South African Journal of Botany 99 (2015) 1–11

Contents lists available at ScienceDirect

South African Journal of Botany

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

Caryopsis micromorphological survey of *Sorghum* (Poaceae)—Taxonomic implications

H. Liu [a,b], X.Y. Hu [a], Y.X. Liu [a], Q. Liu [a,*]

[a] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form 11 February 2015
Accepted 24 February 2015
Available online 31 March 2015

Keywords:
Andropogoneae
Phylogeny
Spermoderm sculpture
Stylopodium

ABSTRACT

Caryopsis micromorphological characters of 25 *Sorghum* species were studied using stereoscopic microscopy and scanning electron microscopy. There are three caryopsis shapes, including obovate-elliptic, ovate-elliptic and subulate; two patterns of stylopodia, including with or without an apical tuft of unicellular microhairs; and five spermoderm sculpture patterns, that is, reticulate with wavy cell walls, reticulate with straight cell walls, substriate, undulating and rugose in *Sorghum*. Caryopsis size and stylopodium persistence pattern have limited taxonomic application at the infrageneric level, while the caryopsis shapes show a certain taxonomic significance at the infrageneric level and spermoderm sculpture patterns are important diagnostic characters at the interspecific level in *Sorghum*. The separation of *Sorghum sudanense* from *S.* × *drummondii* is supported by our result of caryopsis size and spermoderm sculpture patterns. Consistent micromorphological characters of stylopodium persistence, dorsoventral compression, embryo proportion (the ratio of embryo to caryopsis length) and hilum proportion (the ratio of hilum to caryopsis length) are firstly found in *Sorghum*. Caryopsis micromorphological characters provide evidence that *Sorghum* is a highly heterogeneous genus at the micromorphological level.

1. Introduction

Sorghum Moench (Andropogoneae, Poaceae) is a genus of 31 species exhibiting considerable morphological and ecological diversity (Garber, 1950; Snowden, 1955; Celarier, 1958; Chen and Phillips, 2006) in global tropical, subtropical and temperate regions (Lazarides et al., 1991; Spangler, 2003; Liu and Liu, 2014). Five morphological subgenera, *Sorghum*, *Parasorghum*, *Stiposorghum*, *Chaetosorghum* and *Heterosorghum*, were recognized within *Sorghum* based on cytological and traditional taxonomic studies (Garber, 1950; Celarier, 1958; Clayton and Renvoize, 1986; Lazarides et al., 1991). However, molecular phylogenetic studies have indicated that *Sorghum* could be divided into two or three lineages (Dillon et al., 2007; Ng'uni et al., 2010; Liu et al., 2014) or even three distinct genera (Spangler, 2003). Therefore, characters of potential taxonomic value need to be identified for comparisons at the infrageneric level in *Sorghum*.

Caryopsis micromorphology, as a reliable evidence for assessing phylogenetic relationship, has previously been recognized in Poaceae (Bogdan, 1966; Barthlott, 1981; Wang et al., 1986; Liu et al., 2005; Jiang et al., 2011; Gandhi et al., 2013; Zhang et al., 2014). For example, seven caryopsis subtypes for gramineous species were shown to correspond to seven subfamilies in China (Wang et al., 1986). In another example, the caryopsis ventral face and embryo shapes were valuable systematic characters for recognizing three suprageneric groups in the subfamily Chloridoideae (Liu et al., 2005). Scanning electron microscopy (SEM) was shown to be an effective means to investigate caryopsis micromorphology at the familial (Jordan et al., 1983; Wang et al., 1986), tribal (Terrell and Peterson, 1993), generic (Jordan et al., 1985; Jiang et al., 2011; Zhang et al., 2014) and specific (Gandhi et al., 2013) levels in Poaceae. However, there is no detailed study on the caryopsis micromorphology of *Sorghum* except for the cultivated sorghum, *Sorghum bicolor* (L.) Moench (Waniska and Rooney, 2000; Stenhouse et al., 2007). Therefore, the aim of this study was to accurately describe the caryopsis micromorphology, not only to identify characters of taxonomic value to facilitate comparisons at the infrageneric level but also to consider the practical diagnostic value at the interspecific level in *Sorghum*.

2. Materials and methods

Mature caryopses about 3–15 for each species of 25 *Sorghum* species, either from herbarium specimens or germplasm resources center (Table 1), were examined under a Carl Zeiss Stemi SV 11 stereomicroscope (SM) (Carl Zeiss AG, Jena, Germany) and a Jeol JSM-6360LV scanning electron microscope (SEM) (JEOL, Tokyo, Japan).

Caryopsis size measurement and shape observation were examined under the SM. After that, caryopses were sputter-coated with gold in a JFC-1100 sputter coater (JEOL, Tokyo, Japan). Micromorphological

* Corresponding author. Tel. /Fax: +86 20 37252567.
E-mail address: liuqing@scib.ac.cn (Q. Liu).

http://dx.doi.org/10.1016/j.sajb.2015.02.015
0254-6299/

Table 1
Material of *Sorghum* examined in the study [following classifications of Garber (1950) and Lazarides et al. (1991)].

Subgenus	Species	Voucher	Location	Numbers of caryopses	Figures
Sorghum					
	Sorghum × almum Parodi	Waterhouse BMW 7878 (CANB)	Australia	11	1A, 2A, 3A, 4A, 5A
		Parodi 14442a (K)	Argentina		
	S. arundinaceum (Desv.) Stapf	Waterhouse & Tom BMW 7795 (CANB)	Papua New Guinea	15	1B, 2B, 3B, 4B, 5B
		Dos Santos 3925 (K)	Brazil		
		Moutilla s.n. (US)	Venezuela		
	S. bicolor (L.) Moench	PI 17548 (GRIN)	Australia	15	1C, 2C, 3C, 4C, 5C
		Purdie 7863 (CANB)	Australia		
		Goddard s.n. (K)	Sandi Arabia		
		Small 8234 (US)	USA		
	S. × drummondii (Nees ex Steud.) Millsp. & Chase	ILRI 13333 (ILRI)	Kenya	14	1D, 2D, 3D, 4D, 5D, 5E
		Grantham s.n. (CANB)	Australia		
	S. halepense (L.) Pers.	PI 302268 (GRIN)	Tanzania	15	1E, 2E, 3E, 4E, 5 M
		Boyle TPB228 (CANB)	Australia		
	S. miliaceum (Roxb.) Snowden	Chapman s.n. (CANB)	Australia	9	1 F, 2 F, 3 F, 4 F, 5 N
		Gamble 27139 (K)	India		
	S. propinquum (Kunth) Hitchc.	PI 653737 (GRIN)	USA	9	1G, 2G, 3G, 4G, 5 V
		Parry 10 (CANB)	Thailand		
	S. sudanense (Piper) Stapf	Hamasaki s.n. (CANB)	USA	12	1H, 2H, 3H, 4H, 5Q, 5R
		Miller s.n. (K)	Puerto Rico		
	S. virgatum (Hack.) Stapf	Schweinfurth 529 (K)	Sudan	11	1I, 2I, 3I, 4I, 5 L
		Fuller 3146 (US)	USA		
Chaetosorghum					
	S. macrospermum E.D. Garber	Pullen 10611 (CANB)	Australia	4	1P, 2 J, 3 J, 4 J, 5O
Heterosorghum					
	S. laxiflorum F.M. Bailey	PI 562654 (GRIN)	Australia	10	1 K, 2 K, 3 K, 4 K, 5P
		Waterhouse BMW6873 (CANB)	Australia		
		Hartley 11722 (US)	Australia		
Parasorghum					
	S. leiocladum (Hack.) C.E. Hubb.	Blake 19241 (IBSC)	Australia	3	1 L, 2 L, 3 L, 4 L, 5G
		Miller 35 (US)	Australia		
	S. matarankense E.D. Garber & L.A. Snyder	Corfield 2188 (CANB)	Australia	8	1 V, 2 M, 3 M, 4 M, 5H
		Perry 2691 (US)	Australia		
	S. nitidum (Vahl) Pers.	Wang 11094 (IBSC)	China	4	1 J, 2 N, 3 N, 4 N, 5U
		Zhao 290 (IBSC)	China		
	S. purpureosericeum (Hochst. ex A. Rich.) Asch. & Schweinf.	Foticus 2333 (CANB)	Cameroon	3	1 N, 2O, 3O, 4O, 5 J
	S. timorense (Kunth) Büse	Purdie 5825 (CANB)	Australia	3	1Y, 2P, 3P, 4P, 5 W
		Fryxell & Craven 4213 (K)	Australia		
	S. versicolor Andersson	Taylor 221 (CANB)	Zimbabwe	7	1 M, 2Q, 3Q, 4Q, 5Z
		Strohbach & Sheuyange 3198 (K)	Namibia		
		Godfrey SH-1666 (US)	South Africa		
Stiposorghum					
	S. amplum Lazarides	Legge & Murphy 440 (CANB)	Australia	3	1O, 2R, 3R, 4R, 5S
		Start ANS1578 (CANB)	Australia		
	S. angustum S.T. Blake	Clarkson & Simon 7039 (K)	Australia	4	1Q, 2S, 3S, 4S, 5a, 5b
		Clarkson & Neldner 8004 (K)	Australia		
		Blake 18599 (US)	Australia		
	S. brachypodum Lazarides	Corfield 2126 (CANB)	Australia	6	1R, 2 T, 3 T, 4 T, 5 T
	S. ecarinatum Lazarides	Fryxell & Craven 3913 (K)	Australia	4	1S, 2U, 3U, 4U, 5 F
	S. exstans Lazarides	Corfield 2039 (CANB)	Australia	9	1 T, 2 V, 3 V, 4X, 5X
		Corfield 2120 (CANB)	Australia		
	S. intrans F. Muell. ex Benth.	Lazarides 7114 (CANB)	Australia	9	1U, 2 W, 3 W, 4Y, 5Y
		Corfield 2110 (CANB)	Australia		
	S. plumosum (R. Br.) P. Beauv.	Lazarides 7089 (K)	Australia	4	1 W, 2X, 3X, 4 V, 5I
	S. stipoideum (Ewart & Jean White) C.A. Gardner & C.E. Hubb.	Palmer 920 (CANB)	Australia	9	1X, 2Y, 3Y, 4 W, 5 K
		Fryxell & Craven 3942 (US)	Australia		

Voucher specimen abbreviations: CANB = Australian National Herbarium; GRIN = Germplasm Resources Information Network of United States Department of Agriculture at Beltsville; IBSC = South China Botanical Garden Herbarium; ILRI = International Livestock Research Institute at Addis Ababa, Ethiopia; K = Royal Botanic Gardens, Kew; PI = Plant Introduction number; US = United States National Herbarium.

characters were examined and photographed with SEM at 15 kv. Caryopses were orientated with the style pointing up and the embryo pointing down. The dorsal face with embryo was orientated towards the lemma and the ventral face with hilum was orientated towards the palea (Wang et al., 1986; Terrell and Peterson, 1993; Zhang et al., 2014). SEM photos of spermoderm sculpture patterns were taken in the middle area of the ventral face for each sample. Statistical analyses were conducted in Microsoft Excel 2007. Terminology as per Sendulsky et al. (1986), Stearn (1992), Terrell and Peterson (1993) and Liu et al. (2005) was followed.

Caryopsis micromorphological characters (including caryopsis shapes and spermoderm sculpture patterns) were manually optimized onto the redrawn majority rule consensus tree resulting from Bayesian evolutionary analysis by sampling trees (BEAST) of three chloroplast genes (*ndhA* intron, *rpl32-trnL* and *rps16* intron) (Liu et al., 2014). The analysis included 25 *Sorghum* species but excluded *S. bulbosum*,

Table 2
Caryopsis micromorphological characters of *Sorghum*. Abbreviations: OB–EL = obovate to elliptic, OV–EL = ovate to elliptic, SU = subulate; ST = stylopodium persistence patterns ("−" = without an apical tuft of unicellular microhairs, "+" = with an apical tuft of brown or silvery unicellular microhairs); SP = spermoderm sculpture patterns (I = reticulate pattern with wavy walls, II = reticulate pattern with straight walls, III = substriate pattern, IV = undulating pattern, V = rugose pattern); the number range for all species: minimum–(mean) –maximum, mm.

Taxa	Caryopsis shapes	ST	SP	Length of caryopses	Width of caryopses	Thickness of caryopses	Ratio of length to width	Ratio of width to thickness	Ratio of embryo to caryopsis length	Ratio of hilum to caryopsis length
Sorghum × almum	OB–EL	−	I	3.12–(3.53)–3.77	1.86–(2.11)–2.31	1.04–(1.34)–1.47	1.48–(1.68)–1.86	1.27–(1.59)–1.92	0.61–(0.66)–0.71	0.18–(0.20)–0.23
S. arundinaceum	OB–EL	−	I	1.61–(2.82)–4.09	1.19–(1.81)–2.50	0.80–(1.20)–1.75	1.35–(1.55)–1.78	1.31–(1.54)–1.85	0.61–(0.66)–0.73	0.15–(0.23)–0.26
S. bicolor	OB–EL	−	I	3.06–(3.77)–4.43	2.16–(2.65)–3.21	1.11–(1.90)–2.30	1.31–(1.43)–1.70	1.17–(1.42)–2.01	0.52–(0.65)–0.73	0.15–(0.24)–0.28
S. × drummondii	OB–EL	−	I	3.36–(3.99)–4.48	2.06–(2.37)–2.60	1.35–(1.60)–2.12	1.42–(1.69)–1.86	1.23–(1.49)–1.76	0.58–(0.67)–0.76	0.16–(0.19)–0.24
S. halepense	OB–EL	−	II	2.36–(3.19)–3.74	1.45–(1.85)–2.21	1.02–(1.18)–1.30	1.45–(1.71)–1.94	1.36–(1.57)–1.81	0.59–(0.64)–0.70	0.13–(0.17)–0.22
S. miliaceum	OB–EL	−	II	2.45–(2.67)–2.87	1.46–(1.60)–1.67	0.93–(1.05)–1.15	1.55–(1.67)–1.76	1.37–(1.52)–1.59	0.63–(0.72)–0.83	0.15–(0.17)–0.19
S. propinquum	OB–EL	−	III	1.40–(2.64)–3.23	0.74–(1.73)–2.27	0.65–(1.12)–1.48	1.41–(1.57)–1.89	1.14–(1.52)–1.68	0.60–(0.73)–0.84	0.19–(0.22)–0.27
S. sudanense	OB–EL	−	III	2.56–(2.78)–3.00	1.58–(1.68)–1.80	0.80–(1.00)–1.18	1.55–(1.65)–1.85	1.44–(1.71)–2.03	0.56–(0.61)–0.68	0.18–(0.21)–0.24
S. virgatum	OB–EL	−	I	3.01–(3.45)–3.78	1.60–(1.86)–2.14	1.01–(1.25)–1.56	1.70–(1.87)–2.31	1.33–(1.50)–1.65	0.56–(0.64)–0.70	0.17–(0.21)–0.25
S. macrospermum	OV–EL	−	II	4.01–(5.43)–6.18	1.28–(2.15)–2.58	0.52–(1.35)–1.86	2.39–(2.60)–3.13	1.39–(1.75)–2.46	0.52–(0.55)–0.58	0.16–(0.17)–0.18
S. laxiflorum	OB–EL	−	II	2.48–(3.15)–3.58	1.06–(1.59)–1.96	0.55–(1.04)–1.27	1.72–(2.02)–2.45	1.24–(1.55)–1.93	0.56–(0.65)–0.75	0.13–(0.15)–0.17
S. leiocladum	OB–EL	−	I	2.51–(2.55)–2.62	1.06–(1.11)–1.20	0.65–(0.69)–0.73	2.18–(2.30)–2.38	1.47–(1.55)–1.63	0.54–(0.58)–0.64	0.12–(0.13)–0.14
S. matarankense	SU	−	I	1.80–(2.64)–3.06	0.73–(0.96)–1.18	0.39–(0.59)–0.79	2.47–(2.76)–3.17	1.39–(1.67)–1.93	0.50–(0.56)–0.61	0.14–(0.16)–0.20
S. nitidum	OB–EL	−	III	1.89–(1.98)–2.09	1.01–(1.09)–1.20	0.56–(0.66)–0.71	1.58–(1.82)–2.01	1.55–(1.67)–1.80	0.58–(0.61)–0.64	0.20–(0.20)–0.21
S. purpureosericeum	OB–EL	−	I	2.91–(3.84)–4.67	1.18–(1.49)–1.69	0.56–(0.85)–1.09	2.47–(2.57)–2.76	1.55–(1.81)–2.11	0.57–(0.59)–0.61	0.20–(0.21)–0.22
S. timorense	SU	−	III	3.20–(3.41)–3.62	1.24–(1.34)–1.45	0.70–(0.78)–0.83	2.41–(2.55)–2.75	1.51–(1.72)–1.90	0.55–(0.56)–0.58	0.14–(0.15)–0.15
S. versicolor	OB–EL	−	IV	2.05–(2.30)–2.81	0.80–(1.00)–1.41	0.32–(0.38)–0.42	1.99–(2.35)–2.71	1.90–(2.36)–2.65	0.61–(0.63)–0.65	0.20–(0.21)–0.22
S. amplum	OB–EL	−	III	4.42–(4.53)–4.60	1.61–(1.68)–1.74	1.18–(1.21)–1.26	2.64–(2.71)–2.75	1.28–(1.39)–1.46	0.61–(0.62)–0.63	0.14–(0.14)–0.15
S. angustum	SU	−	V	2.45–(2.95)–3.38	0.98–(1.11)–1.22	0.62–(0.69)–0.76	2.31–(2.69)–3.45	1.58–(1.60)–1.63	0.64–(0.67)–0.71	0.18–(0.19)–0.20
S. brachypodum	SU	−	III	4.58–(5.48)–6.05	1.73–(1.85)–1.98	1.25–(1.51)–1.67	2.65–(2.95)–3.16	1.09–(1.27)–1.58	0.55–(0.61)–0.67	0.16–(0.18)–0.19
S. ecarinatum	SU	−	I	3.73–(4.07)–4.42	1.47–(1.55)–1.68	1.10–(1.22)–1.33	2.54–(2.62)–2.76	1.19–(1.36)–1.53	0.55–(0.56)–0.59	0.18–(0.20)–0.21
S. exstans	SU	+	IV	2.46–(3.27)–4.53	0.81–(1.10)–1.31	0.50–(0.81)–1.03	2.41–(2.97)–3.46	1.16–(1.37)–1.62	0.53–(0.61)–0.68	0.15–(0.18)–0.21
S. intrans	SU	+	IV	2.43–(3.81)–4.18	0.79–(1.13)–1.25	0.61–(0.76)–0.92	3.08–(3.36)–3.63	1.32–(1.55)–1.73	0.45–(0.52)–0.59	0.08–(0.14)–0.17
S. plumosum	SU	−	I	3.31–(3.65)–4.12	1.13–(1.20)–1.30	0.93–(0.96)–1.02	2.79–(3.05)–3.37	1.22–(1.25)–1.29	0.56–(0.59)–0.61	0.14–(0.15)–0.15
S. stipoideum	SU	−	I	3.26–(3.90)–4.38	0.99–(1.12)–1.33	0.78–(0.88)–1.06	2.61–(3.54)–4.02	1.15–(1.26)–1.42	0.51–(0.57)–0.64	0.12–(0.14)–0.17

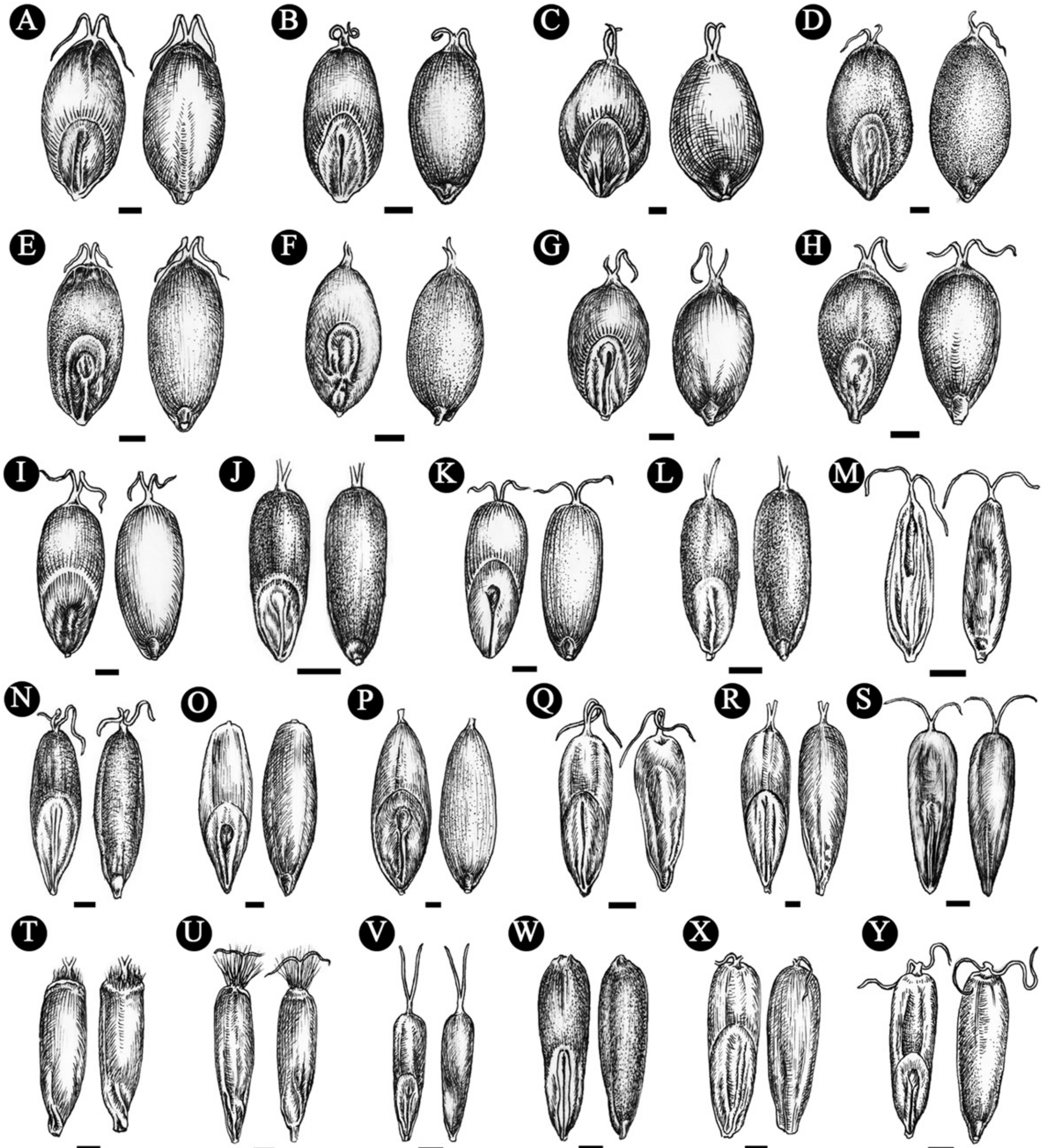

Fig. 1. Caryopsis shapes of *Sorghum* (SM). Obovate-elliptic: (A) *S.* × *almum*; (B) *S. arundinaceum*; (C) *S. bicolor*; (D) *S.* × *drummondii*; (E) *S. halepense*; (F) *S. miliaceum*; (G) *S. propinquum*; (H) *S. sudanense*; (I) *S. virgatum*; (J) *S. nitidum*; (K) *S. laxiflorum*; (L) *S. leiocladum*; (M) *S. versicolor*; (N) *S. purpureosericeum*; (O) *S. amplum*. Ovate-elliptic: (P) *S. macrospermum*. Subulate: (Q) *S. angustum*; (R) *S. brachypodum*; (S) *S. ecarinatum*; (T) *S. exstans*; (U) *S. intrans*; (V) *S. matarankense*; (W) *S. plumosum*; (X) *S. stipoideum*; (Y) *S. timorense*. Dorsal and ventral face located on left and right side of each figure member, respectively. Scale bars = 0.5 mm. Artist: Yunxiao Liu.

S. grande, *S. interjectum* and *Cleistachne sorghoides* due to absence of mature caryopses.

3. Results

Selected micromorphological characters of *Sorghum* including caryopsis size, caryopsis shapes, stylopodium persistence patterns, spermoderm sculpture patterns, embryo proportion (the ratio of embryo to caryopsis length) and hilum proportion (the ratio of hilum to caryopsis length) are summarized in Table 2. A detailed description is given below.

3.1. Caryopsis size

Caryopsis size varies greatly among the examined taxa: the mean length of caryopses ranges from 1.98 mm in *Sorghum nitidum* to 5.48 mm in *S. brachypodum*, and the mean width of caryopses ranges from 0.96 mm in *S. matarankense* to 2.65 mm in *S. bicolor*, and the mean thickness of caryopses varies from 0.38 mm in *S. versicolor* to 1.90 mm in *S. bicolor* (Table 2). The caryopsis size range of length × width × thickness is 1.40–6.18 × 0.73–3.21 × 0.32–2.30 mm. The ratio of length to width (L/W) and width to thickness (W/T) are 1.31–4.02 and 1.09–2.65, respectively (Table 2).

3.2. Caryopsis shapes

Three caryopsis shapes are found in the examined taxa according to the combination data of the ventral face outline and the ratio of length to width (L/W): (I) the broad type of obovate-elliptic caryopses with the mean L/W from 1.43 to 2.02 in *Sorghum* × *almum* (Fig. 1A), *S. arundinaceum* (Fig. 1B), *S. bicolor* (Fig. 1C), *S.* ×

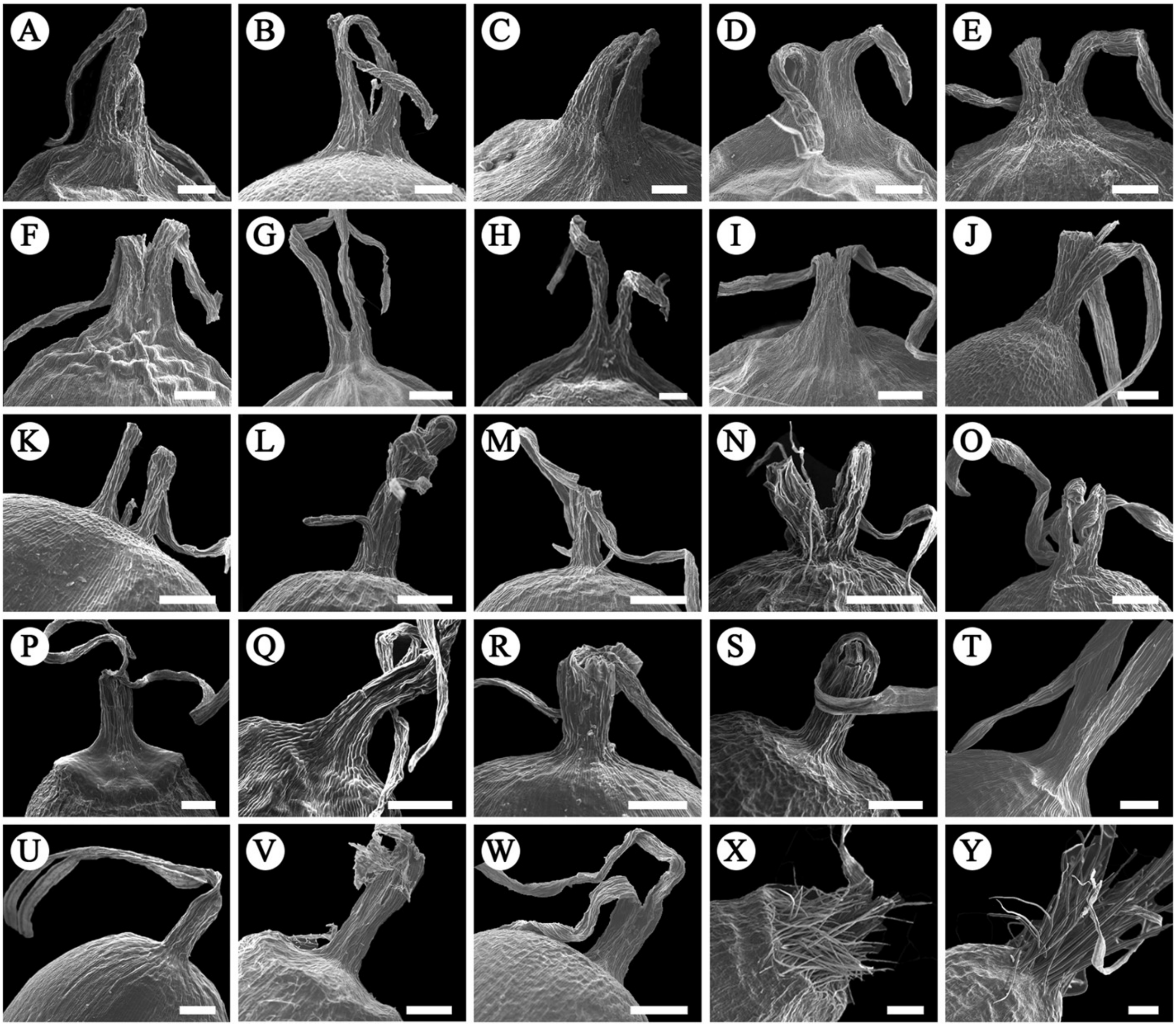

Fig. 2. Embryo shapes of *Sorghum* (SEM). (A) *S.* × *almum*; (B) *S. arundinaceum*; (C) *S. bicolor*; (D) *S.* × *drummondii*; (E) *S. halepense*; (F) *S. miliaceum*; (G) *S. propinquum*; (H) *S. sudanense*; (I) *S. virgatum*; (J) *S. macrospermum*; (K) *S. laxiflorum*; (L) *S. leiocladum*; (M) *S. matarankense*; (N) *S. nitidum*; (O) *S. purpureosericeum*; (P) *S. timorense*; (Q) *S. versicolor*; (R) *S. amplum*; (S) *S. angustum*; (T) *S. brachypodum*; (U) *S. ecarinatum*; (V) *S. exstans*; (W) *S. intrans*; (X) *S. plumosum*; (Y) *S. stipoideum*. Scale bars = 200 μm.

drummondii (Fig. 1D), *S. halepense* (Fig. 1E), *S. miliaceum* (Fig. 1F), *S. propinquum* (Fig. 1G), *S. sudanense* (Fig. 1H), *S. virgatum* (Fig. 1I), *S. nitidum* (Fig. 1J), *S. laxiflorum* (Fig. 1K), *S. leiocladum* (Fig. 1L) and *S. versicolor* (Fig. 1M), *S. purpureosericeum* (Fig. 1N) and *S. amplum* (Fig. 1O); (II) the broad type of ovate-elliptic caryopses with L/W from 2.39 to 3.13 in *S. macrospermum* (Fig. 1P); (III) the slender type of subulate caryopses with the mean L/W from 2.69 to 3.54 in *S. angustum* (Fig. 1Q), *S. brachypodum* (Fig. 1R), *S. ecarinatum* (Fig. 1S), *S. exstans* (Fig. 1T), *S. intrans* (Fig. 1U), *S. matarankense* (Fig. 1V), *S. plumosum* (Fig. 1W), *S. stipoideum* (Fig. 1X) and *S. timorense* (Fig. 1Y).

3.3. Embryo and hilum shapes

All examined taxa have dorsoventrally compressed caryopses with either a plano-convex or a convex dorsal face. An elliptic embryo is observed in *Sorghum* × *almum* (Fig. 2A), *S. arundinaceum* (Fig. 2B), *S. bicolor* (Fig. 2C), *S.* × *drummondii* (Fig. 2D), *S. halepense* (Fig. 2E), *S. miliaceum* (Fig. 2F), *S. propinquum* (Fig. 2G), *S. sudanense* (Fig. 2H), *S. virgatum* (Fig. 2I), *S. macrospermum* (Fig. 2J), *S. laxiflorum* (Fig. 2K), *S. leiocladum* (Fig. 2L), *S. matarankense* (Fig. 2M), *S. nitidum* (Fig. 2N), *S. purpureosericeum* (Fig. 2O), *S. timorense* (Fig. 2P), *S. versicolor* (Fig. 2Q), *S. amplum* (Fig. 2R), *S. angustum* (Fig. 2S), *S. brachypodum* (Fig. 2T), *S. ecarinatum* (Fig. 2U), *S. exstans* (Fig. 2V), *S. intrans* (Fig. 2W), *S. plumosum* (Fig. 2X) and *S. stipoideum* (Fig. 2Y).

All examined taxa have caryopses with a relatively flat ventral face. The more or less concave hilum is observed in *Sorghum* × *almum* (Fig. 3A), *S. arundinaceum* (Fig. 3B), *S. bicolor* (Fig. 3C), *S.* × *drummondii* (Fig. 3D), *S. halepense* (Fig. 3E), *S. miliaceum* (Fig. 3F), *S. propinquum* (Fig. 3G), *S. sudanense* (Fig. 3H), *S. virgatum* (Fig. 3I), *S. macrospermum* (Fig. 3J), *S. laxiflorum* (Fig. 3K), *S. leiocladum* (Fig. 3L), *S. matarankense* (Fig. 3M), *S. nitidum* (Fig. 3N), *S. purpureosericeum* (Fig. 3O), *S. timorense* (Fig. 3P), *S. versicolor* (Fig. 3Q), *S. amplum* (Fig. 3R), *S. angustum* (Fig. 3S), *S. brachypodum* (Fig. 3T), *S. ecarinatum* (Fig. 3U), *S. exstans*

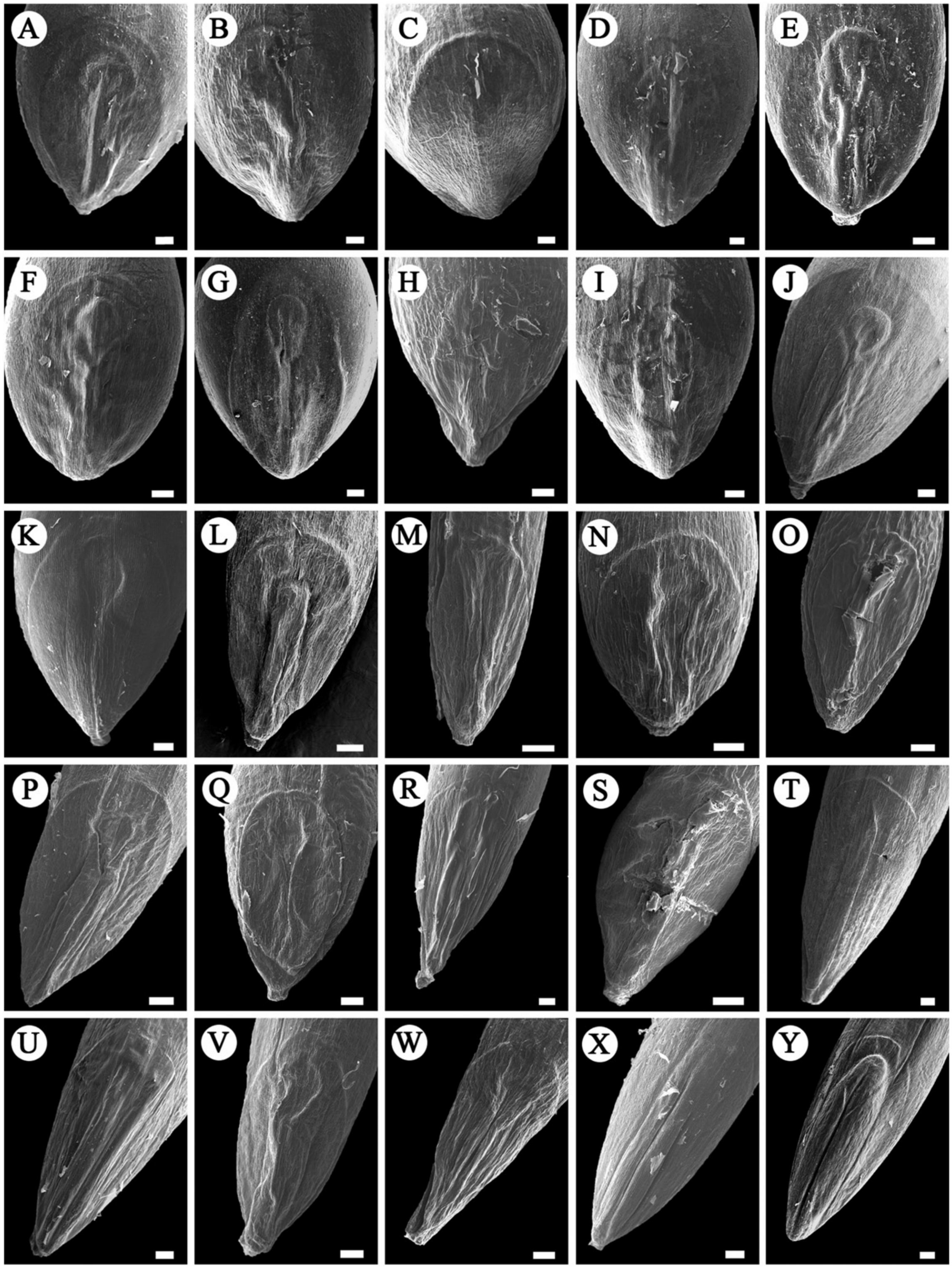

Fig. 3. Hilum shapes of *Sorghum* (SEM). (A) *S. × almum*; (B) *S. arundinaceum*; (C) *S. bicolor*; (D) *S. × drummondii*; (E) *S. halepense*; (F) *S. miliaceum*; (G) *S. propinquum*; (H) *S. sudanense*; (I) *S. virgatum*; (J) *S. macrospermum*; (K) *S. laxiflorum*; (L) *S. leiocladum*; (M) *S. matarankense*; (N) *S. nitidum*; (O) *S. purpureosericeum*; (P) *S. timorense*; (Q) *S. versicolor*; (R) *S. amplum*; (S) *S. angustum*; (T) *S. brachypodum*; (U) *S. ecarinatum*; (V) *S. exstans*; (W) *S. intrans*; (X) *S. plumosum*; (Y) *S. stipoideum*. Scale bars = 100 μm.

(Fig. 3V), *S. intrans* (Fig. 3W), *S. plumosum* (Fig. 3X) and *S. stipoideum* (Fig. 3Y).

3.4. Stylopodium persistence patterns

Two patterns of stylopodium persistence are observed in examined taxa based on the apical tuft of unicellular microhairs: (I) without an apical tuft of microhairs in *Sorghum × almum* (Fig. 4A), *S. arundinaceum* (Fig. 4B), *S. bicolor* (Fig. 4C), *S. × drummondii* (Fig. 4D), *S. halepense* (Fig. 4E), *S. miliaceum* (Fig. 4F), *S. propinquum* (Fig. 4G), *S. sudanense* (Fig. 4H), *S. virgatum* (Fig. 4I), *S. macrospermum* (Fig. 4J), *S. laxiflorum* (Fig. 4K), *S. leiocladum* (Fig. 4L), *S. matarankense* (Fig. 4M), *S. nitidum* (Fig. 4N), *S. purpureosericeum* (Fig. 4O), *S. timorense* (Fig. 4P), *S. versicolor* (Fig. 4Q), *S. amplum* (Fig. 4R),

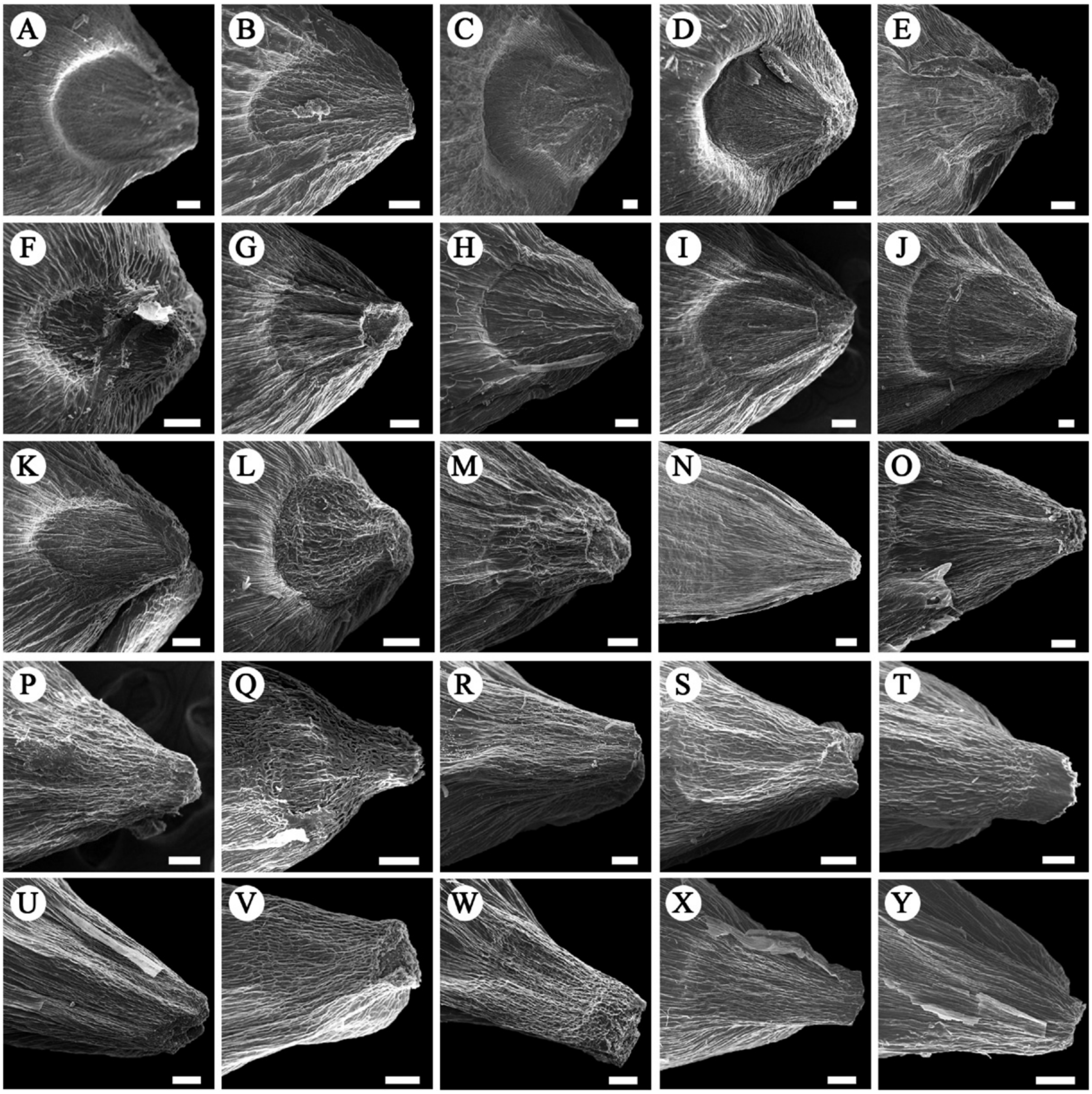

Fig. 4. Stylopodium persistence patterns of *Sorghum* (SEM). Without an apical tuft of microhairs: (A) *S.* × *almum*; (B) *S. arundinaceum*; (C) *S. bicolor*; (D) *S.* × *drummondii*; (E) *S. halepense*; (F) *S. miliaceum*; (G) *S. propinquum*; (H) *S. sudanense*; (I) *S. virgatum*; (J) *S. macrospermum*; (K) *S. laxiflorum*; (L) *S. leiocladum*; (M) *S. matarankense*; (N) *S. nitidum*; (O) *S. purpureosericeum*; (P) *S. timorense*; (Q) *S. versicolor*; (R) *S. amplum*; (S) *S. angustum*; (T) *S. brachypodum*; (U) *S. ecarinatum*; (V) *S. plumosum*; (W) *S. stipoideum*. With an apical tuft of brown or silvery unicellular microhairs: (X) *S. exstans*; (Y) *S. intrans*. Scale bars = 200 μm.

S. angustum (Fig. 4S), *S. brachypodum* (Fig. 4T), *S. ecarinatum* (Fig. 4U), *S. plumosum* (Fig. 4V) and *S. stipoideum* (Fig. 4W); and (II) with an apical tuft of brown or silvery unicellular microhairs in *S. exstans* (Fig. 4X) and *S. intrans* (Fig. 4Y).

3.5. Spermoderm sculpture patterns

Five different spermoderm sculpture patterns are observed in examined caryopses: (I) a reticulate pattern with wavy walls in *Sorghum* × *almum* (Fig. 5A), *S. arundinaceum* (Fig. 5B), *S. bicolor* (Fig. 5C), *S.* × *drummondii* (Fig. 5D, E), *S. ecarinatum* (Fig. 5F), *S. leiocladum* (Fig. 5G), *S. matarankense* (Fig. 5H), *S. plumosum* (Fig. 5I), *S. purpureosericeum* (Fig. 5J), *S. stipoideum* (Fig. 5K) and *S. virgatum* (Fig. 5L); (II) a reticulate pattern with straight walls in *S. halepense* (Fig. 5M), *S. miliaceum* (Fig. 5N), *S. macrospermum* (Fig. 5O) and *S. laxiflorum* (Fig. 5P); (III) a substriate pattern in *S. sudanense* (Fig. 5Q, R), *S. amplum* (Fig. 5S), *S. brachypodum* (Fig. 5T), *S. nitidum* (Fig. 5U), *S. propinquum* (Fig. 5V) and *S. timorense* (Fig. 5W); (IV) an undulating pattern in *S. exstans* (Fig. 5X), *S. intrans* (Fig. 5Y) and *S. versicolor* (Fig. 5Z); and (V) a rugose pattern in *S. angustum* (Fig. 5a, b).

3.6. Embryo proportion and hilum proportion

The embryo proportion varies from 0.45 in *Sorghum intrans* to 0.84 in *S. propinquum*, the mean embryo proportion varies from 0.55 in *S. macrospermum* to 0.73 in *S. propinquum*. The hilum proportion varies from 0.12 in *S. leiocladum* and *S. stipoideum* to 0.28 in *S. bicolor*, and the mean hilum proportion varies from 0.13 in *S. leiocladum* to 0.24 in *S. bicolor*. In general, there is a positive correlation between caryopsis length and embryo length (Fig. 6A): the longer caryopsis tends to have the longer embryo. This kind of correlation between caryopsis and embryo length is also found in other taxa of Poaceae, which may reflect a functional rather than a phylogenetic relationship (Wang et al., 1986). We detect a positive correlation between caryopsis length and hilum length (Fig. 6B), but the positive correlation between caryopsis and embryo length ($R^2 = 0.826$) is stronger than that between caryopsis and hilum length ($R^2 = 0.571$).

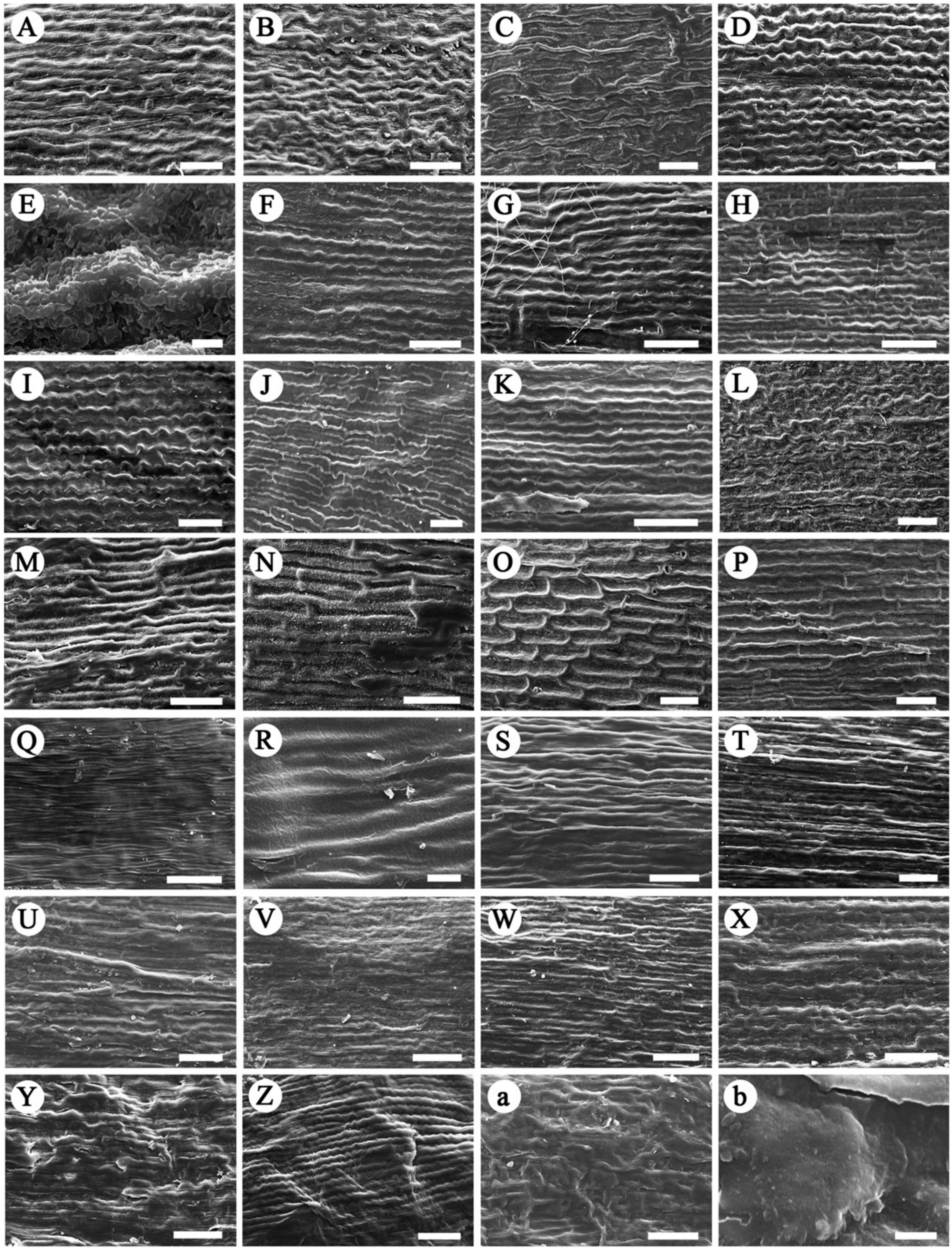

Fig. 5. Spermoderm sculpture patterns of *Sorghum* (SEM). Reticulate pattern with wavy walls: (A) *S.* × *almum*; (B) *S. arundinaceum*; (C) *S. bicolor*; (D) *S.* × *drummondii*; (E) Detail of 5D; (F) *S. ecarinatum*; (G) *S. leiocladum*; (H) *S. matarankense*; (I) *S. plumosum*; (J) *S. purpureosericeum*; (K) *S. stipoideum*; (L) *S. virgatum*. Reticulate pattern with straight walls: (M) *S. halepense*; (N) *S. miliaceum*; (O) *S. macrospermum*; (P) *S. laxiflorum*. Substriate pattern: (Q) *S. sudanense*; (R) Detail of 5Q; (S) *S. amplum*; (T) *S. brachypodum*; (U) *S. nitidum*; (V) *S. propinquum*; (W) *S. timorense*. Undulating pattern: (X) *S. exstans*; (Y) *S. intrans*; (Z) *S. versicolor*. Rugose pattern: (a) *S. angustum*; (b) Detail of 5a. Scale bars = 50 μm for 5A–D, 5 F–Q, 5S–Z, 5a; 10 μm for 5E, R, b.

3.7. Character evolution in Sorghum

Caryopsis micromorphological characters—caryopsis shapes and spermoderm sculpture patterns—were plotted on the redrawn majority rule consensus tree based on three chloroplast genes (*ndhA* intron, *rpl32-trnL* and *rps16* intron) of *Sorghum* (Liu et al., 2014). In the topology (Fig. 7), the obovate-elliptic caryopses occur in all the members of subg. *Sorghum* (clade II) and *S. nitidum, S. leiocladum*, *S. versicolor*, *S. purpureosericeum*, *S. amplum* of clade III and *S. laxiflorum* of clade I, whereas subulate caryopses and ovate-elliptic caryopses occur exclusively in clade III and in *S. macrospermum* of clade I, respectively. Among the five spermoderm sculpture patterns

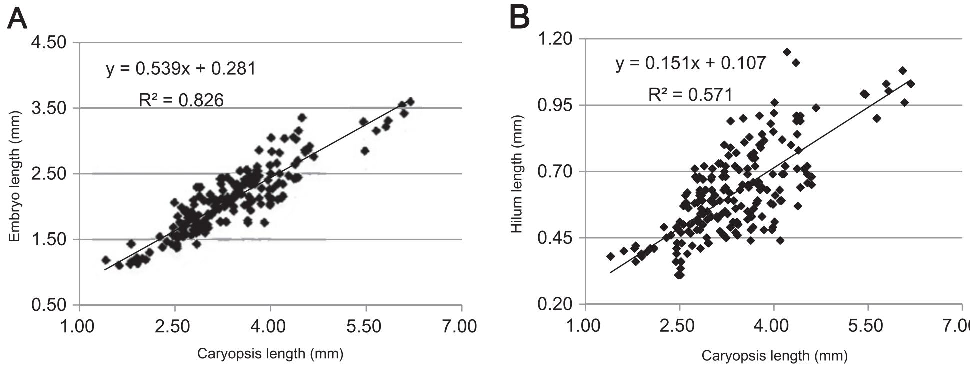

Fig. 6. Correlation analyses between caryopsis length and embryo or hilum length. (A) Correlation between caryopsis and embryo length. (B) Correlation between caryopsis and hilum length.

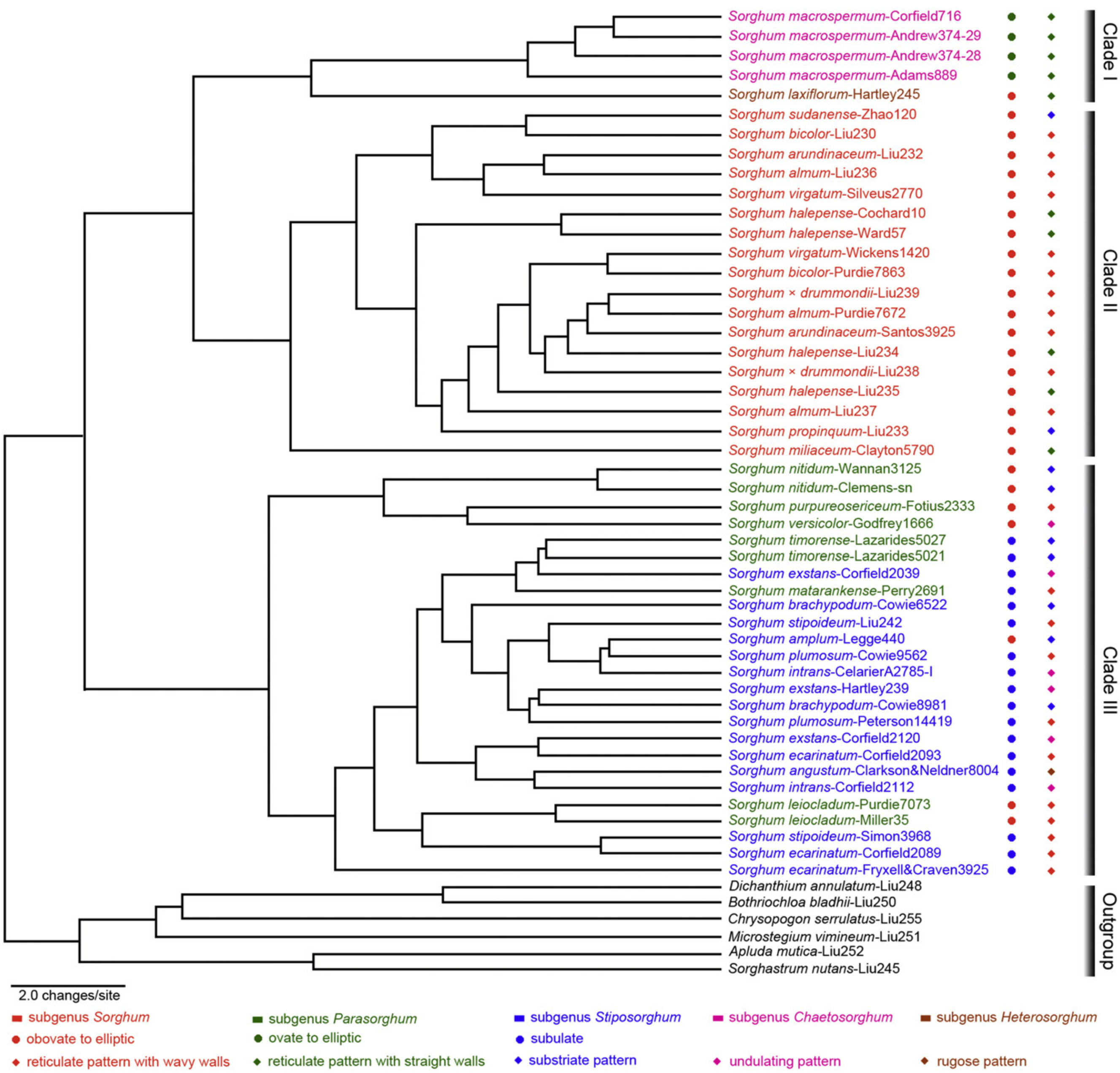

Fig. 7. Caryopsis shapes and spermoderm sculpture patterns mapped onto the redrawn majority rule consensus tree of three chloroplast genes (*ndhA* intron, *rpl32-trnL* and *rps16* intron) of *Sorghum* (Liu et al., 2014). Rectangles, circles and rhombuses represent subgenera, caryopsis shapes and spermoderm sculpture patterns, respectively.

observed, the rugose pattern is only seen in *S. angustum* of clade III. Caryopses of members of clade I exhibit the reticulate pattern with straight walls, and the reticulate with wavy cell walls, the reticulate with straight cell walls, substriate and undulating patterns were observed in clade II and reticulate with wavy cell walls, substriate, undulating and rugose patterns in clade III (Fig. 7).

4. Discussion

The caryopses of *Sorghum* have the following diagnostic characters: (1) mean length × width × thickness is 1.98–5.48 × 0.96–2.65 × 0.38–1.90 mm; (2) obovate-elliptic, ovate-elliptic or subulate caryopsis shapes; (3) stylopodia with or without an apical tuft of unicellular microhairs; (4) five spermoderm sculpture patterns including the reticulate with wavy walls, the reticulate with straight walls, substriate, undulating and rugose patterns.

Caryopsis size thus has limited taxonomic value as a diagnostic character at the infrageneric level in *Sorghum*. The caryopsis size varies considerably within the same species in *Sorghum*. For example, the caryopsis length variation range of *S. arundinaceum* is 1.61–4.09 mm, that of *S. propinquum* is 0.74–2.27 mm, and 0.52–1.86 mm for *S. macrospermum*. In general, the caryopsis lengths of *S. miliaceum* (2.45–2.87 mm), *S. leiocladum* (2.51–2.62 mm) and *S. nitidum* (1.89–2.09 mm) are shorter than that of *S. macrospermum* (4.01–6.18 mm), *S. amplum* (4.42–4.60 mm) and *S. brachypodum* (4.58–6.05 mm), which is useful to diagnose the former species from the latter ones.

The caryopsis shapes show a certain taxonomic significance at the infrageneric level in *Sorghum*. Caryopses of all members in clade I (subgenera *Chateosorghum* and *Hetersorghum*) and clade II (subg. *Sorghum*) are of the broad type of obovate/ovate-elliptic. Garber (1950) proposed that the subg. *Parasorghum* was characterized by obovoid caryopses and the subg. *Stiposorghum* by broad subulate caryopses exclusively; however, the relatively slender type of subulate caryopses was observed in the majority members of the clade III containing these two subgenera here. Although Lazarides et al. (1991) proposed that caryopsis ventral face outline had no taxonomic significance in Australian *Sorghum* species, in our study, the slender type of caryopsis is uniquely observed in the majority members of clade III (the subg. *Parasorghum* and *Stiposorghum* lineage), providing new evidence for the combination of the two subgenera *Stiposorghum* and *Parasorghum* (clade III) into a single subg. *Parasorghum* (Liu et al., 2014).

Spermoderm sculpture patterns are important diagnostic characters at the interspecific level in *Sorghum*. *Sorghum sudanense* (sudan grass), sharing a similar ventral face shape (Table 2) with *S.* × *drummondii*, has always been treated as the synonym of the latter by traditional taxonomists (De Wet, 1978; The Plant List, 2013). Here we suggest that *S. sudanense* can be treated as an independent species based on the spermoderm sculpture patterns: *S. sudanense* has relatively smaller caryopses [2.56–(2.78)–3.00 mm] with substriate spermoderm sculptures (Fig. 5Q, R), while *S.* × *drummondii* has relatively larger caryopses [3.36–(3.99)–4.48 mm] with reticulate spermoderm sculptures with wavy walls (Fig. 5D, E). The separation of *S. sudanense* from *S.* × *drummondii* is also supported by phylogenetic analysis of nuclear *Pepc4* and *GBSSI* sequence data, which suggest that the former species is genetically distant from the latter (Liu et al., 2014). Therefore, the recognition of these two taxa at the specific level is compatible with our results.

Consistent micromorphological characters of stylopodium persistence, dorsoventral compression, embryo proportion larger than 1/2 and a narrow range of hilum proportion are firstly found in *Sorghum*, and are therefore of limited taxonomic value at the infrageneric level in the genus. Stylopodium persistence and dorsoventral compression of caryopses are consistent in the genus *Sorghum* and the spathaceous genera in the Andropogoneae, and might reflect the consequence of an adaptive process that increased the pollen collection efficiency of stigma through the extended space range around 2 style branches stretching out from two sides of the wide and flat lemma and palea (Zhang et al., 2014). The mean embryo proportion is in the range of 0.52 to 0.73 (>1/2), which belongs to the panicoid type, being larger than those of the ehrhartoid, bambusoid and pooid types (<1/2) in Poaceae (Reeder, 1957; Wang et al., 1986). It is easy to understand that the trend of larger embryo proportion in Andropogoneae could reflect the selection pressures which existed in the seasonally hydrothermal rhythm during the short growing season (Stebbins, 1986; Wang et al., 1986; Liu and Liu, 2014). Hilum proportion is described for the first time for *Sorghum*, and its variation range is shown to be narrow in *Sorghum*.

The apical tuft of unicellular microhairs has been observed in caryopses of the majority of species in tribe Triticeae (Pooideae, Poaceae), but it has rarely been observed in the Panicoideae (Wang et al., 1986; Terrell and Peterson, 1993). The presence or absence of an apical tuft of unicellular microhairs on the caryopsis has been proposed as a diagnostic character at familial or tribal levels rather than at generic or specific levels (Watson et al., 1985; Sendulsky et al., 1986). The apical tuft of unicellular microhairs is observed in only two species, i.e., *Sorghum exstans* and *S. intrans* (Fig. 4X, Y) in *Sorghum*. We infer that the caryopsis apical microhairs are the carpel appendages and are an adaptation to unfavorable conditions (Sendulsky et al., 1986), as they promote the absorption of water before caryopsis germination in arid regions (Stebbins, 1986).

In conclusion, the caryopsis shapes show a certain taxonomic significance at the infrageneric level in *Sorghum*, whereas caryopsis size and stylopodium persistence are of limited taxonomic value at the infrageneric level. In addition, spermoderm sculpture patterns are important diagnostic characters at the interspecific level in *Sorghum*. The recognition of the species *S. sudanense* and *S.* × *drummondii* at the specific level is compatible with our results. Additionally, an apical tuft of unicellular microhairs is observed in two *Sorghum* species examined thus far. Caryopsis micromorphological characters provide evidence that *Sorghum* is a highly heterogeneous genus at the micromorphological level (Spangler et al., 1999).

Acknowledgments

The authors thank the USDA-Beltsville National Germplasm System for caryopsis collection and curators of CANB, IBSC, K and US for lending specimens. The project was supported by the National Natural Science Foundation of China (31270275), Special Basic Research Foundation of Ministry of Science and Technology of the People's Republic of China (2013FY112100), and the Foundation of Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences (201212ZS). The authors also thank the editor and two anonymous reviewers for their valuable comments.

References

Barthlott, W., 1981. Epidermis and seed surface characters of plants: systematic applicability and some evolutionary aspects. Nord. J. Bot. 1, 345–355.
Bogdan, A.V., 1966. Seed morphology of some cultivated African grasses. Proc. Int. Seed Test. Assoc. 31, 789–799.
Celarier, R.P., 1958. Cytotaxonomy of the Andropogoneae: III. sbtribe *Sorgheae*, genus *Sorghum*. Cytologia 23, 395–418.
Chen, S.L., Phillips, S.M., 2006. *Sorghum* Moench. In: Wu, Z.Y., Raven, P.H., Hong, D.Y. (Eds.), Flora of China vol. 22. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp. 602–605.
Clayton, W.D., Renvoize, S.A., 1986. Genera graminum: grasses of the world. Kew Bull. Add. Ser. 13, 320–375.
De Wet, J.M.J., 1978. Systematics and evolution of *Sorghum* sect. *Sorghum* (Gramineae). Am. J. Bot. 65, 477–484.
Dillon, S.L., Lawrence, P.K., Henry, R.J., Price, H.J., 2007. *Sorghum* resolved as a distinct genus based on combined ITS1, *ndhF* and *Adh1* analyses. Plant Syst. Evol. 268, 29–43.
Gandhi, D., Albert, S., Pandya, N., 2013. Morphometric analysis of caryopsis in some species of *Eragrostis* (Poaceae). Telopea 15, 87–97.
Garber, E.D., 1950. Cytotaxonomic studies in the genus *Sorghum*. Univ. Calif. Publ. Bot. 23, 283–361.

Jiang, B., Peterson, P.M., Liu, Q., 2011. Caryopsis micromorphology of *Eleusine* Gaertn. (Poaceae) and its systematic implication. J. Trop. Subtropical Bot. 19, 195–204.
Jordan, L.S., Jordan, J.L., Jordan, C.M., 1983. Prominent spermoderm patterns of Poaceae. Bot. Mag. Tokyo 96, 269–272.
Jordan, L.S., Jordan, J.L., Jordan, C.M., 1985. Comparison of *Dactyloctenium* Willd and *Eleusine* Gaertn spermoderm patterns. Bot. Mag. Tokyo 98, 199–202.
Lazarides, M., Hacker, J.B., Andrew, M.H., 1991. Taxonomy, cytology and ecology of indigenous Australian sorghums (*Sorghum* Moench: Andropogoneae: Poaceae). Aust. Syst. Bot. 4, 591–635.
Liu, H., Liu, Q., 2014. Geographical distribution of *Sorghum* Moench (Poaceae). J. Trop. Subtropical Bot. 22, 1–11.
Liu, Q., Zhao, N.X., Hao, G., Hu, X.Y., Liu, Y.X., 2005. Caryopsis morphology of the Chloridoideae (Gramineae) and its systematic implications. Bot. J. Linn. Soc. 148, 57–72.
Liu, Q., Liu, H., Wen, J., Peterson, P.M., 2014. Infragenetic phylogeny and temporal divergence of *Sorghum* (Andropogoneae, Poaceae) based on low-copy nuclear and plastid sequences. PLoS One 9, e104933.
Ng'uni, D., Geleta, M., Fatih, M., Bryngelsson, T., 2010. Phylogenetic analysis of the genus *Sorghum* based on combined sequence data from cpDNA regions and ITS generate well-supported trees with two major lineages. Ann. Bot. 105, 471–480.
Reeder, J.R., 1957. The embryo in grass systematics. Am. J. Bot. 44, 756–768.
Sendulsky, T., Filgueiras, T.S., Burman, A.G., 1986. Fruits, embryos, and seedlings. In: Soderstrom, T.R., Hilu, K.W., Campbell, C.S., Barkworth, M.E. (Eds.), Grass Systematics and Evolution. Smithsonian Institution Press, Washington DC, USA, pp. 31–36.
Snowden, J.D., 1955. The wild fodder sorghums of the section *Eu-sorghum*. J. Linnean Soc.Lond. 55, 191–260.
Spangler, R.E., 2003. Taxonomy of *Sarga*, *Sorghum* and *Vacoparis* (Poaceae: Andropogoneae). Aust. J. Bot. 16, 279–299.
Spangler, R.E., Zaitchik, B., Russo, E., Kellogg, E., 1999. Andropogoneae evolution and generic limits in *Sorghum* (Poaceae) using *ndhF* sequences. Syst. Bot. 24, 267–281.
Stearn, W.T. (Ed.), 1992. Botanical Latin, 4th ed. David and Charles, Newton Abbot.
Stebbins, G.L., 1986. Grass systematics and evolution: past, present and future. In: Soderstrom, R., Hilu, C.S., Campbell, C.S., Barkworth, M.E. (Eds.), Grass Systematics and Evolution. Smithsonian Institution Press, Washington DC, pp. 359–367.
Stenhouse, J.W., Prasada Rao, K.E., Gopal Reddy, V., Appa Pao, S., 2007. Sorghum. In: Fuccillo, D., Sears, L., Stapleton, P. (Eds.), Biodiversity in Trust: Conservation and Use of Plant Genetic Resources in CGIAR Centers. Cambridge University Press, Cambridge, pp. 292–308.
Terrell, E.E., Peterson, P.M., 1993. Caryopsis morphology and classification in the Triticeae (Pooideae: Poaceae). Smithson. Contrib. Bot. 83, 1–25.
The Plant List, 2013. Version 1.1. Published on the Internet. http://www.theplantlist.org/ (date of accession: 01.01.2013).
Wang, S.J., Guo, P.C., Li, J.H., 1986. The major types of caryopses of the Chinese Gramineae in relation to systematics. Acta Phytotaxonomica Sin. 24, 327–345.
Waniska, R.D., Rooney, U.W., 2000. Structure and chemistry of the *Sorghum* caryopsis. In: Smith, C.W., Frederiksen, R.A. (Eds.), *Sorghum*: Origin, History, Technology, and Production vol. 2. John Wiley & Sons, USA, pp. 649–679.
Watson, L., Clifford, H.T., Dallwitz, M.J., 1985. The classification of Poaceae: subfamilies and supertribes. Aust. J. Bot. 33, 433–486.
Zhang, Y., Hu, X.Y., Liu, Y.X., Liu, Q., 2014. Caryopsis micromorphological survey of the genus *Themeda* (Poaceae) and allied spathaceous genera in the Andropogoneae. Turk. J. Bot. 38, 665–676.