INTRODUCTION

Seed morphology often provides useful characters for generic and species identifications, and can also help understand taxonomic relationships, such as in the following families: Acanthaceae (Rueangsawang et al., 2012), Asteraceae (Chehregani & Mahanfar, 2007; Inceer et al., 2012), Iridaceae (Erol et al., 2006), Orobanchaceae (Plaza et al., 2004) and Scrophulariaceae (Kaplan et al., 2007).

Various seed morphological characters in Fabaceae have been studied several times and some seed characters are very useful for faboid generic identifications (Kirkbride et al., 2003). Studies based on various genera in the Fabaceae indicated that seed morphology provides valuable taxonomic characters for distinguishing some taxa in the tribe Genisteae (López et al., 2000) and the genera Colutea L. (Mirzaei et al., 2015), Entada Adans. (Rodrigues, 2015), Lathyrus L. (Günes & Ali, 2011; Günes, 2013), Indigofera L. (Al-Ghamdi, 2011), Trigonella L. (Turki et al., 2014), and Vigna Savi (Nath & Dasgupta, 2015).

Seed morphology of nine Indian Crotalaria L. species was studied by Gandhi et al. (2011) using light and scanning electron microscopy. The seeds varied significantly in size, colour, surface, and hilum characters. Seeds of Crotalaria were typically kidney-shaped. Seed colour appeared to be of less diagnostic and systematic value. All species of Crotalaria had a smooth surface, except C. albida Heyne ex Roth and C. spectabilis Roth. The study showed that seed coat ornamentation pattern can be helpful for species identification. Moreover, the shape of Thai Crotalaria seed has been described by Niyomdham (1978). However, he overlooked seed coat sculpturing. Because previous data on seed morphology of Thai Crotalaria species are insufficient, the current study evaluates the taxonomic significance of seed morphology of the Crotalaria species in Thailand for application in classification and identification of the species.

MATERIALS AND METHODS

Mature seeds of 19 Crotalaria species from five sections of Le Roux et al. (2013) were obtained from living specimens, vouchers are stored in the KKU herbarium (Table 1). The seed were examined with microscopes and measurements were based on a sample size of 10 grains. Seeds were cleaned by ultrasonic cleaner for 2–5 minutes and dehydrated.

Seed morphology of nineteen Crotalaria L. (Fabaceae) species in Thailand

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ABSTRACT

Seed morphology of 19 Crotalaria species in Thailand was studied using stereoscopic microscopy and scanning electron microscopy. Five different morphological types are described based on differences in shape, aril, fracture lines, size and seed coat surface. Each type is morphologically described, compared, illustrated, and the taxonomic implications are discussed. A key to identify the different types or some species is presented.

KEYWORDS: Crotalaria, Leguminosae, Papilionoideae, micromorphology, Thailand.

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with an alcohol series (70%, 95%, 100%), and subsequently studied by stereoscopic microscope (SM) and scanning electron microscopy (SEM). The seed measurements were investigated under SM. For SEM, the seed was adhered onto an aluminum stub with double-sided cellophane tape and air-dried at room temperature. Finally, the samples were sputter-coated with a gold-palladium mixture under vacuum and examined with Leo 1450 VP SEM. The terminology of seed morphology mainly follows Kirkbride et al. (2003) and Bojňanský & Fargašová (2007). The measurement of length and width are represented in Figure 1.

Table 1. List of specimens examined of *Crotalaria* for seed morphology

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher Collector</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>C. acicularis</em></td>
<td>S. Ninkaew 259 (KKU)</td>
<td>Phetchabun</td>
</tr>
<tr>
<td>2. <em>C. alata</em></td>
<td>S. Ninkaew 263 (KKU)</td>
<td>Phetchabun</td>
</tr>
<tr>
<td>3. <em>C. albida</em></td>
<td>S. Ninkaew 305 (KKU)</td>
<td>Chiang Mai</td>
</tr>
<tr>
<td>4. <em>C. assamica</em></td>
<td>S. Ninkaew 312 (KKU)</td>
<td>Nan</td>
</tr>
<tr>
<td>5. <em>C. bracteata</em></td>
<td>S. Ninkaew 253 (KKU)</td>
<td>Nan</td>
</tr>
<tr>
<td>7. <em>C. chinensis</em></td>
<td>S. Ninkaew 261 (KKU)</td>
<td>Phetchabun</td>
</tr>
<tr>
<td>8. <em>C. dubia</em></td>
<td>S. Ninkaew 307 (KKU)</td>
<td>Chiang Mai</td>
</tr>
<tr>
<td>9. <em>C. filiformis</em></td>
<td>S. Ninkaew 306 (KKU)</td>
<td>Chiang Mai</td>
</tr>
<tr>
<td>10. <em>C. gorrensis</em></td>
<td>S. Ninkaew 287 (KKU)</td>
<td>Sakon Nakhon</td>
</tr>
<tr>
<td>11. <em>C. juncea</em></td>
<td>S. Ninkaew 280 (KKU)</td>
<td>Nakhon Ratchasima</td>
</tr>
<tr>
<td>12. <em>C. lejoloba</em></td>
<td>S. Ninkaew 318 (KKU)</td>
<td>Phetchabun</td>
</tr>
<tr>
<td>13. <em>C. medicaginea</em></td>
<td>S. Ninkaew 281 (KKU)</td>
<td>Khon Kaen</td>
</tr>
<tr>
<td>14. <em>C. montana</em></td>
<td>S. Ninkaew 329 (KKU)</td>
<td>Khon Kaen</td>
</tr>
<tr>
<td>15. <em>C. neriifolia</em></td>
<td>S. Ninkaew 180 (KKU)</td>
<td>Sakon Nakhon</td>
</tr>
<tr>
<td>16. <em>C. pallida</em></td>
<td>S. Ninkaew 273 (KKU)</td>
<td>Bueng Kan</td>
</tr>
<tr>
<td>17. <em>C. sessiliflora</em></td>
<td>S. Ninkaew 291 (KKU)</td>
<td>Sakon Nakhon</td>
</tr>
<tr>
<td>18. <em>C. spectabilis</em></td>
<td>S. Ninkaew 271 (KKU)</td>
<td>Sa Kaeo</td>
</tr>
<tr>
<td>19. <em>C. verrucosa</em></td>
<td>S. Ninkaew 298 (KKU)</td>
<td>Loei</td>
</tr>
</tbody>
</table>

Figure 1. The measurement of length and width in each shape of *Crotalaria* seeds: A) reniform; B) harp-shaped (L = Length; W = Width).
RESULTS

The micromorphological characters of Crotalaria seeds in Thailand including shape, colour, size, seed coat surface, fracture lines, hilum shape and aril features, were studied and summarized in Table 2. Among the taxa examined, two basic morphological seed shape types can be distinguished, which are reniform and harp-shaped. Both show a wide range of variation in the characters of shape, aril, fracture lines, size and seed coat surface. Based on our SM and SEM studies we distinguish five different seed morphological types. However, the seed morphological features of each type cannot be used for species identification except in Type IV. The types are keyed out and described here, for type IV identification to species level is provided:

KEY TO SEED TYPES

1. Type I

Seeds reniform, 1.8–3.5 × 1–3 mm, brown-black, with aril; seed coat surface smooth with or without fracture lines; hilum circular or oval, ruminate. This type occurs in C. alata Buch.-Ham. ex D. Don (seeds slightly larger, 3–3.5 × 2.5–3 mm) and C. lejoloba Bartl. (seeds slightly smaller, 1.8–3 × 1–2 mm) (Figs. 2 & 3). The aril was already reported by Niyomdham (1978).

2. Type II

Seeds reniform, 1.3–2.8 × 1–2.2 mm, brown, without aril; seed coat surface smooth with fracture lines; hilum oval or circular, ruminate. This type occurs in C. acicularis Buch.-Ham. ex Benth., C. chinensis L. and C. montana Heyne ex Roth (Figs. 2, 3 & 4), of which the seeds are similar in colour, size and seed coat surface. The hilum is different, circular in C. acicularis and C. chinensis and oval in C. montana.

3. Type III

Seeds reniform, 3–5 × 2.8–5 mm, brown, yellow brown or brown-black, without aril; seed coat surface smooth and irregularly wrinkled or colliculate and with fracture lines; hilum circular or oval, ruminate. This type is found in C. assamica Benth., C. bracteata Roxb. ex DC., C. neriifolia Wall. ex Benth. and C. verrucosa L. (Figs. 2 & 4). The variation in colour, seed coat surface and hilum shape cannot be used to identification of species. The hilum shape of C. assamica is oval, circular in the other three species. Seed coat surfaces are either smooth and irregularly wrinkled (C. assamica and C. neriifolia) or colliculate (C. bracteata and C. verrucosa).

4. Type IV

Seeds reniform, 3–5 × 2–4.5 mm, orange-brown, brown, brown-green, brown-black, without aril; seed coat surface smooth and irregularly wrinkled, colliculate or tuberculate, without fracture lines; hilum circular or oval, ruminate. This type is found in C. goreensis Guill. & Perr., C. juncea L., C. pallida Aiton and C. spectabilis. The species can be keyed out using colour, size, seed coat surface and hilum shapes of the seeds (Figs. 2 & 5).

KEY TO SPECIES
Table 2. A comparison of seed characters studied for *Crotalaria* species

<table>
<thead>
<tr>
<th>Seed types</th>
<th>Species</th>
<th>Shape</th>
<th>Colour</th>
<th>Size (Length (mm) Width (mm))</th>
<th>Seed coat surface</th>
<th>Fracture lines</th>
<th>Hilum shape</th>
<th>Aril</th>
</tr>
</thead>
<tbody>
<tr>
<td>I C. alata</td>
<td>reniform</td>
<td>brown-black</td>
<td>3–3.5 (3.23±0.19) 2.5–3 (2.68±0.14)</td>
<td>smooth</td>
<td>−</td>
<td>oval</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>C. lejoloba</td>
<td>reniform</td>
<td>brown-black</td>
<td>1.8–3 (2.29±0.53) 1–2 (1.60±0.46)</td>
<td>smooth</td>
<td>+</td>
<td>oval</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>II C. acicularis</td>
<td>reniform</td>
<td>brown</td>
<td>1.3–1.5 (1.39±0.09) 1–1.2 (1.10±0.08)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. chinensis</td>
<td>reniform</td>
<td>brown</td>
<td>2–2.3 (2.13±0.14) 1.5–1.7 (1.58±0.09)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. montana</td>
<td>reniform</td>
<td>brown</td>
<td>2–2.8 (2.43±0.35) 1.5–2.2 (1.88±0.30)</td>
<td>smooth</td>
<td>+</td>
<td>oval</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>III C. assamica</td>
<td>reniform</td>
<td>brown-black</td>
<td>3–5 (4±0.94) 3–5 (4.2±0.79)</td>
<td>smooth &amp; irregularly wrinkled</td>
<td>+</td>
<td>oval</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. bracteata</td>
<td>reniform</td>
<td>yellow-brown</td>
<td>3–3.2 (3.11±0.10) 2.8–3 (2.91±0.10)</td>
<td>smooth &amp; colliculate</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. neriifolia</td>
<td>reniform</td>
<td>brown-black</td>
<td>3.5–3.8 (3.66±0.14) 2.8–3 (2.9±0.09)</td>
<td>smooth &amp; irregularly wrinkled</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. verrucosa</td>
<td>reniform</td>
<td>brown</td>
<td>3.5–4.3 (3.95±0.30) 3–3.5 (3.25±0.21)</td>
<td>smooth &amp; colliculate</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>IV C. gorrensis</td>
<td>reniform</td>
<td>orange-brown</td>
<td>4–5 (4.55±0.44) 2.5–2.8 (2.67±0.13)</td>
<td>smooth &amp; irregularly wrinkled</td>
<td>−</td>
<td>oval</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. juncea</td>
<td>reniform</td>
<td>brown-green</td>
<td>4.5–5 (4.74±0.24) 4–4.5 (4.26±0.24)</td>
<td>smooth &amp; tuberculate</td>
<td>−</td>
<td>oval</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. pallida</td>
<td>reniform</td>
<td>brown</td>
<td>3–4 (3.5±0.47) 2.2–5 (2.26±0.24)</td>
<td>smooth &amp; colliculate</td>
<td>−</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. spectabilis</td>
<td>reniform</td>
<td>brown-black</td>
<td>4.5–5 (4.77±0.22) 3.5–4 (3.76±0.23)</td>
<td>smooth &amp; irregularly wrinkled</td>
<td>−</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>V C. albida</td>
<td>harp-shaped</td>
<td>brown</td>
<td>1–1.5 (1.28±0.22) 1.3–2 (1.63±0.29)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. calycina</td>
<td>harp-shaped</td>
<td>brown</td>
<td>1.5–2 (1.76±0.21) 2.5–3 (2.76±0.21)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. dubia</td>
<td>harp-shaped</td>
<td>yellow-brown</td>
<td>1–1.5 (1.27±0.19) 1.6–1.8 (1.49±0.26)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. filiformis</td>
<td>harp-shaped</td>
<td>brown</td>
<td>0.8–1 (0.94±0.08) 1–1.3 (1.22±0.12)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. medicaginea</td>
<td>harp-shaped</td>
<td>brown-black</td>
<td>1.5–1.7 (1.61±0.09) 1.7–2 (1.90±0.12)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>C. sessiliflora</td>
<td>harp-shaped</td>
<td>brown</td>
<td>1.3–1.5 (1.38±0.08) 1.5–1.7 (1.57±0.08)</td>
<td>smooth</td>
<td>+</td>
<td>circular</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

Notes: + = present; − = absent
5. Type V

Seeds harp-shaped, 0.8–2 × 1–3 mm, yellow-brown, brown, brown-black, without aril; seed coat surface smooth with fracture lines; hilum circular, ruminate. This type is found in *C. albida*, *C. calycina* Schrank, *C. dubia* Graham ex Benth., *C. filiformis* Wall. ex Benth., *C. medicaginea* Lam. and *C. ses-siliflora* L. (Figs. 2 & 6). The seed of *C. filiformis* is smallest (0.8–1 × 1–1.3 mm) and of *C. calycina* largest (1.5–2 × 2.5–3 mm). The smooth seed coat surface of *C. albida* is in contrast with the undulating lines reported by Gandhi et al. (2011). The taxa cannot be keyed out with seed characters alone.

DISCUSSION AND CONCLUSION

Based on external morphology, two groups of species were traditionally distinguished, reniform or harp-shaped, which agrees well with Niyomdham (1978). The seed colour varies from yellow-brown, orange-brown, brown, brown-green to brown-black. Seed sizes vary between 0.8–5 mm in both length and width. The seed of *C. filiformis* is the smallest (0.8–1 × 1–1.3 mm), while that of *C. juncea* is the largest (4.5–5 × 4–4.5 mm).

Gandhi et al. (2011) reported that seed colour is not diagnostic, but in contrast, we found it to be an important character for identification of the species with Type IV.

Seed morphology has proven to provide useful characters for the identification and delimitation of species or species groups within *Crotalaria* as the seeds of *Crotalaria* can be divided into five types.

The seeds shape, colour and seed coat surface are quite similar in each species while seeds size is slightly variable based on standard deviation (SD; Table 2). The three species with the highest variation in seed sizes are: *C. assimica* (3–5 (4±0.94) × 3–5 (4.2±0.79)), *C. lejoloba* (1.8–3 (2.29±0.53) × 1–2 (1.60±0.46)) and *C. montana* (2–2.8 (2.43±0.35) × 1.5–2.2 (1.88±0.30)), respectively. However, this study is based on few specimens per species. It can be that the variability appears to greater than described here if more specimens from other areas were collected too. However, we are confident that especially the qualitative characters used in the key are stable.

The seed types do not agree with the division of *Crotalaria* into sections that are based on morphological characters of Polhill (1982) and molecular evidence of Le Roux et al. (2013) and Rockinger et al. (2017). Seed morphological characters alone are insufficient for a full taxonomic resolution of *Crotalaria* species as the variability in morphology is too great. Nevertheless, in combination with other characters seed morphology can help to resolve taxonomic problems. Therefore, as in other Leguminosae, pollen morphology (Ridder-Numan & Van der Ham, 1997), anatomy (Ninkaew & Chantaranothai, 2015) and molecular characters (The Legume Phylogeny Working Group, 2017) should be combined to clarify the taxonomy of *Crotalaria* in Thailand.

ACKNOWLEDGEMENTS

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REFERENCES


Figure 3. SEM micrographs of Crotaaria seeds: Type I: A & B) *C. alata*, C & D) *C. lejoloba*; Type II: E & F) *C. acicularis*. Scale bars: 1 mm (A, C, E); 200 µm (B, D, F); Ar = Aril; white arrows = fracture lines.
Figure 4. SEM micrographs of Crotalaria seeds: Type II: A & B) C. montana; Type III: C & D) C. assamica, E & F) C. bracteata. Scale bars: 1 mm (A, C, E); 200 µm (B, D, F); Hi = hilum; Co = colliculate; Ir = irregularly wrinkled; white arrows = fracture lines.
Figure 5. SEM micrographs of Crotalaria seeds: Type IV: A & B) C. goreensis, C & D) C. juncea, E & F) C. pallida. Scale bars: 1 mm (A, C, E); 200 µm (B, D, F); Co = colliculate; Ir = irregularly wrinkled; Tu = tuberculate.
Figure 6. SEM micrographs of *Crotalaria* seeds, Type V: A & B) *C. albida*, C & D) *C. calycina*, E & F) *C. sessiliflora*. Scale bars: 1 mm (A, C, E); 200 µm (B, D, F); white arrows = fracture lines.


