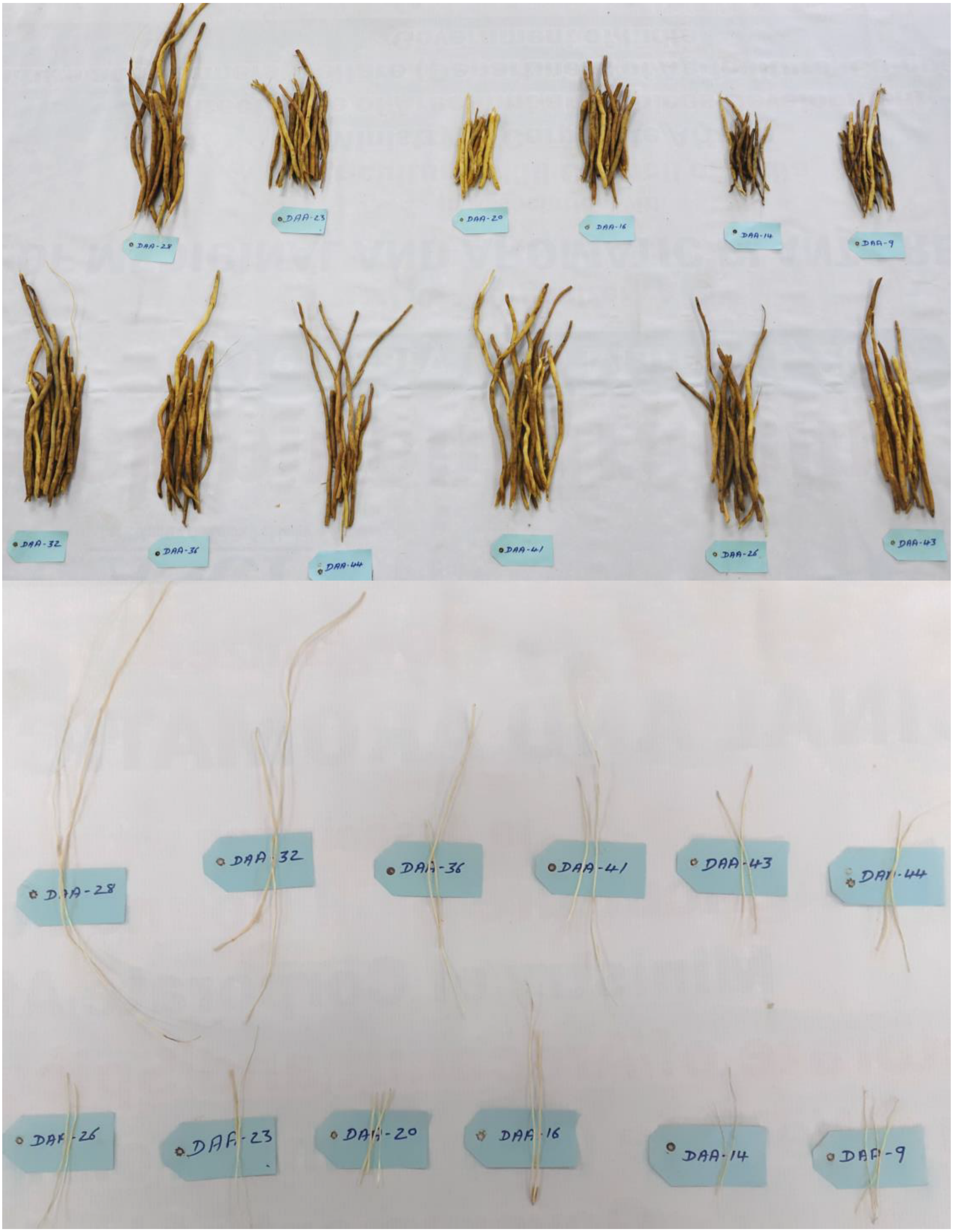


**Figure 4.** Variation for root morphology in different AA accessions.

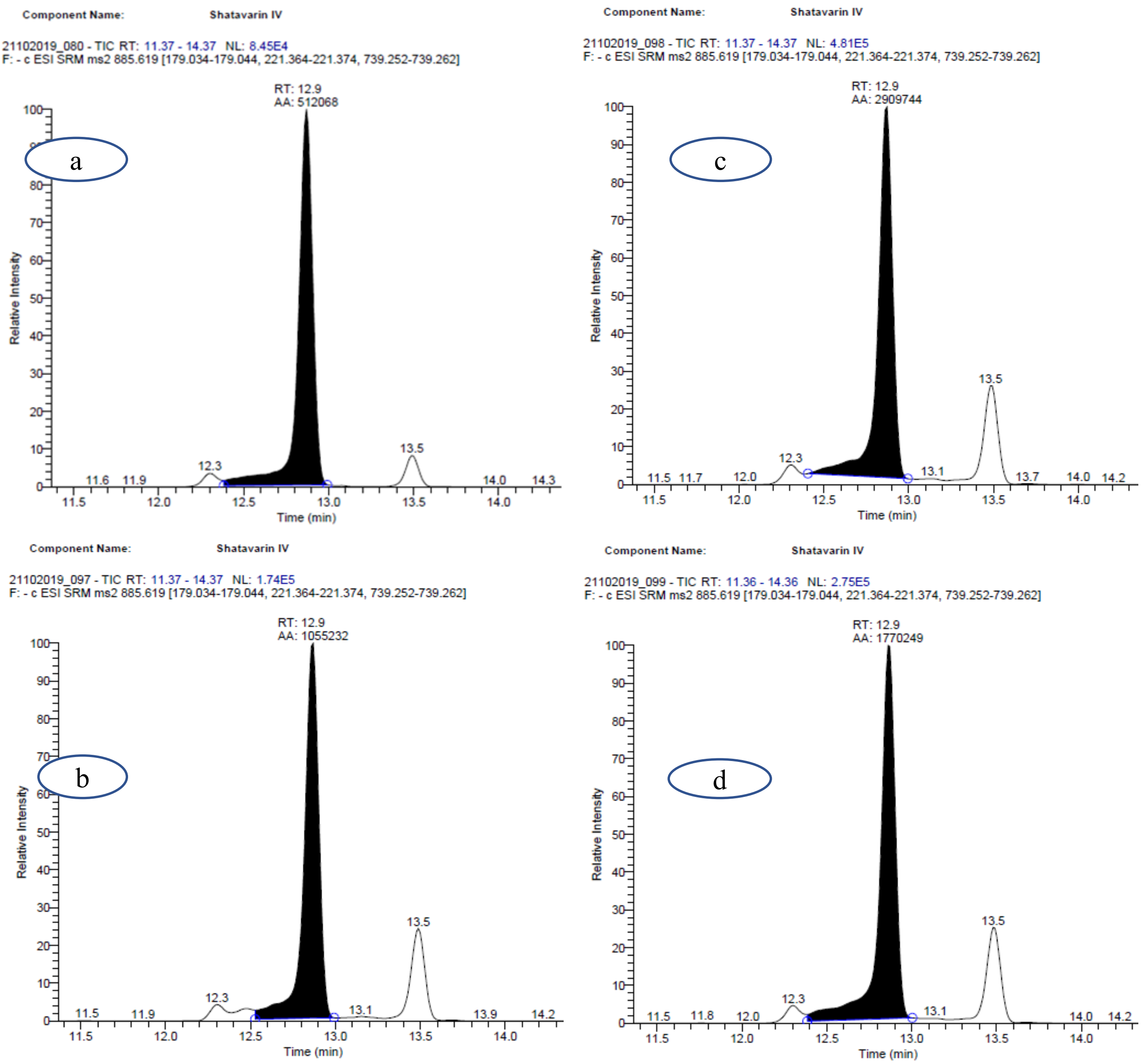


**Figure 5.** LC–MS/MS chromatograms of AA accessions. 5a) DAA-9. 5b) DAA-26. 5c) DAA-28. 5d) DAA-32.

varying in herbage, root yield and shatavarin IV content, therefore, they can be used further in crop improvement programs.

## Conflict of interest

The authors declare that they have no competing interests.

## Acknowledgment

The authors are grateful to the Indian Council of Agricultural Research for providing the resources for conducting the experiments. This study was supported by the NMPB, New Delhi and NAHEP-CAAST, ICAR, New Delhi.

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