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Comparative Anatomy and Histochemical Study of the Seeds of *Sedum formosanum* N. E. Br. and *Sedum morrisonense* Hayata

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ABSTRACT: Both seeds of *Sedum formosanum* and *Sedum morrisonense* are oblong in shape. The seed size of *S. morrisonense* is 1.5-2 times of that of *S. formosanum* and the seed weight of *S. morrisonense* is four times of that of *S. formosanum*. On the outer surfaces of these two kinds of seeds, there are thick cuticular layers. The surface view of seed coats showed that the epidermal cells were all tuberculate but their shapes and arrangements were different between species. The inner spaces of these seeds are almost filled by the embryos. Between the radicle and seed coat there is a layer of endosperm cells. These cells contain predominantly lipid as a nutrient reserve. In the embryo the insoluble reserves are lipid and protein. The ratio of areas of oil bodies and areas of protein bodies in the cotyledonous cells of *S. morrisonense* (3.57) is higher than that of *S. formosanum* (1.87).

KEY WORDS: **Seeds, Comparative anatomy, Insoluble reserves, *Sedum formosanum*, *Sedum morrisonense*.**

INTRODUCTION

In angiosperm, seed develops from ovule after fertilization. The fertilized egg becomes embryo and the fertilized polar cell produces endosperm. However, the seed coat drives from the integuments which do not pass through fertilization (Esau, 1965).The integument belongs to sporophyte, the maternal cells, so the seed coat will have the same genome of maternal cells and does not get the characters of the paternal cells. It means that the characters of the seed coat will be very stable and there is a practical value about the seed coat in plant classification (Corner, 1976). According to the seed coat morphology, Knapp (1994) grouped the seeds of Crassulaceae into four types.

Based on the inner structure of the seeds, including the size, position and morphology of embryo and the relative size of embryo and endosperm, Martin (1946) classified the seeds of 1278 genera into three major types. In the basal type, the embryo is quite small and located in the bottom of the seeds. Otherwise, the embryo is curved in one side of the seed (peripheral type) or is in the central position of the seed (axial type).

Both of *Sedum formosanum* (Fig. 1 a) and *Sedum morrisonense* (Figs. 1 b, c) belong to Crassulaceae. Their habitats are quite different. *S. formosanum* is widely distributed along

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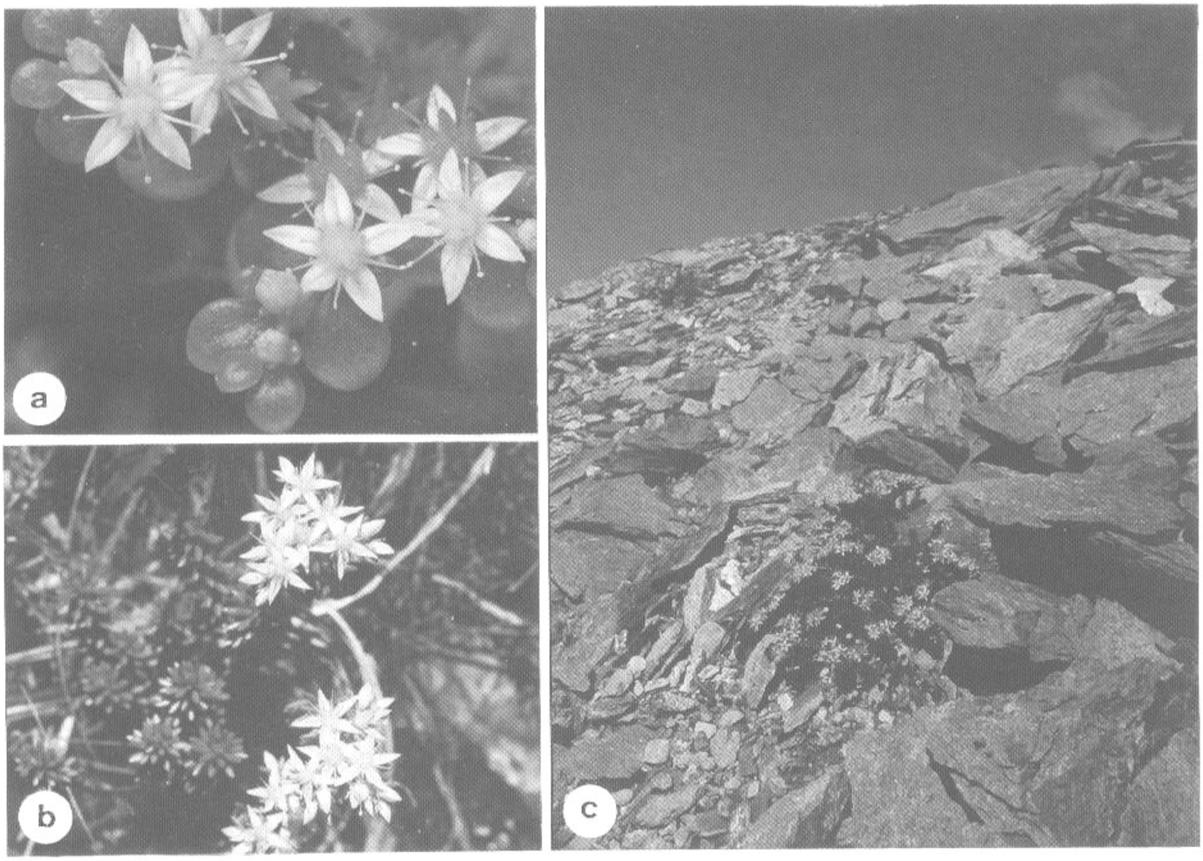

Fig. 1. (a) Plants of *S. formosanum* showing the flowers with 5 petals, 10 stamens, and 5 carpels. The leaves are obovate in shape. (b) Plants of *S. morrisonense*. The leaves are rosulate and approximately oblong-lanceolate in shape. (c) Plants of *S. morrisonense* growing in the Dabachienshan at an altitude of 3000m.

the sea shore all over Taiwan island, but *S. morrisonense* is distributed only on the rocky places of high mountains above 2500m (Tang and Huang, 1993). In this study we investigated the morphology, anatomy, and the insoluble reserves of these two kinds of seeds by light and electron microscopy, and histochemistry.

MATERIALS AND METHODS

The seeds of *Sedum morrisonense* Hayata were collected from the plants growing in the Dabachienshan at an altitude of 3000m in September of 1997. In the field we put mature fruits in the bags, and then stored them in 4℃ in laboratory. The seeds of *Sedum formosanum* N. E. Br., collected from northeast coast of Taiwan, were offered from Dr. Kuo in the Department of Agronomy of National Taiwan University. The seeds were cut in various sites and then fixed in 2.5% glutaraldehyde in 0.1 phosphate buffer (pH=7) for 2-4 hours and followed by post-fixation with 1% OsO_4 in the same buffer for 2-4 hours. For SEM, the fixed samples were dehydrated in an ethanol-acetone series. The samples were then dried with a Hitachi Critical Point Dryer (HCP-1), coated with an IB-2 Ion Coater (Kuo-Huang, 1990), and then examined with a Hitachi S-550 SEM.

Some fixed samples were dehydrated in an acetone series and embedded in Spurr's resin (Spurr, 1969). Thin sections were made with a diamond knife, stained with uranyl acetate and lead citrate and then photographed with a Hitachi H-7100 TEM (Tokyo, Japan). The 1-1.5μm semi-thin sections were stained with toluidine blue and examined and photographed with a Nikon Optiphot microscope. Some semi-thin sections were stained with Schiff reagent, Coomassie Brillant Blue R, and Sudan Black B for the examination of the polysaccharide, protein, and lipid (Gahan, 1984).

RESULTS

Both seeds of *Sedum formosanum* and *Sedum morrisonense* are oblong in shape (Figs. 2 a, e). The length and width of the seeds of *S. formosanum* are 0.42±0.02mm and 0.21±0.16mm respectively, and the seed weight is 0.006g per 1000 seeds. For *S. morrisonense*, the seeds are 0.63±0.02mm and 0.31±0.02mm in length and width, and 0.023g per 1000 seeds in weight (Table 1).

Table. 1. The characters of the seeds of *Sedum formosanum* and *Sedum morrisonense*.

Taxa / characters	*Sedum formosanum*	*Sedum morrisonense*
seed shape	oblong	oblong
length of seed (mm)	0.42±0.02	0.63±0.02
width of seed (mm)	0.21±0.16	0.31±0.02
seed weight (g/1000 seeds)	0.006	0.023
length of embryo (mm)	0.40±0.30	0.60±0.34
length of cotyledon (mm)	0.22±0.16	0.31±0.21
mean percentage of areas of protein bodies	35.47%	21.92%
mean percentage of areas of oil bodies	64.53%	78.18%

The surface view of most epidermal cells of the seed coat of *S. formosanum* is hexagon in shape (Fig. 2 b). But in *S. morrisonense*, they are almost in the shape of square (Fig. 2 f). Both the epidermal cells of the seeds of these two species have a tuberculate on the outer surface. The size and shape of the tuberculates are similar, but on the apex of the seeds of *S. formosanum* the tuberculates are more accumulated together than those of *S. morrisonense* (Figs. 2 c, g). The hilum of *S. formosanum* is square in form (Fig. 2 d), and it extends more or less to the seed coat. Besides, the arrangement of the epidermal cells on the hilum is massive. However, the hilum of *S. morrisonense* extends to the seed coat very much and the arrangement of cells on it is more orderly (Fig. 2 h).

On the outer surfaces of these two kinds of seeds, there are thick cuticular layers. These cuticular layers were stained by Schiff reagent (Figs. 3 b, d, h, j), but not by Sudan Black B (Figs. 3 a, c, g, i). However, the cell lumens in the tuberculate and the seed coat cells of *S. morrisonense* were stained densely by Sudan Black B (Fig. 3g).

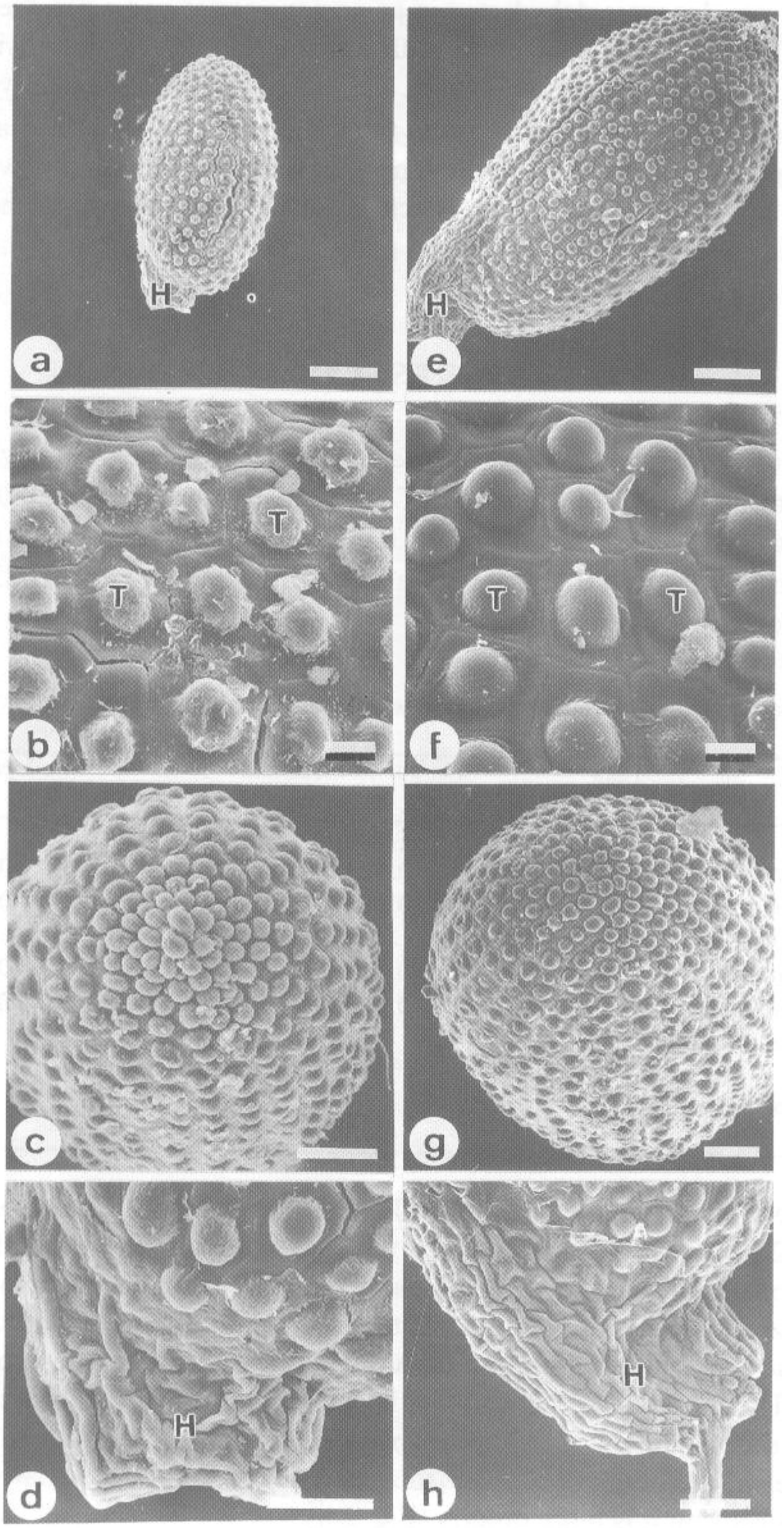

Fig. 2. SEM photographs of seeds. a-d: *Sedum formosanum*. e-h: *Sedum morrisonense*. (a, e) Surface view of the whole seed. Bars=100 μm. (b, f) Seed coat sculpture showing the shape of epidermal cells. Bars=15 μm. (c, g) Apex view of seed showing the arrangement of the tuberculate epidermal cells. Bars=50 μm. (d, h) Side view of hilum showing the arrangement of the epidermal cells. Bars=25 μm. H: Hilum. T: Tuberculate.

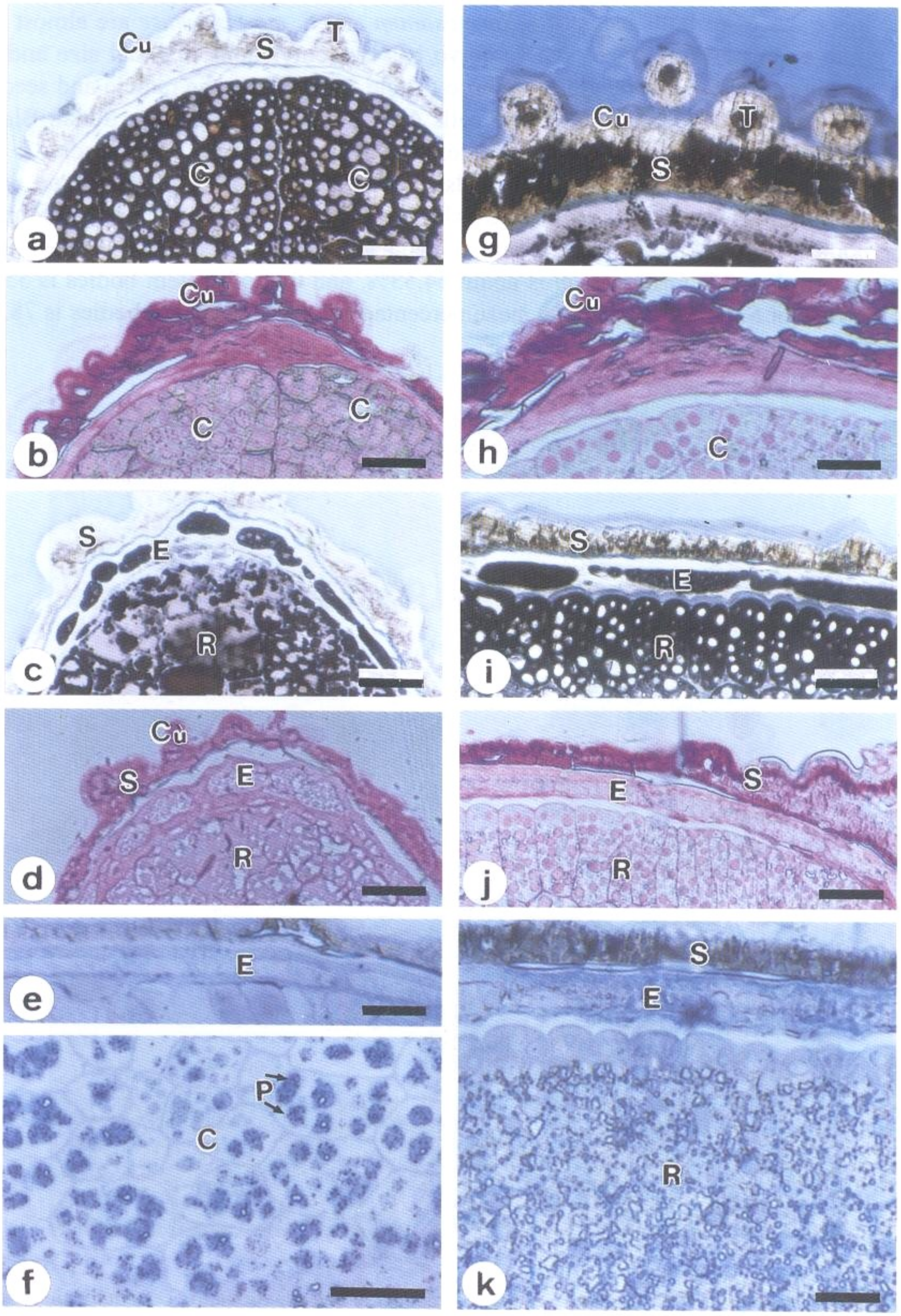

Fig. 3. Histochemical tests on the sections of seeds. a-f: *Sedum formosanum*. G-k: *Sedum morrisonense*. (a, c, g, i) Section stained by Sudan Black B. (b, d, h, j) Section stained by Schiff reagent. (e, f, k) Section stained by Coomassie Brillant Blue R. Bars=20 μm. C: Cotyledon. Cu: Cuticular layer. E: Endosperm. P: Protein bodies. R: Radicle. S: Seed coat. T: Tuberculate.

The inner spaces of both seeds of *S. formosanum* and *S. morrisonense* are almost filled by the embryo (Figs. 4 a, b, d, e). The embryo has two cotyledons in equal size and their lengths are almost half of the length of embryo (Table 1). Between the radicle and seed coat there is a layer of endosperm cells. These cells were obviously stained by Sudan Black B (Figs. 4 c-e, i-k). It indicates that the lipid is stored in the endosperm of these seeds.

In the embryo of these two kinds of seeds, the insoluble reserves are lipid and protein (Figs. 3 a-k). Lipid is stored in the oil bodies which are along the plasmalemma or around the protein bodies (Figs. 4 c, f). The mean percentage of the areas of oil bodies in the cotyledonous cells of *S. formosanum* is about 64.53%, and that of protein bodies is 35.46% (Table 1). But for *S. morrisonense*, the mean percentage of the areas of oil bodies is 78.18%, and that of protein bodies is 21.92%.

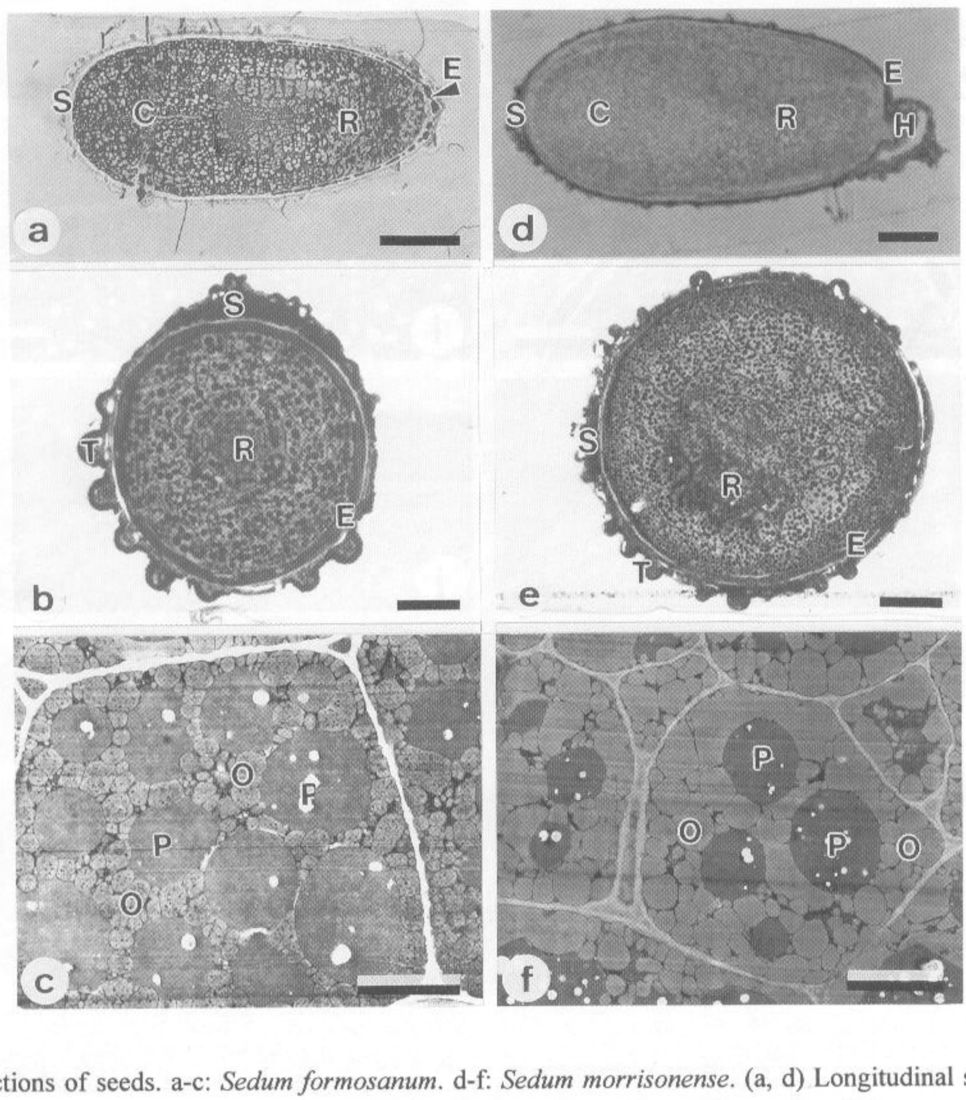

Fig. 4. Sections of seeds. a-c: *Sedum formosanum*. d-f: *Sedum morrisonense*. (a, d) Longitudinal section of a whole seed showing the inner space almost filled with the embryo. Bars=100 μm. (b, e) Transverse section of seed showing a layer of endosperm cells located between the radicle and the seed coat. Bars=50 μm. (c, f) TEM photograph of the cotyledonous cell showing the accumulation of oil bodies and protein bodies. Bars=2 μm. C: Cotyledon. E: Endosperm. H: Hilum. O: Oil bodies. P: Protein bodies. R: Radicle. S: Seed coat. T: Tuberculate.

DISCUSSION

The mature angiosperm seed is usually comprised of an embryo (sometimes underdeveloped), endosperm, perisperm, and the seed coat. The extent of the persistence of endosperm or perisperm varies between species (Fahn, 1990). Besides, the variability in the morphology and anatomy of the seed coat between species is considerable, so that, it has been taxonomically used to distinguish the different genera and species (Corner, 1976).

Knapp (1994) investigated the seeds of 225 species of 31 genera in the family of Crassulaceae. He defined the surface morphology of the epidermal cells of the seed coat to four types, *i.e.* Leitermodell, Wabenmodell, Puzzlemodell, and Warzenmodell. These types are individually restricted to the different genera. However, in the genus of *Sedum*, the outer morphology of the epidermal cells of the seed coats may be Leitermodel or Wabenmodell. According to the results of our observation, both seeds of *S. formosanum* and *S. morrisonense* are belonging to the type of Wabenmodell. Besides, the seeds of *S. formosanum* collected from Japan have also the same type of seed coat (Knapp, 1994).

There is no single structure which abstracts as the best structure for all the plant species. In general, for each plant species the size and structure of its tissues and organs are optimally determined (Mauseth, 1988). In our study, the seeds of *Sedum* are all oblong in shape. However the seed size of *S. morrisonense* (from Dabachienshan) is 1.5-2 times of that of *S. formosanum* (from Keelong coast) and the seed weight of *S. morrisonens* is four times of that of *S. formosanum* (Table 1). If plants live in a more or less stable environment with few seed predators, the large seeds with enough food reserves are preferable. While the plants live in the heterogeneous environments, such as coast areas, which prefer to produce enough small seeds to enhance the chances of arrival at a proper microhabitat (Parker and Leck, 1985; Stanton, 1984).

Based on the ratio of the size of embryo and endosperm and the position of embryo and endosperm, Martin (1946) defined the seeds of 1278 genera to 12 types. The results of our investigations showed that the seeds of *S. morrisonense* and *S. formosanum* are dwarf types. They are quite small and the inner spaces are almost filled with embryo. Between the radicle and the seed coat there is only a layer of endosperm cells. These cells contain lipid as a nutrient reserve.

The major storage materials in the cotyledons or the endosperm of seeds are starch, protein, and lipid. The amount of storage materials in the soybean seeds changes with the altitudes of their habitats (Watanabe and Nagasawa, 1990). The amount of protein is declined with the increasing of altitude. However the amount of lipid increases with the increasing of altitude. Seeds from the plants growing at high altitude may need more efficient storage material, such as lipid with more unsaturated fatty acids, for the germination in the beginning of the following spring. According to our results (Table 1), the mean percentage of the areas of protein bodies in the cotyledonous cells of *S. morrisonense* (from Dabachienshan at an altitude of 3000m) is also lower than that of *S. formosanum* (from Keelong coast). Meanwhile the mean percentage of the areas of oil bodies in the cells of *S. morrisonense* is higher than that of *S. formosanum*. Besides, the ratio of the areas of oil bodies and the areas of protein bodies in the cotyledonous cells of *S. formosanum* is 1.87 (Table 1). But for *S. morrisonense*, the ratio is 3.57. However the futher study of the correlation between the amounts of storage materials in seeds of different plant species and the altitudes of their habitats is necessary.

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石板菜與玉山佛甲草種子之比較解剖與組織化學的研究

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摘 要

石板菜與玉山佛甲草的種子均為長橢圓形，玉山佛甲草種子的大小為石板菜種子的 1.5 至 2 倍，而玉山佛甲草種子的重量則為石板菜種子的 4 倍。這兩種種子的外表均有厚的角皮層。而其種皮細胞亦各具一個突起，然而兩種種子種皮細胞的形狀與排列方式稍有不同。這兩種種子的內部空間幾乎均為胚胎所佔滿，而在種皮與胚根之間具一層胚乳細胞，這些細胞主要儲存物質為油脂。胚胎內的不可溶性儲存物質則為脂質與蛋白質，其中玉山佛甲草子葉細胞內的油脂體佔其細胞切面面積與蛋白質佔其細胞切面面積的比率較石板菜為高。

關鍵詞：種子、比較解剖、不可溶性儲存物質、石板菜、玉山佛甲草。

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