



Delineating taxonomic identity of two closely related *Vigna* species of section *Aconitifoliae*: *V. trilobata* (L.) Verdc. and *V. stipulacea* (Lam.) Kuntz in India

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Received: 18 December 2018 / Accepted: 18 March 2019 / Published online: 27 March 2019
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Abstract *Vigna trilobata* (L.) Verdc. and *V. stipulacea* (L.) Kuntz. of section *Aconitifoliae* were studied based on morphological characters to address taxonomic delineation. We have attempted to resolve the identities of these two species by studying a representative set of collections (125 accessions) across diverse eco-geographical zones of India. Agro-morphological traits were recorded for 47 descriptor states to differentiate the two species. Remarks on some additional characters not reported in earlier studies were highlighted with an aim to facilitate field identification of these taxa and use in their genetic resource management. Key diagnostic characters essential for delineating identities of these two species were presented.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10722-019-00767-9>) contains supplementary material, which is available to authorized users.

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Keywords Comparative characters · Morphology · Taxonomic delineation · *Vigna trilobata* · *V. stipulacea*

Introduction

Food legume production is challenged by a number of biotic and abiotic stresses and edaphic factors (Ojiewo et al. 2017; Umdale et al. 2018; Tripathi et al. 2013). This is leading to search for newer sources of nutrition to meet the protein malnutrition. Some wild underexploited or underutilised vignas have been reported with superior agronomic characteristics (Oyatomi et al. 2016), important nutritional elements (Macorni et al. 1997; Difo et al. 2015) nutraceutical value (Bhat and Karim 2009) and adaptability to unusual edaphic conditions such as sandy beaches, acid soils, limestone rocks, deserts and wetlands (Tomooka et al. 2011).

The genus *Vigna* is categorized into seven subgenera (Marechal et al. 1978). Among these, Asia represents a center of diversity only for subgenus *Ceratotropis*, popularly known as the Asiatic *Vigna*. It includes 24 species, distributed in three sections viz., section *Ceratotropis* Tomooka and Maxted, section *Aconitifoliae* Tomooka and Maxted and section *Angulare* Tomooka and Maxted (Tateishi 1996; Bisht et al. 2005; Tomooka et al. 2002; Yadav et al. 2014).

Section *Aconitifoliae* consists of six species: *Vigna aconitifolia* (Jacq.) Maréchal, *V. aridicola* N.

Tomooka et al. (2002), *V. indica* T.M. Dixit, K.V. Bhat and S.R. Yadav, *V. khandalensis* (Santapau) Raghvan et al. (2014), *V. trilobata* (L.) Verdc. and *V. stipulacea* (Lam.) Kuntze. The important traits of this section which make it more important, especially during the climate change regime are adaptation to hot, dry, and tropical lowland habitats (Tomooka et al. 2002). Tomooka et al. (2014) have reported *V. trilobata* and *V. stipulacea* as candidates for neo-domestication for drought tolerance and disease and pest resistance, respectively. Various other workers also reported resistance to diseases and pests in both the species (Nagaraj et al. 1981; Chandel et al. 1984; Tomooka et al. 2006; Pandiyan et al. 2008).

Morphologically *V. trilobata* and *V. stipulacea* are closely related (Tomooka et al. 2002) and often misidentified in the field due to gross morphological similarity (Dixit 2014). *V. stipulacea*, newly recognized as a domesticated Indian *Vigna* species (Difo et al. 2018), was earlier included in the description of *V. trilobata* (Tomooka et al. 2006). Nevertheless, inadequate published information demands further study to reveal the correct identity to unveil the possible potential of these species.

Studies undertaken by various workers have considered morphological characters important to distinguish species in the genus *Vigna* (Corner 1976; Alsina 1988; Tomooka et al. 2002; Dixit et al. 2011; Gaikwad et al. 2014; Latha et al. 2014; Yadav et al. 2014; Pratap et al. 2015). For example, *Vigna trilobata* (L.) Verdc. var. *pusilla* Naik and Pokle was raised to the rank of species as *V. indica* (Dixit et al. 2011) and several new species were identified, including *V. sahyadriana* Aitwade et al. and *V. konkanensis* Latha et al. (Umdale et al. 2017) and *V. yadavii* Gaikwad et al. (Gaikwad et al. 2014).

Verdcourt (1970) while studying the Papilionidae stated that “the whole matter (of taxonomy of *Vigna*) is very confused and no sound decision can (we) come to until a great deal of work has been done”. Most of the previous studies were performed by studying a limited number of accessions. In contrast to this, the present study was carried out on 125 accessions available in the Indian National Genebank for 47 agromorphological traits. This study mainly focused on the distinct characters useful for delimiting these two underutilized *Vigna* species with an objective to assess the taxonomic significance of morphological and micro-morphological traits with respect to the species

delineation; and to develop an identification key for the benefit of botanists, plant explorers, crop curators, breeders and other researchers.

Materials and methods

Plant material

A total of 125 accessions of *V. stipulacea* and *V. trilobata* collected from diverse eco-geographical zones of India and conserved at Indian National Genebank were grown at two locations in the northern plains of India viz., New Area Farm, Indian Council of Agricultural Research (ICAR)—National Bureau of Plant Genetic Resources (NBPGR), New Delhi and wide hybridization garden at ICAR- Indian Institute of Pulses Research (IIPR), Kanpur during *Kharif* 2018–19. Accessions were sown under natural field conditions on 19th July, 2018 at both the locations. At ICAR-NBPGR, each accession was sown in a plot of two rows of 4 m length, each spaced 60 cm apart. At ICAR-IIPR these were grown as unreplicated in a 1-m row plot each. As most of the wild accessions show staggered germination due to hard seed coat, scarification was done to ensure proper water imbibition for facilitating good germination in all accessions. For scarification, individual seed was held between the thumb and index finger and excision was made on opposite side of the hilum using a sharp surgical blade. The scarred seeds were incubated on moist filter paper at room temperature for 24 h in Petri plates, before their direct seeding in the field following method by Pratap et al. (2015).

Recording observations: A total of 47 traits were recorded following descriptors developed by International Board of Plant Genetic Resources (IBPGR), Rome (1980) and International Institute of Tropical Agriculture, Nigeria (data presented in Table 1). Ten individual plant samples from each accession were used to examine morphological characters. While plant parts were observed with the help of naked eye and a hand lens (10x), the floral characters were recorded using stereo-microscope (Lmi, SZM 12) with separated floral parts. Growth habit was recorded when first pod changed colour. Plant height was measured by using metric scale. For seed and pod characters ten samples from each accession were used. Length and width of seed, hilum and pod were

Table 1 Comparison of characters^a between *Vigna stipulacea* and *V. trilobata*

S. no	Characters	<i>Vigna stipulacea</i>	<i>Vigna trilobata</i>
<i>Vegetative characteristics</i>			
1	Germination	Intermediate	Epigeal
2	First leaf (shape and size)	Petiolate, simple, broad, elliptic (1.1 × 1.0 cm)	Petiolate, cordate (0.8 × 0.8 cm)
3	Habit	Trailing, semi-erect to erect	Trailing
4	Root	Shallow root	Deep root
5	Stem angle	Angular	Slender
6	Plant height (cm) (at 30 days)	4–28	4–11
7	Branch length (cm)	45–152	180–210
8	Petiole length (cm)	6–16	18–22
9	Peduncle length (cm)	6.5–62	6–10
10	Stipule length (cm)	1.1–2.6	0.2–0.4
11	Stipule width (cm)	1.1–1.9	0.1–0.2
12	Stipule shape	Ovate and broad at base	Lanceolate
13	Leaflet shape and pubescence	Oval-trilobed and sparsely hairy	Trilobed, hairy and densely hairy
14	Epidermal cell structure	6–8 angle, not unit	5 angle, unit size
15	Stomata shape	Guard cell unequal	Guard cell equal
16	Trichome length (cm)	0.77	1.68
17	Pubescence	Glabrous	Pubescent
<i>Flower characteristics</i>			
18	Raceme position	Above canopy	Under canopy
19	Calyx colour	Greenish-purple	Green
20	Inflorescence	Compact and glabrescence	Loosely bind and hairy
21	Flower colour	Shiny yellow	Golden yellow
22	Standard (mm)	12	8
23	Wing (mm)	3	2
24	Colour of keel pocket (at tip)	Purple	Whitish
25	Ovary (mm)	2.4	2.6
26	Style (mm)	6.6	10.4
27	Style beak shape (mm)	Pear (0.2)	Pointed (0.4)
<i>Pod characteristics</i>			
28	Pod length (cm)	4–6	2–3
29	Seed per pod	10–15	4–6
30	Constriction on pods	Not prominent	Prominent
31	Colour of ventral suture of immature pod	Dark purple	Green
32	Pod colour at maturity	Tan-black	Straw
33	Beak shape	Pointed	Blunt
34	Trichome colour, hairiness	Brown short hairs	Glabrescent to white short hairs
35	Pod section (placenta)	Swollen	Invisible
36	Pod dehiscence pattern	Remain cling at the base	Two valves separate
<i>Seed characteristics</i>			
37	Seed colour at immaturity	Green	Light orange
38	Seed colour at maturity	Black	Light orange

Table 1 continued

S. no	Characters	<i>Vigna stipulacea</i>	<i>Vigna trilobata</i>
39	Lustre on the seed surface	Shiny	Dull
41	Seed shape	Round	Oblong
42	Seed length (mm)	2.1–2.5	2.3–3.27
	Seed width (mm)	2–2.3.7	1.9–2.51
43	Seed thickness (mm)	2.0–2.1	2.1–2.3
44	100 seed weight (gm)	0.6–1.0	1–1.4
45	Aril	Slightly developed	Well developed
46	Hilum colour	Off-white	Light-brown
47	Hilum shape	Ellipsoidal, oblong, protruding	Broadly ovate/orbicular, protruding

^aBased on ten sample of each accession

measured with a Vernier caliper. Seed and pod colour were measured using Royal Horticultural Society (RHS) colour chart. Number of the pods/peduncle and number of seeds/pod were recorded by manual counting. Selected micro traits viz., trichome length (10x), epidermal cell structure and stomata shape (40x) were recorded by using a compound microscope. Trichome density was measured by using hand lens (10x). Herbarium based study was done using 120 sheets in global herbaria (Kew (K), Paris (P), Global Biodiversity Information Facility (GBIF), British Museum of Natural History (BMNH) and National Herbarium of Cultivated Plants (NHCP).

Results and discussion

Utmost care was taken to grow all the *Vigna* accessions at most favourable locations during the season when these had best phenotypic expression. While northern plains lying in North West Plain Zone (NWPZ) are the major mungbean and urdbean growing areas in India, *Kharif* (July–October) is the main season for growing these crops which is typically characterized by high precipitation (up to 400 mm), high humidity (> 70%), warmer temperatures (up to 20° min. and 36 °C max.) and long photo-periods (day length up to 14 h). The other eco-geographic regions suitable for growing *Vigna* crops in India include

North East Plain Zone (NEPZ), North Hill Zone (NHZ), Central Zone (CZ), and the South Zone (SZ). Likewise, the other *Vigna* growing seasons include Spring (February end to middle of May) and Summer (April to June) (Pratap et al. 2013) which are comparatively warmer (temperatures reaching up to 43 °C) and drier months (< 70 mm rainfall) of the year and require assured irrigation facilities to raise a crop. India is considered to be endowed with rich diversity of cultivated as well as wild and weedy types of Asiatic *Vigna*, predominantly in the Western Ghats and the Himalayan region, and is also considered as the region of first domestication of some of the important cultivated *Vigna* species of the world (Bisht et al. (2005). While most of the accessions studied in the present investigation were also collected from SZ, especially the Western Ghats, a few represented the NEPZ, CZ and NHZ (Fig. 1 and Supplementary Table 1).

Data on all the traits were recorded when these had full expression. The morphological and anatomical characters of the studied taxa are presented in Table 1 and Figs. 2, 3, and 4.

Seedling characteristics

Seed germination was a major differentiating character between these two species that can be observed at an early stage during viability test. An intermediate



Fig. 1 Distribution map of of *V. stipulacea* and *V. trilobata*

type of germination was observed in *V. stipulacea* and epigeal in *V. trilobata*. Intermediate type is when germination occurs between hypogeal and epigeal type (Schmidt, 2000). In most of the *V. stipulacea* accessions cotyledons were above the ground surface but hypocotyl was underground. These observations were similar to reports of Dixit et al. (2011); Dixit

(2014). In contrast to this, Tomooka et al. (2002) reported hypogeal germination in *V. stipulacea*.

At two leaf stage, *V. stipulacea* showed the cordate leaf tip broadly elliptic in shape with petiole (1.1×1.0 cm) while *V. trilobata* showed pointed leaf tip cordate at base with petiole (0.8×0.8 cm) (Fig. 2). Tomooka et al. (2002) reported that seedling characters like germination type and presence of first

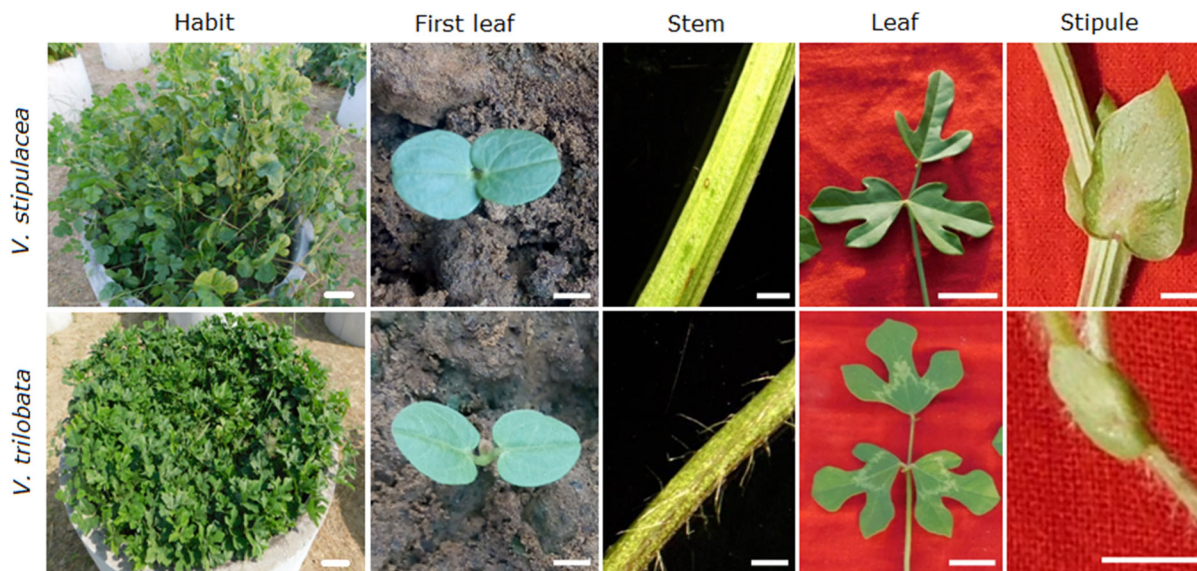


Fig. 2 Vegetative characteristics of *V. stipulacea* and *V. trilobata*. (Scale bars are 5 mm)



Fig. 3 Floral characteristics of *V. stipulacea* and *V. trilobata*. (Scale bars are 5 mm)

and second leaf petiole are important for taxonomic delineation.

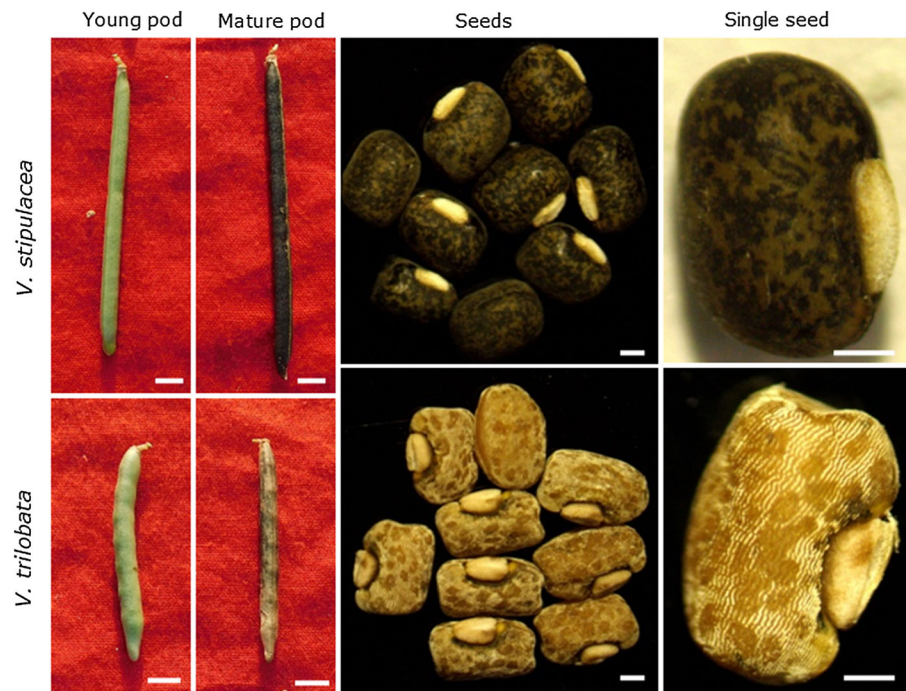
Vegetative characteristics

Growth habit of *V. stipulacea* varied from erect, semi-erect to trailing. Accessions from Tamil Nadu region were trailing type while Andhra Pradesh accessions showed erect to semi-erect habit. Tomooka et al. (2002) and Yadav et al. (2014) observed *V. stipulacea* with trailing habit based on only one accession. On the

other hand, in this study all accessions of *V. trilobata* irrespective of eco-geographical region showed trailing habit.

Vigna stipulacea accessions were observed to have shallow root with many secondary branches while *V. trilobata* had deep tap root system with less secondary branches. Tomooka et al. (2014) also reported *V. trilobata* having a deep root system which is a desirable character for drought tolerance. Stem was angular and hollow in *V. stipulacea* whereas slender

Fig. 4 Pod and seed characteristics of *V. stipulacea* and *V. trilobata*. (Scale bars are 5 mm)



and solid in the *V. trilobata*. Very sparse hairs were observed in *V. stipulacea* than *V. trilobata* (Fig. 2).

Plant height ranged from 4 to 28 cm in *V. stipulacea* and the longest branch varied from 45 to 152 cm while plant height ranged from 4 to 11 cm with 180–210 cm branch length in *V. trilobata*. Leaf characters of taxonomic significance include stipule shape and size (Tomooka et al. 2002; Dixit 2014). Stipules were comparatively large ($1.1\text{--}2.6 \times 1.1\text{--}1.9$ cm) in *V. stipulacea* being ovate and broad at base while they were small ($0.2\text{--}0.3 \times 0.1\text{--}0.2$) and lanceolate in *V. trilobata* (Fig. 2). Larger variation for petiole length was observed in *V. stipulacea* ranging from 2 to 16 cm. Trailing type accessions showed very short petiole while semi-erect to erect accessions showed larger petiole, whereas, in *V. trilobata* petiole length varied between 18 and 22 cm. In *V. stipulacea*, peduncle length ranged from 6.5 to 62 cm with up to 14 pods per peduncle while it ranged from 6 to 10 cm in *V. trilobata* with up to 5 pods per peduncle.

In *V. stipulacea*, the leaf was observed variously shaped. Terminal leaflet shape varied from oval to trilobed, obtuse at apex, obtuse to rounded at base, $1.5\text{--}7.1 \times 1.8\text{--}6.1$ cm in size and the lateral leaflets were oblique, oval, bi- or tri-lobed, obtuse or rounded

at apex, rounded at base and $1.5\text{--}5.7 \times 1.1\text{--}5.8$ cm in size (Fig. 2). In *V. trilobata*, terminal leaflet was always deeply tri-lobed, $1.4\text{--}2.5 \times 1.6\text{--}2.5$ cm in size, while lateral leaflets were ovate, deeply tri-lobed and $1.2\text{--}2.1 \times 1.0\text{--}2.2$ cm. In size. Vegetative and reproductive plant parts were glabrous in *V. stipulacea* whereas pubescent in *V. trilobata* (Fig. 2). The epidermal cell structure was observed to be 6–8 angled in *V. stipulacea*, while they were 5 angled in *V. trilobata*. Guard cells were unequal in size in *V. stipulacea* and equal in *V. trilobata*. Very large trichomes were observed in the leaves of *V. trilobata* with trichome length 1.6 cm while they were short (0.77 cm) in *V. stipulacea*. Tri-lobed leaf shape were found to be mainly confusing characters between these two species.

Flower characteristics

Flowers and pods were always located above the canopy in *V. stipulacea*, while these were not visible above the canopy in *V. trilobata*. Inflorescence was compact and glabrous in *V. stipulacea* while loosely bound and hairy in *V. trilobata* (Fig. 3). In *V. stipulacea*, shiny yellow flowers with purple tinge on the standard petal in the center and purple tip of keel

pocket was the prominent character to distinguish this species from *V. trilobata*. On the other side, golden yellow flowers with purple stripes on the standard petal and whitish tip of keel pocket were observed in *V. trilobata* (Fig. 3).

The breadth of standard petal and wing petal in *V. stipulacea* was 12 mm and 3 mm, respectively while in *V. trilobata* standard petal was 10 mm and wing petal was about 2 mm. Ovary was 2.4 mm long in *V. stipulacea* and 2.6 mm in *V. trilobata*. Style was comparatively smaller (6.6 mm) in *V. stipulacea* than *V. trilobata* (10.4 mm). Previous workers (Tomooka et al. 2002; Dixit 2014) reported the same findings. Style grows beyond the stigma to form a peculiar beak shape that serves as one of the most distinguishing characters in both species. Pear shaped beak was observed in *V. stipulacea* whereas it was pointed in *V. trilobata* (Fig. 3). Calyx colour was greenish-purple and green in *V. stipulacea* and *V. trilobata*, respectively.

Pod characteristics

Pod was also one of the most distinguishing characters in both the species. It was longer (4–6 cm × 2.86–3.0 cm) in *V. stipulacea* with 10–15 seeds while smaller (2–3 cm × 2.9–3.1 cm) in *V. trilobata* with 4–6 seeds in each pod. *V. stipulacea* had round shaped pod with a hard surface and no prominent constriction on fruit wall in contrast to constricted fruit wall with soft surface in *V. trilobata*. Ventral suture of immature pod in *V. stipulacea* were dark purple in colour while it was green in *V. trilobata*. Pod colour was tan-black in *V. stipulacea* and straw coloured in *V. trilobata* (Fig. 4). In pod section, placenta was small in *V. stipulacea* whereas swollen and prominent in *V. trilobata*.

Seed characteristics

The seed morphological characters of the both the species observed under light microscopy are presented in Table 1 and Fig. 4. The seeds of the genus *Vigna* varied in their shape, colour, size and hilum structure. Colour of immature seed was green in *V. stipulacea* and light orange in *V. trilobata*. Colour of mature seeds varying from tan to black, with or without mottling, was recorded in *V. stipulacea* while more uniform seed pattern (light orange with variegation) was observed in *V. trilobata*. Seed shape was oblong to

round in *V. stipulacea* while oblong in *V. trilobata*. These findings were in concurrence with the results of Tomooka et al. (2002); Dixit (2014) and Yadav et al. (2014). In contrast to this, Bisht et al. (2005) reported *V. trilobata* possessing more roundish, black seeds with slightly raised hilum, probably due to confusion of identity between *V. stipulacea* and *V. trilobata*.

The smooth testa with lustre was observed in *V. stipulacea* whereas, *V. trilobata* seed coat surface was found to be rough. The hilum was observed as whitish, oblong and slightly protruding in *V. stipulacea* whereas it was creamish, broadly obovate or orbicular and protruding in *V. trilobata*. The hilum was located in a central position in both these species. Aril was found to be less developed in *V. stipulacea* than *V. trilobata*.

The seed length in *V. stipulacea* ranged from 2.1 to 2.5 mm, width from 2.2 to 3.7 mm and thickness from 2.0 to 2.1 mm while in *V. trilobata*, seed length ranged from 2.3 to 3.27 mm, width from 1.9 to 2.51 mm and thickness from 2.1 to 2.3 mm. 100-seed weight was higher (1–1.4 g) in *V. trilobata* than in *V. stipulacea* (0.6–1.0 g).

The detailed study of Asiatic *Vigna* species showed that the species-specific nature of the seed coat and hilum morphology can be potentially useful in taxonomic differentiation (Chandel and Laster 1991; Nath and Dasgupta 2015). Seed morphological characters have provided reliable information in systematic studies of various legume genera (Manning and van Staden 1987; Kirkbride et al. 2003; de Queiroz et al. 2013). Seed size and shape, as well as hilum shape, had diagnostic value at the species level, in accordance with the findings of Alsina (1988) and Paulino et al. (2010) for other genera of legumes. Likewise, several other investigations have reported importance of morphological characters in taxonomy for differentiating related taxa (Abou-El-Enain et al. 2007; Al-Ghamdi and Al-Zahrani 2010; Chernoff et al. 1992). Dixit et al. (2011) suggested that seed characters viz., texture, presence or absence of an aril, and testa ornamentation are important to delineate *V. trilobata* and *V. stipulacea*. The potential taxonomic significance of ultrastructural patterns of seed coat morphology has been recognized in several taxa (Koul et al. 2000; de Queiroz et al. 2013; Patil et al. 2015; Umdale et al. 2017). However, in our study, we focussed mainly on the morphological characters.

Previous studies based on meagre diversity study did not point out the range of the variation. However, in the present study, characterization of 125 accessions for 47 agro-morphological characters facilitated observations on variability in two taxa. Additional characters included in the study were anatomy of leaf epidermal, stomatal and trichome characters that were also very distinct in the two taxa.

Herbarium study

Lectotypification of *Dolichos stipulaceus* more than 225 years after its first documentation is a good example of the importance of herbarium database (Dixit 2014). Herbarium specimens and data from e-resources available in K, P, GBIF, BMNH and NHCP revealed a lack of flower and pod characters and information on status as wild or cultivated. Only stipule characters were clearly visible in the herbarium specimen (P00296830) as the flowers were too small. Vouchers of the experimental study deposited in the NHCP, ICAR-NBPGR, New Delhi, India (HS no. 23531—*V. trilobata*; 23532 and 23,533—*V. stipulacea*) are likely to serve as good resource for identification.

Field key for identification

On the basis of above observations, clear cut differentiation between *V. stipulacea* and *V. trilobata* was established and accordingly, the following key was developed to differentiate these two taxa:

- Intermediate germination, angular stem, large stipule, raceme above canopy, compact inflorescence, purple keel pocket, aril slightly developed, seed with hilum oblong, slightly Protruding: *V. stipulacea*.
- Epigeal germination, solid stem, small stipule, raceme below canopy, loose inflorescence, whitish keel pocket, aril well developed, seed with hilum broadly ovate or orbicular, much Protruding: *V. trilobata*.

Conclusion

The present study demonstrated remarkable morphological variation in diverse accessions of these two

taxa and added novel traits to the key described earlier, including stem angles, colour of the keel pocket, raceme position, inflorescence type. The results corroborated the findings of Maréchal et al. (1978); Tomooka et al. (2002); Yadav et al. (2014) and Umdale et al. (2017). Study on seed germination, hard seededness and other physiological aspects are required to help in domesticating them as desired.

Acknowledgements We are thankful to the Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi, and Dean, ICAR-IARI, New Delhi for their support and encouragement in undertaking this work. We thank the Director, ICAR-IIPR, Kanpur for facilitating field trial. Dr Anuradha Agrawal, ICAR-NBPGR, Dr M Latha and Dr Joseph John, Regional Station (RS) Thrissur and Dr Kamala Venkateswaran, RS, Hyderabad, ICAR-NBPGR are acknowledged for the help rendered by them in different ways.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the content of manuscript and study undertaken.

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