Industrial Crops and Products 35 (2012) 199–202

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Multivariate analysis and genetic diversity for morphometric and root textural quality traits in ashwagandha (*Withania somnifera* Dunal)

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ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form 23 June 2011
Accepted 28 June 2011
Available online 26 July 2011

Key words:
Withania somnifera
Morphometric traits
Root textural quality
Root starch content
Multivariate analysis
Genetic diversity

ABSTRACT

Ashwagandha (*Withania somnifera*) is an important medicinal plant and its dried roots are used in traditional systems of medicine. The market price of roots is determined by physical (textural) quality. Brittle roots with high starch and low fiber are considered to be superior because of ease in grinding. Genetic diversity studies based on root textural parameters have not been done so far. So the present study was designed to assess genetic diversity for morphometric traits and root textural quality parameters among two morphologically distinct groups: Poshita and Nagore. The PCA separated the morphometric and root texture variables distinctly into two different principal components: PC-1 and PC-2 respectively, indicating that both are negatively associated. All the morphotypes in Poshita group showed high positive loadings in PC-1 indicating that component genotypes are high root yielding. Nagore morphotypes were low yielding but the root texture was good. Clustering of morphotypes grouped Poshita and Nagore separately with high inter-cluster distances indicating that both groups are highly divergent from each other, suggesting that there is sufficient scope for varietal improvement through hybridization.

1. Introduction

The genus *Withania* comprises of 23 species among which only two (*Withania somnifera* and *Withania coagulans*) are reported from India. The dried roots of W. somnifera commonly known as ‘Ashwagandha’ are widely used in Indian traditional systems of medicine such as Ayurveda, Unani and Siddha. The quality parameters of ashwagandha roots can be broadly classified into chemical quality and physical (textural) quality. The medicinal properties of the root are attributed to the chemical quality, *i.e.* alkaloids and withanolides content (Gupta and Rana, 2007). On the other hand the commercial value of roots depends upon the physical (textural) quality, which is determined by starch and fiber content. Roots having high starch and low fiber were quoted to be characteristic root textural feature of commercial ashwagandha (Atal and Schwarting, 1962). This is because, majority of the ashwagandha roots are consumed as semi-processed drug (the dried roots are powdered and consumed) and starchy roots fetch premium price because of ease in grinding them into powder.

W. somnifera encompasses rich variability for plant morphology, root yield, chemical contents and root textural quality. Kaul (1957) reported that cultivated types differ from wild ones not only for therapeutic properties but also for morphological characters and suggested new specific name *Withania ashwagandha* for the cultivated plants. Dhalla et al. (1961) observed that roots of cultivated types contains abundance of starch grains and breaks easily with even fracture, whereas wild type roots with less starch grains breaks with difficulty having uneven fracture. Atal and Schwarting (1962) studied several populations in the Indian region and concluded existence of 5 different morphological forms among which one morphotype with starchy roots was designated as cultivated form and remaining four were stated to be wild types.

The nature and extent of genetic diversity among the different morphotypes based on morphometric data have been undertaken (Jain et al., 2007; Misra et al., 1998). Further Negi et al. (2000, 2006) grouped the morphotypes into Nagori and Kashmiri groups based on AFLP and SAMPL markers. Dhar et al. (2008) assessed genetic diversity among *Withania* accessions belonging to different eco-geographical locations using ISSR and RAPD markers. Cultivated and wild accessions were reported to be divergent not only for morphometric traits and withanolides markers (Arun Kumar et al., 2007) but also for intra-specific variation (ITS) region of rDNA (Mir et al., 2010). Dhar et al. (2006) reported correlation of withanolide markers with molecular (AFLP) markers in different chemotypes of *W. somnifera*.

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doi:10.1016/j.indcrop.2011.06.032

For any organized plant improvement programme, the trait of focal importance depends on utility of the plants. In ashwagandha, apart from root yield and chemical components, root textural quality that determines the market price of the root is also a trait of focal importance. So there is a need for genetic characterization for textural quality parameters. However, genetic diversity studies on *W. somnifera* were limited to morphometric traits, chemical quality, molecular markers and so far no attempt has been made to study the genetic variation for root physical quality. So the present investigation was carried out to study the genetic diversity for root textural quality features in *W. somnifera*.

2. Materials and methods

2.1. Experimental material and design of experiment

Poshita (variety developed by CIMAP) and Nagore (traditionally cultivated cultivar) are morphologically distinct cultivars that are widely cultivated in dryland districts of Andhra Pradesh, India. Due to mixed mating (occurrence of both selfing and out-crossing) nature of the species (Lattoo et al., 2007) Poshita and Nagore developed some amount of variability during the course of cultivation in farmers field. Selections were executed in the populations of Poshita and Nagore to identify the morphological variants (morphotypes). 21 morphotypes were identified and evaluated for morpho-metric traits and root textural quality parameters along with 3 cultivated varieties (Poshita, JS-134, JS-20) and two traditionally cultivated cultivars (Nagore and Neemuch), in a RBD trial with three replications at the research farm of the Central Institute of Medicinal and Aromatic Plants (CIMAP) – Research Centre, Hyderabad, India during 2009–2010.

Based on morphological features the experimental materials were categorized into two morphologically distinct groups: Poshita and Nagore. Poshita group (tall, with red coloured berries and broadly ovate, entire leaves) comprised of 9 morphotypes (Prefix–WSP) selected in variant population of Poshita and cultivars: Poshita and JS-134. Nagore group (short, with orange berries and narrowly ovate, wavy leaves) included 7 Nagore morphotypes (with Prefix-ET and WSN), variety: JS-20 and traditionally cultivated cultivars: Nagore and Neemuch. Each treatment was accommodated in 1.5 m × 1.5 m plot. Seeds were sown in nursery during last week of June 2009. The seedlings were transplanted in lines 30 cm apart with a distance of 15 cm between plant to plant during second week of August 2009. Normal cultural practices were followed throughout the crop season.

2.2. Observation on morphometric traits

Data on 8 morphometric traits, *i.e.* plant height (cm), leaf area (cm), number of leaves/plant, number of branches/plant, root length (cm), root diameter (cm), number of secondary roots/plant, dry root yield/plant (g) were recorded on ten randomly selected plants in each experimental material in each replication. Leaf length and width were measured in 10 leaves/plant and leaf area was arrived by multiplying leaf length and width by constant value (0.7028) as suggested by Patidar et al. (1990).

2.3. Observations on root textural quality parameters

The root textural quality parameters in ashwagandha include starch content, fiber content and starch–fiber ratio. For quantification of starch and fiber contents the root materials of each morphotype were bulked replication wise and dried under shade. The dried root material was grinded in pulverizer, sieved and fine root powder was used for analysis. Root starch Content was determined as per methodology by Hodge and Hofrieter (1962) and crude fiber content was quantified according to Maynard (1970). Starch–fiber ratio was arrived by dividing starch content with fiber content as follows:

$$\text{starch–fiber ratio (SFR)} = \frac{\text{starch content (\%)}}{\text{fiber content (\%)}}$$

2.4. Statistical analysis

Since the variables were measured in different units, they were standardized to eliminate bias due to different magnitude of units. Each observation on each variable was standardized by subtracting the mean of that variable from the value for the observation on the variable and dividing this by the standard deviation for the corresponding variable. The standardized data were subjected to principal component analysis (PCA) and clustering was performed based on minimum variance method of Ward (1963) utilizing statistical software Ky plot.

3. Results

Analysis of variance showed significant differences among the treatments for all the characters (data not shown) indicating that PCA and clustering are worthwhile.

3.1. Principal component analysis

The first three principal components (PCs) with eigenvalues >1 contributed 84.31% of the variability among the genotypes evaluated for morphometric traits and root quality parameters (Table 1). The first PC was more related to root yield and its attributes with high positive loadings for all the morphometric traits except number of branches/plant. In PC-1, root diameter showed the highest positive loading (0.966) followed by root yield (0.925) and leaf area (0.916). Among the root textural quality parameters, fiber content showed high positive loading (0.821) in PC-1, wherein starch content had low positive loading (0.118) and loading value of SFR was negative (−0.758). The second PC was more relevant to root textural quality with high positive loadings for starch content (0.970) and SFR (0.521). The PC-2 loading for fiber content was low (0.106). Number of branches/plant showed high positive PC loading in PC-3 (0.805).

The scores for first two PCs of the morphotypes along with their grouping are presented in Table 2. With respect to first PC related with morphometric traits and fiber content all the genotypes in Poshita group showed positive scores and the genotypes of Nagore group showed negative scores. In PC-2 related with root texture, Nagore selections – WSN-3, ET-2, WSN-4 and Poshita selections – WSP-10 and WSP-3 showed higher positive scores.

Table 1
Principal component loadings for morpho-metric traits and root textural quality parameters in *W. somnifera*.

Traits	PC-1	PC-2	PC-3
Plant height (cm)	0.884	0.002	0.074
Leaf area (cm)	0.916	−0.070	0.035
No. of leaves/plant	0.817	−0.074	0.238
No. of branches/plant	0.535	0.050	0.805
Root length (cm)	0.802	0.129	−0.208
Root diameter (cm)	0.966	0.072	−0.139
No. of secondary roots/plant	0.803	0.131	0.308
Dry root yield/plant (g)	0.925	0.013	−0.253
Root starch content (%)	0.118	0.970	−0.117
Root fiber content (%)	0.821	0.106	−0.291
SFR	−0.758	0.521	0.239
Eigen values	6.912	1.275	1.087
% of variation	62.84	11.59	9.88
% of cumulative variation	62.84	74.43	84.31