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Seed Coat Variation in Genus *Glycine* Surveyed by Scanning Electron Microscopy

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We aimed to analyse the seed coat of *Glycine* species accessions, in particular, the morphology and fine structure, by scanning electron microscopy. The seed coat makes up about 6.7% and 22.9% of the seed weight in the *Glycine max* cv. Shishi and *Glycine soja*, respectively, and 30% to 40% in other species (*Glycine canesences*, *Glycine latifolia*, *Glycine tabacina*, *Glycine tomentella*) surveyed in this study. The seed coat surface of the cultivar contained pores and deposits, and the bloom formed by these deposits coming from the endocarp was present in all of the species examined except the cultivar Shishi. As well, the cavity sizes of the bloom were significantly different among these accessions. These characters, including seed weight, seed length, seed coat proportion and cavity density, might be helpful in *Glycine* species taxonomy.

***Key word*: bloom, deposit, *Glycine* species, pore, scanning electron microscopy, seed coat.**

Introduction

The genus *Glycine* Willd. has been divided into 2 subgenera: *Glycine* and *Soja*. The subgenus *Glycine* contains 21 wild perennial species and is largely indigenous in Australia. Most species in this subgenus carry diploid (2n = 40) chromosomes, whereas 3 species -- *Glycine hirticaulis* Tind. and Craven, *Glycine tabacina* (Labill.) Benth., and *Glycine tomentella* Hayata -- carry diploid (2n = 40) and tetraploid (2n = 80) forms. In addition, *G. tomentella* is composed of aneudiploid (2n = 38) and aneutetraploid (2n = 78) cytotypes [1]. The subgenus *Soja*, with its center of diversity in Asia, includes the cultivated soybean *G. max* (L.) Merr., the cultivated soybean and its wild annual progenitor *Glycine soja* Sieb. and Zucc. Both species are diploid (2n = 40) and cross-compatible.

Of the perennial species, *G. tomentella* Hayata is the most widely distributed and diverse, although it has long been recognized as polytypic. Besides being collected in Australia, the species was also collected in Papua New Guinea, Timor, the Philippines and Taiwan. Early cytogenetic studies of this

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species detected four distinct chromosome numbers, including 2n = 38, 40, 78, 80 [1] with a wide range of morphology. The relations among these accessions are complicated, and the grouping method based on the isozyme pattern detailed in 1985 may facilitate further classification of *G. tomentella* [2].

Numerical analysis of isozyme variation detected 12 distinctive major groups of *Glycine* both at the diploid and tetraploid levels [2]. These isozyme groups were designated D1, D2, D3, D4, D5, D6, D7 and T1, T2, T3, T4, T5. The groups D1 and D2 are aneuploid (2n = 38) and the remainder euploid (2n = 40). In the tetraploids, T1, T5 and the later identified T6 are aneuploid (2n = 78), whereas plants belonging to T2, T3 and T4 are euploid, with 2n = 80 [3].

Soybean is a major source of protein and vegetable oil for animal and human nutrition. The main edible part of soybean seed is the cotyledon, which constitutes about 90% of total seed weight. The seed coat, constituting less than 8% of the seed, is usually wasted in food manufacturing [4]. Observations of soybean seed coat (see review by Carlson [5]) indicated that it was derived mainly from the outer integument. The integuments often undergo considerable cell proliferation as they expand and thicken into seed coat. The seed coat of the mature soybeans has been well characterized and contains features in common with most of the legumes: an epidermal layer of palisade cells, a sub-epidermal layer of hourglass cells, a few layers of parenchyma, and an aleurone and compressed fiber-like cells on the inner surface [6, 7, 8].

In addition to providing a protective covering for the embryo at maturity, soybean seed coat also provides support and nourishment for the developing embryo [9]. Wolf and Baker [10] studied the soybean seed coat by scanning electron microscopy (SEM) and observed that the outer surface was covered with numerous pores (pits) that appeared to terminate in elongated, slot-like structures. Pitting of the seed coat surface occurred in many cultivars, but a number of them were free of pores (i.e., smooth; [11]).

Besides observing pores, authors have also observed other seed coat structural features such as deposits and cracks [11] and morphologically distinct deposits on the surface referred to as “bloom” [8]. The bloom consists of ridges that form polygonal outlines of 20 to 30 μm in diameter. Examination of the seed coat surface revealed that the bloom may separate from the seed coat in certain areas, particularly near the raphe [8].

Large amounts of materials on the seed coat can affect the luster of the seed [11]. Even after rubbing the seed with a clean cotton towel or soaking the seeds with different solvents such as water, hexane or chloroform:methanol (2:1), the bulk of the material remains on the seed coat. Thus, the deposits are not water-soluble proteins, lipids or waxes [11, 12], but staining with Cellufluor suggests that they consist of cellulose [13, 14].

The external and internal seed coat structures are stable in species and may be used for classification and identification. Newell and Hymowitz [15] surveyed the seed coat variation of different subgeners of *Glycine* by SEM and found the endocarp of the inner pod wall of some species adhered to the seed coat. The endocarp imprinted a reticulated network on the seed coat, which ranged in appearance from alveolate to stellate depending on the shape of the endocarp cells. The authors also paid attention to the difference between the ploidy levels in *G. tomentella*.

In the last 2 decades, we have collected annual and perennial *Glycine* species in Taiwan and the adjacent islands and studied the evolution and relations between the 2 subgenera by restriction fragment length polymorphism, gene sequencing, seed germination behavior, and seed protein

profiles [16, 17, 18, 19]. In the present study, we observed seed coat phenotypes by SEM, with a focus on the *G. tomentella* species complex and other species collected in Taiwan. We addressed seed weight, seed length, the proportion of seed coat to seed, which may affect the storage and quality of seeds. We also offer detailed information on the cavity number in seed coat bloom to gain a better understanding of structural characteristics related to taxonomy.

Materials and Method

Plant materials

The accessions of *Glycine* species used in this study are listed in Table 1. The seeds of cultivar variety Shishi were obtained from Kaohsiung Agriculture Station in Pintung. Accessions from Taiwan were collected during the past 20 years, and those out of Taiwan were kindly provided from Dr. Hymowitz, Illinois University, in 1997. The wild soybean accessions and their perennial relatives were planted in the greenhouse under natural light condition at the Institute of Plant and Microbial Biology, Academia Sinica, Taiwan and the mature seeds were harvested.

Seed weight and seed coat proportion

For seed weight examination, data from 3 duplications and 10 seeds from each replicate were recorded. For seed coat proportion, seeds were separated into seed coat and embryos and weighed, with 3 duplications. ANOVA was used to test for significant differences between species, with Duncan's multiple range test used to compare means. The relation between seed weight and seed coat proportion was also estimated.

SEM observation

SEM was used to observe seed coat characteristics such as bloom and cavity number in the *Glycine* species. The surface of 3 intact mature seeds from each sample was examined. Intact seeds were glued onto aluminum stubs, with the abaxial side facing up, and viewed under an environmental SEM (FEI Quanta 200, Philip, Netherlands) operated at 15 or 20 kV, at 0.45 to 0.68 Torr.

Seed length and cavity number count

The seed length and cavity numbers on seed coats were determined from SEM photographs. The length of 3 seeds form each accession was measured along the long axis of the seed, without regard to hilum position. A surface of 1 mm^2 of both sides of the seed coat was used to determine cavity number per unit area. The location measured was approximately at the center of the seed. Three duplications of each sample were processed. ANOVA was used to test for significance between species, with Duncan's multiple range test used to compare means.

Results

Seed weight, seed coat proportion and seed length

Table 1 lists the 15 *Glycine* accessions we studied, with information on collection sites, genome classification, isozyme group (if available) and plant introduction (PI) number. The average seed weight of *Glycine* species varied, from 163.8 mg for *G. max* cv, Shishi as the highest to 3.2 mg (Table 2). Next in weight were *G. canescens* (Can007) and *G. latifolia* (Lat), at above 10 mg, then *G. tomentella* (Tom056, Tom048) and *G. soja* (Soja001), at above 7 mg. The seed weight of Soja001 was 8.2 mg, smaller than most of the other *G. soja* accessions, such as Soja039, collected in South Korea (19.7 mg). The seed weight of other accessions ranged from 4.0 to 6.0 mg, with seeds of most of the *G. tomentella* accessions having this weight. The smallest one, Tom062, was only 3.2 mg in weight.

Table 1. Accessions of the wild soybean and their relatives used in this study and their collection site.

Accession	Genome	Isozyme group	PI number	Collection site
Max	GG			Cultivar
Soja 001	GG			Shimen, Taoyuan, Taiwan
Tab 004	AAB'B'			Hoaliaw, Penghu, Taiwan
Lat	B_1B_1		378709	New S. Wales, Australia
Can 003	AA		446935	New S. Wales, Australia
Can 007	AA		440932	South Australia
Tom 039	DDD_3D_3	T 2		Tungho, Taitung, Taiwan
Tom 048	DDD_1D_1	T 4		Kingmen, Taiwan
Tom 049	DD	D 3	505222	North Queensland, Australia
Tom 052	D_3D_3	D 4	441000	North Queensland, Australia
Tom 054	DDEE	T 1	339657	New S. Wales, Australia
Tom 055	EE	D 1	440998	South Queensland, Australia
Tom 056	DDD_3D_3	T 2	441005	Queensland, Australia
Tom 061	DDD_2D_2	T 3	446988	Papua New Guinea
Tom 062	DD	D 3	446993	Papua New Guinea

PI = plant introduction
Max = *Glycine max* cv. Shishi; Soja001 = *G. soja*; Tab004 = *G. tabacina*; Lat = *G. latifolia*; Can003, Can007 = *G. canescens*; Tom039, Tom048, Tom049, Tom052, Tom054, Tom055, Tom056, Tom061, Tom062 = *G. tomentella*

Table 2 also lists the proportion of seed coat and seed length of all accessions. The seed coat proportion of *G. max* cv. Shishi and Soja001 were 6.7% and 22.9%, respectively. Among the perennial accessions, the proportion ranged from 31.2% (*G. tabacina*) to 44.7% (Can003). Thus, the annual species had larger seeds and smaller ratios of seed coat to seed. In fact, negative correlations existed between seed size and seed coat proportion ($r = -0.78$, $p<0.0001$) and the tendency was also in common in the perennial species.

The seed outline for most of accessions was elliptic to circular, except for 2 *G. canescens* accessions, which had oblong forms (Fig 2B and 2C). Seed length and seed weight were correlated, at $r = 0.97$ and 0.78 for all accessions and only these 2 *G. canescens* accessions, respectively. After removing Can003 and Can007, the correlation between seed length and seed weight was $r = 0.98$ ($p<0.0001$).

SEM observation of seeds

Fig. 1 shows the SEM observation of texture of the seed coats. We found indurate endocarp remnants adhering to the testa in the seeds of the *Glycine* genus. The seed surface of *G. max* cv. Shishi, *G. soja* and *G. tabacina* looked smooth on naked-eye observation. However, their testa were either pitted or wrinkled under high magnification. The formation of depressions (pores) appeared only in the seeds of *G. max* cv. Shishi. Fig. 1A shows the seed coat surface of *G. max* cv. Shishi, with a portion of the hilum. The seed coat surface is covered with round or elongated pores and with some deposits. These pores are abundant across the whole surface of the seed coat and ranged from 25 to 45 μm (Fig. 1B). Deposits on the

Table 2. Dry weight of seeds, proportion of seed coat to seed in dry weight, and seed length of the *Glycine* species.

Accession	Seed weight (mg)	Seed coat proportion (%)	Seed length (mm)
Max	163.8 ± 2.7 A*	6.7 ± 0.1 I	7.97 ± 0.18 A
Soja001	8.2 ± 0.7 C	22.9 ± 0.9 H	3.04 ± 0.28 D
Tab004	4.5 ± 0.3 EF	31.2 ± 0.7 G	2.10 ± 0.13 FG
Can003	5.9 ± 0.3 D	44.7 ± 1.7 A	3.51 ± 0.06 C
Can007	12.5 ± 0.4 B	34.6 ± 0.9 EF	3.80 ± 0.19 B
Lat	11.2 ± 0.6 B	37.9 ± 1.7 CD	3.21 ± 0.12 D
Tom039	4.3 ± 0.7 EF	33.2 ± 0.8 FG	2.23 ± 0.06 F
Tom048	7.6 ± 0.2 C	35.7 ± 1.0 DEF	2.72 ± 0.17 E
Tom049	5.3 ± 0.1 DE	34.5 ± 0.5 EF	2.27 ± 0.15 F
Tom052	5.3 ± 0.4 DE	37.6 ± 4.4 CDE	2.54 ± 0.07 E
Tom054	5.3 ± 0.0 DE	42.4 ± 2.2 AB	2.30 ± 0.09 F
Tom055	4.0 ± 0.1 EF	38.8 ± 2.2 CD	2.29 ± 0.04 F
Tom056	8.7 ± 0.2 C	44.3 ± 0.5 A	2.60 ± 0.03 E
Tom061	4.4 ± 0.1 EF	34.7 ± 0.4 EF	2.26 ± 0.04 F
Tom062	3.2 ± 0.1 F	40.5 ± 2.3 BC	1.88 ± 0.10 G

Data are Mean ± SD

* Duncan's multiple range test (α = 0.05)

Max = *Glycine max* cv. Shishi; Soja001 = *G. soja*; Tab004 = *G. tabacina*; Lat = *G. latifolia*; Can003, Can007 = *G. canescens*; Tom039, Tom048, Tom049, Tom052, Tom054, Tom055, Tom056, Tom061, Tom062 = *G. tomentella*

surface of *G. max* seeds were embedded at different densities, although not as distinct as the bloom covering other species. Soja001 had a dull seed coat luster and the surface of its seed coat had a fairly heavy deposit with a network pattern, also called as bloom (Fig. 1C). The bloom was abundant on the surface of the seed coat, except in areas immediately adjacent to the hilum region (Fig. 1D). The cavity number per unit area of the bloom in *G. soja* seeds was the highest, more than 2400/mm^2 (Table 3).

Seeds of the perennial *Glycine* species typically exhibited a muriculate appearance, resulting from adherence to the inner pod wall layer, endocarp, a papillose surface was produced by short outer projections of the epidermal cells, and the seed thus acquired a velvety appearance. (Figs. 2, 3, 4A and 4B). Seeds of *G. canescens* (Can003, Can007), *G. latifolia* (Lat), *G. tabacina* (Tab004) and all of the *G. tomentella* members also had heavy large deposits in the form of a honeycomb. Their average cavity numbers ranged from 139 to 571/mm^2 (Table 3). The density among different isozyme groups of *G. tomentella* was 571, 139, 256, 231, 197, 166, 195, 189 and 213 for Tom055 (D1), Tom049 (D3), Tom062 (D3), Tom052 (D4), Tom054 (T1), Tom039 (T2), Tom056 (T2), Tom061 (T3), Tom048 (T4), respectively. Except for Tom049, the tetraploid accessions had smaller cavity density than the diploids. As well, the cavity density of the accessions from the same isozyme group differed significantly, for instance, Tom049 (Fig. 3C) and Tom062 (Fig. 3H) in the D3 isozyme group, or Tom039 (Fig. 3A) and Tom056 (Fig. 4A) in the T2 isozyme group.

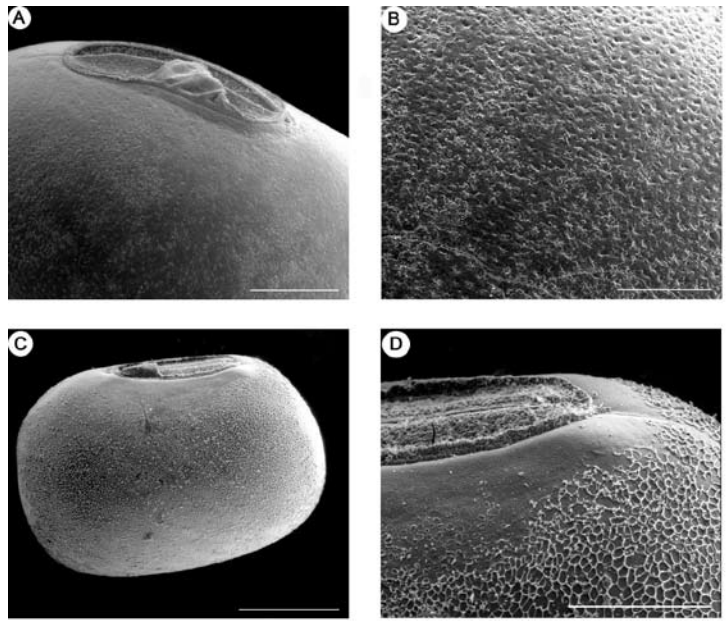

Fig. 1. Scanning electron micrograph of seed coat surface of the 2 *Glycine* species in subgenus *Soja*. A and B, numerous pores and pores of *G. max* cv. Shishi, from Taiwan, on higher magnification, respectively. C and D, bloom and an area beside the hilum in higher magnification, respectively, of Soja001, a *G. soja* collected in Taiwan. (A,C) Bars = 1 mm; (B) Bar = 500μm; (D) Bar = 400μm.

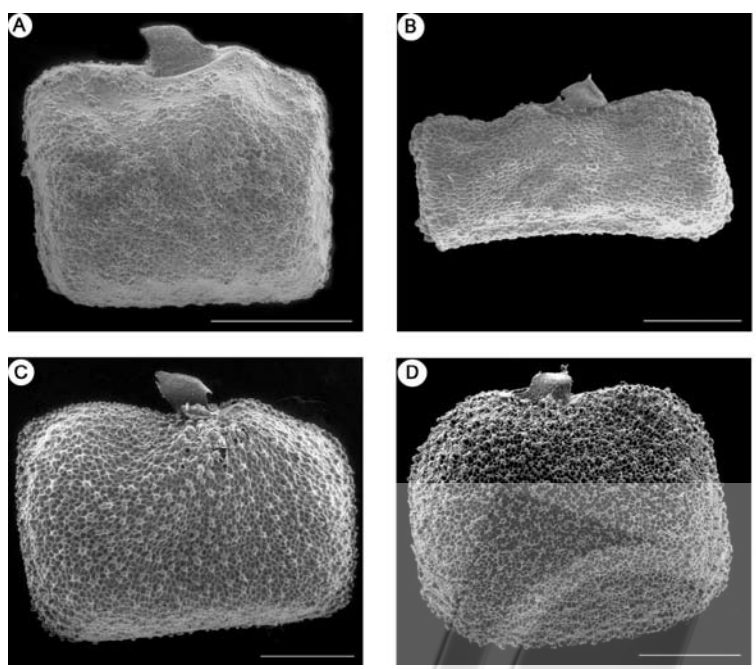

Fig. 2. Seed coat surface of perennial *Glycine* species. A. *G. tabacina* (Tab004). B. *G. canescens* (Can003). C. *G. canescens* (Can007). D. *G. latifolia* (Lat). Bars = 1 mm.

Table 3. Cavity density on the seed coat surface of *Glycine* species. The isozyme grouping of *G. tomentella* species complex is also indicated.

Accession	Isozyme group	Cavity number / mm^2
Max		ND
Soja001		2467 ± 105 A*
Tab004		342 ± 13 D
Can003		287 ± 45 E
Can007		248 ± 40 EF
Lat		481 ± 41 C
Tom039	T2	166 ± 32 GH
Tom048	T4	213 ± 13 EFGH
Tom049	D3	139 ± 8 H
Tom052	D4	231 ± 13 EFG
Tom054	T1	197 ± 10 FGH
Tom055	D1	571 ± 48 B
Tom056	T2	195 ± 24 FGH
Tom061	T3	189 ± 21 FGH
Tom062	D3	256 ± 8 EF

Data are Mean ± SD

ND: Not detected because of no endocarp

* Duncan's multiple range test (α = 0.05)

Max = *Glycine max* cv. Shishi; Soja001 = *G. soja*; Tab004 = *G. tabacina*; Lat = *G. latifolia*; Can003, Can007 = *G. canescens*; Tom039, Tom048, Tom049, Tom052, Tom054, Tom055, Tom056, Tom061, Tom062 = *G. tomentella*

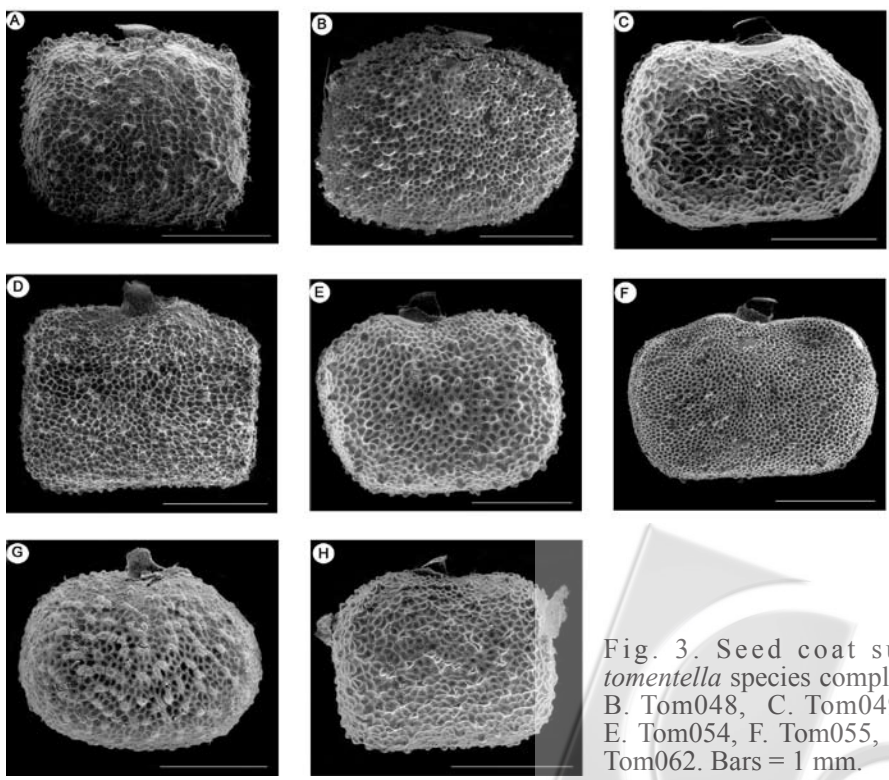

Fig. 3. Seed coat surface of *G. tomentella* species complex. A. Tom039, B. Tom048, C. Tom049, D. Tom052, E. Tom054, F. Tom055, G. Tom061, H. Tom062. Bars = 1 mm.

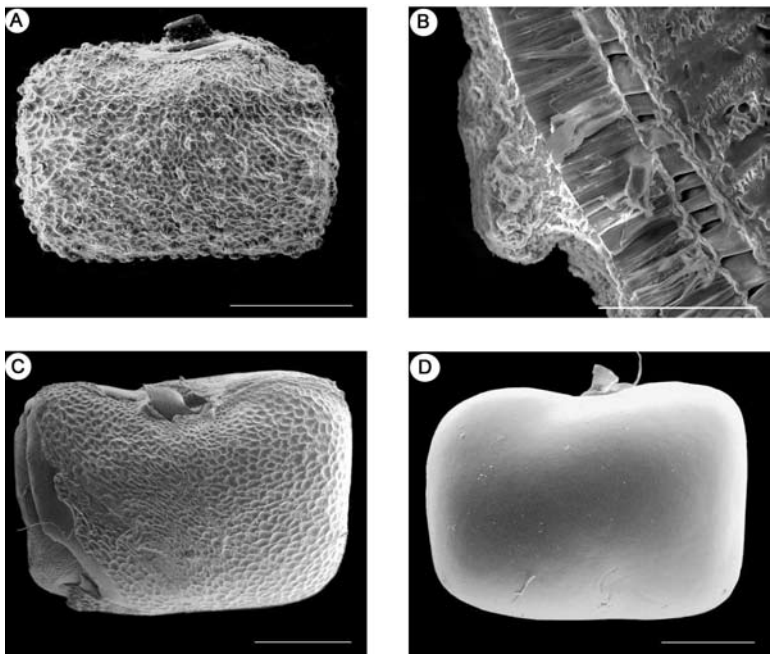

Fig. 4. *G. tomentella* (Tom056) A. Mature seed. B. Pipallose in seed coat surface. C and D. Immature seeds with and without endocarp, respectively. (A,C-D) Bars = 1 mm; (B) Bar = 100 μm.

The papery tissue

A membranaceous, papery, scale-like flap was attached to the hilum of seed from each *Glycine* accession observed by scanning electron microscopy (Fig. 1, 2, 3 and 4). This thin tissue is part of the funiculus, the tissue that attaches the seed to the pod [10]. Newell and Hymowitz [15] following Hermann [20] applied the term "caruncle" to the papery tissue. The sizes of the caruncle were similar among the perennial accessions, and occupied approximately one-third of the hilum of these accessions but approximately one-sixth of the hilum among annual accessions because of the larger seeds.

Deposit from endocarp

To determine whether the deposits in bloom originated from endocarp of the inner wall of pods or from the epidermal layer of palisade cells, we observed fresh immature seeds. The morphological difference between the fresh seed of Tom056 with and without endocarp shown in Fig. 4C and 4D, reveals that the bloom of mature seeds derived from endocarp, since the muriculate appearance was similar between mature and fresh immature seeds with endocarp (Fig. 4A and 4C).

Discussion

Taiwan is the southernmost of the distribution for annual wild soybean and the northernmost of the distribution of the perennial relatives. Four *Glycine* species are collected in Taiwan, including *G. soja* (formerly *G. formosana*), *Glycine dolichocarpa* (divided from *G. tomentella* species complex [21]), *G. tabacina* (as *Glycine pescadrensis* [22]) and *G. tomentella*. All of the perennial accessions collected in Taiwan contain 80 chromosomes (Tsai *et al.*, unpublished). Seed protein profile and isozyme group classification

reveals 2 isozyme groups -- T2 and T4 -- in Taiwan. The accessions of *G. dolichocarpa* belong to the T2 isozyme group and those of *G. tomentella* belong to the T4 group [23]. In order to test if seed coat surface character such as seed weight, seed length, seed coat proportion, and cavity density of bloom may be used to assess the degree of genetic variability between and within different *Glycine* species, we employed SEM to analyze these seed coat surface characters. In the current survey, the seeds of the T4 group (Tom048) were heavier than those of the T2 group (Tom039). However, within the T2 group, one accession from Taiwan (Tom039) contained lighter seeds than that from Australia (Tom056).

In the Leguminosae family, the morphology of seed coats is highly differentiated. The hardening of cell walls and the accumulation of new substances in the lumen and wall can serve in protection and may also cause impermeability. Hardened seed coats are common in *Glycine* species; they may also be impermeable to water and gases. Such walls often contain a high proportion of hemicellulose and pectic substances, which provide the hardness. This hardness is most conspicuous in the palisade cells of leguminous seeds [24]. The perennial *Glycine* species have hard seeds with thick walls that are cutinized, suberized, or lignified. To plant these materials, the seed coat must be cut with a razor blade to facilitate seed germination, since the perennial *Glycine* species contain harder seeds than the annual relatives. In our experience, when seeds were stored within sealed bags at 4℃, *G. soja* seeds lost their germination ability within 5 years. However, the longevity of other perennial species was still high, with germination rates at 90-100% (data not shown).

As well, small seeds have higher proportion of seed coat in terms of weight, whereas large seeds have a lower proportion of seed coat [25]. In general, seed viability is maintained better in small rather than large seeds, possibly because smaller seeds tend to have more seed coat tissue. The results shown in Table 2 support the assumption that large seeds have a lower proportion of seed coat. Within accessions, the seed coat proportion was negatively correlated with dry weight. *G. soja* accessions collected in Taiwan were smaller than those in China, Japan, and Korea. From its distinct morphology, a new nomenclature, G. *formosana*, had been suggested [26]. The seed weight and seed size were about two-thirds that of the other *G. soja* accessions; our preliminary data also suggested that its seed viability was better than that of larger seeds.

Detailed structure of seed coat

The pore-like structures in the soybean seed coat were first reported by Wolf and Baker [10]. Pitting of the seed coat surface occurred in many cultivars, although a number of them were free of pores. The distribution and size of these indentions varied among soybean varieties [11]. In our study, the seed coat surface of the cultivar (Shishi) but none of the other *Glycine* species tested contained pores. These naturally occurring pores on the surface of the seed coat were suggested to provide a means for entry into the seeds. These pores frequently appeared to be closed slots [11]. They were found to penetrate deeply into the palisade layer, about 20% to 35% of the thickness of the palisade cell, providing a passage into the hourglass layer [27]. The function of these pores is still unknown, although they would increase the seed coat surface area; no further evidence suggests that they play a role in imbibition since they do not occur in all varieties of soybean [11]. Hill and West [28] postulated that fungal mycelia could enter the seed via these pores. The number of pores varied with the position on the seed coat surface. We did not try to quantitate the number of pores because

some cultivars with severe surface deposits. A method for removing the surface deposits should be developed for detailed studies.

The cultivars showed differences in the amount of a deposit on the seed coat surface which appeared to be a fingerprint of the endocarp, the innermost layer of the pod wall [11]. However, the seed coats of wild relatives were densely covered. Newell and Hymowitz [15] reported that the seed coats of species of the subgenus *Glycine* typically exhibited a reticulated appearance on their surfaces, which appeared to result from adherence of the membraneous endocarp to the seed coat [5]. Newell and Hymowitz [15] and Yeh [29] followed Hermann [20] to call this material "perisperm". However, perisperm develops from nucellus and is located under the seed coat and therefore cannot be this material, as discussed by Wolf *et al.* [11]. In our study, using fresh seeds from immature pods, we also showed deposits on the seed coat surface, which appeared to be fingerprints of the endocarp. These deposits are not protein, lipid or wax, and may be fibrous materials such as cellulose, hemicellulose, lignin, and cutin, which may contain calcium or silicate.

The cavity densities of bloom on seed coat surface can influence the luster of the seed surface. High cavity density makes seed coat surface smooth, such as *G. soja* with more than 2400 / mm^2, however when the density decrease to about 600 / mm^2, the seed coat surface displays reticulated. As well, the cavity densities of bloom on seed coat surface among these *G. tomentella* accessions are varied: the higher ploidy seemed to have smaller cavity density. However, this character may not be a good tool for further grouping of these *G. tomentella* accessions, if we consider the grouping by isozyme profiles.

In summary, *G. max* is significantly different in seed size when compare to other *Glycine* species, annual or perennial, the pore-like character is also specific to some of *G. max* accessions. *G. soja* with high cavity density displays smooth seed coat surface and has less seed coat proportion than other perennial relatives do. *G. tabacina* with severe deposits that covering the bloom and make it smooth when observed with naked-eye, this species also has light seed weight. With oblong outline, *G. canescens* is easy to distinguish with other species. *G. latifolia* has more heavy seed weight than any other perennial relatives and also a higher cavity density. Accessions within the *G. tomentella* species complex display different degree of genetic diversities, they are similar in seed size, but different in seed weight, seed coat proportion and cavity density. Hence, these seed coat characters seem useful to distinguish between different *Glycine* species.

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References

[1] Newell CA, Hymowitz T: A reappraisal of the subgenus *Glycine*. Amer J Bot, 1978; 65: 168-179.

[2] Doyle MJ, Brown AHD: Numerical analysis of isozyme variation in *Glycine tomentella*. Biochem Syst Ecol, 1985; 13: 413-419.

[3] Kollipara KP, Singh RJ, Hymowitz T: Genomic diversity and multiple origins of tetraploid (2n = 78, 80) *Glycine tomentella*. Genome, 1994; 37**:** 448-459.
[4] Saio K, Arai K, Watanabe T: Fine structure of soybean seed coat and its changes on cooking. Cereal Sci Today, 1973; 18: 197-201, 205.
[5] Carlson JB: Morphology. *In*: Soybeans: Improvement, production, and uses. Ed. Caldwell BE. American Society of Agronomy, Madison, WI. pp.11-22, and 81-87. 1973.
[6] Williams LF: Structure and genetic characteristics of soybean. *In*: Soybeans and soybean products: Volume 1. Ed. Markley KS. New York: Interscience Publishers, pp. 111-134. 1950.
[7] Corner EJH: The leguminous seed. Phytomorphology, 1951; 1: 117-150.
[8] Yaklich RW, Vigil EL, Wergin WP: Scanning electron microscopy of soybean seed coat. Scanning Electron Microsc, 1984; 1984II: 991-1000.
[9] Murray DR: Nutritive role of seed coats in developing legume seeds. Amer J Bot, 1987; 74: 1122-1137.
[10] Wolf WJ, Baker FL: Scanning electron microscopy of soybeans. Cereal Sci Today, 1972; 17: 124-126, 127-130, 147.
[11] Wolf WJ, Baker FL, Bernard RL: Soybean seed-coat structural features: pits, deposits and cracks. Scanning Electron Microsc, 1981; 1981III: 531-544.
[12] Wolf WJ, Baker FL: Scanning electron microscopy of soybeans and soybean protein products. Scanning Electron Microsc, 1980; 1980III: 621-634.
[13] Hahalis D, Cochrane MP, Smith ML: Water penetration sites in the testa of soybeans (*Glycine max* L. Merril) during seed imbibition. Sci Legumes, 1996; 3: 218-226.
[14] Ma F, Cholewa E, Mohamed T, Peterson CA, Gijzen M: Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. Annals Bot, 2004; 94: 213-228.
[15] Newell CA, Hymowitz T: Seed coat variation in *Glycine* Willd. subgenus *Glycine* (Leguminosae) by SEM. Brittonia, 1978; 30:76-88.
[16] Hsing YIC, Hsieh KL, Huang YC, Hsieh JS: Premature drying and germination in wild soybean seeds. Taiwania, 1995; 40: 73-81.
[17] Hsing YIC, Hsieh KL, Huang YC, Hsieh JS: The relationships of cultivated soybeans and their wild relatives collected from Taiwan: revealed by seed proteins. Bot Bull Acad Sin, 1995; 36: 65-72.
[18] Hsing YIC, Hsieh JS, Peng CI, Chou CH, Chiang TY: Systematic status of the *Glycine tomentella* and *G. tabacina* species complexes based on ITS sequences of nuclear ribosomal DNA. J Plant Res, 2001; 114: 435-442.
[19] Hsieh JS, Hsieh KL, Tsai YC, Hsing YIC: Each species of *Glycine* collected in Taiwan has a unique seed protein pattern. Euphytica, 2001; 118:67-73.
[20] Hermann FJ: A revision of the genus *Glycine* and its immediate allies. USDA Techn Bull, 1962; 1268: 1-79.
[21] Ohashi H, Tateshi Y, Nemoto T, Hoshi H: Taxonomic studies on the Leguminosae of Taiwan IV. Sci Rep Tohoku Univ 4 th ser (Biol), 1991; 40: 1-37.
[22] Doyle JJ, Doyle JL, Brown AHD, Palmer RG: Genomes, multiple origins and lineage recombination in the *Glycine tomentella* (Leguminosae) polyploidy complex: Histone H3-D gene sequences. Evolution, 2002; 56: 1388-1402.
[23] Tsai YC, Hsieh TH, Chen YL, Peng YC, Hsing YIC, Hsieh JS: Using seed protein electrophoresis profile and western blotting to analyze the classification of *Glycine* species. Crop Environ Bioinform, 2006; 3: 159-176.
[24] Werker E: Seed Anatomy. (Encyclopedia of plant anatomy) Gebruder Borntraeger, Berlin, Stuttgart. 1997.
[25] Calero E, West SH, Hinson K: Water absorption of soybean seeds and associated causal factors. Crop Sci, 1981; 21: 926-933.
[26] Tateishi Y, Ohashi H: Taxonomic studies on *Glycine* of Taiwan. J Jpn Bot, 1992; 67: 127-147.
[27] Wallis TE: The structure of the soya bean. Pharm J, 1913; 37:120-123.
[28] Hill HJ, West SH: Fungal penetration of soybean seed through pores. Crop Sci, 1982; 22: 602-605.
[29] Yeh MS: Studies on external morphology and variation of surface structure in seed of *Glycine*, Leguminosae. J Agriculture and Forestry, 1987; 36: 1-14.