Foliar Adaptations in *Aster hispidus* var. *insularis* (Asteraceae)

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Abstract

A comparative study of leaf morphology of *Aster hispidus* and its coastal variety, *A. hispidus* var. *insularis* revealed that the latter has larger and thicker leaves than the former. The anatomical analysis indicated that the cell number of the leaf was lower but the cell size was larger in *A. hispidus* var. *insularis*. The reduction of cell number in the leaves of *A. hispidus* var. *insularis* was compensated by an increase in cell volume while the expansion of cells could be considered an adaptive strategy to coastal environments that allowed saving of water in the plant body. Salinity and drought seemed to have played a crucial role in the ecotypic differentiation.

Key words: anatomical analysis, *Aster hispidus* var. *insularis*, cell numbers, cell width, leaf morphology

1. Introduction

The existence of locally adapted ecotypes implies that disruptive selection favours alternative trait combinations in different environments; this suggests that trade-offs which are generated by ecological, morphological, physiological and genetic constraints are important determinants of diversification (Phillimore & Price, 2009). Such variation in plant populations is frequently found where water availability varies abruptly over short spatial scales (Jackson & Jobbgy, 2005). For example, coastal areas are characterised by open, sandy, or rocky habitats with weakly developed soils, and plants in these areas face severe droughts. Because of the accumulation of Na^+ in soils of coastal areas, plants are also impeded in their water uptake owing to osmotic stress between the soil and plant roots (Matoh, 2001). Moreover, the absorbed Na^+ in plant bodies is concentrated in the leaves and causes a decrease in the osmotic potential of cells, thereby causing dehydration. In other words, this salt stress induces water stress (Kasperi et al., 2009). In such situations, roots, stems, or leaves can store water and the development of storage abilities is associated with environments subjected to drought. Thus, species with such drought-tolerant ecotypes can provide excellent systems for studying the mechanisms of local adaptations.

Aster hispidus Thunb. is widely distributed across China, Korea and Japan, and is found in lowland open fields and grasslands (Figure 1 I). It has four intraspecific taxa in Japan, namely, *A. hispidus* var. *leptocladus* (Makino) Okuyama, *A. hispidus* var. *koidzumianus* (Kitam.) Okuyama, *A. hispidus* var. *tubulosus* K.Asano ex T. Shimizu and *A. hispidus* var. *insularis* (Makino) Mot.Ito (Kitamura, 1982; Yonekura & Kajita, 2003). Of these, *A. hispidus* var. *leptocladus* is found in serpentine areas of central Honshu, Kyushu and Shikoku, *A. hispidus* var. *koidzumianus* occurs along the riverside in Kyushu, *A. hispidus* var. *tubulosus* grows in central Honshu with the characteristic tubular flowers (homogamous heads with disc florets) and *A. hispidus* var. *insularis* is restricted to coastal areas of western Shikoku and eastern Kyushu (Kitamura, 1982; Ito & Soejima, 1995; Shimizu, 1997). Conversely, these varieties could be result of ecotypic differentiation in *A. hispidus* in Japan driven by different ecological factors prevailing in the habitats it occupied. To substantiate the premise, the study by Hayakawa et al. (2012) involving *A. hispidus* and of the stenophyllous *A. hispidus* var. *leptocladus* showed decreased cell number of the horizontal axis of a leaf based as an adaptation. To clarify the whole aspect of ecotype differentiation in *A. hispidus*, there is a need to know the morphological and anatomical characteristics of other ecotypes as well.

Figure 1. *Aster hispidus* varieties. (I) *Aster hispidus* var. *hispidus,* (II) *A. hispidus* var. *insularis,* and (III) Habitat of *A. hispidus* var. *insularis*.

Aster hispidus var. *insularis* has thicker stems and leaves than *A. hispidus* var. *hispidus* (Kitamura, 1982). Anatomical analyses are indispensable to discover whether the bases of the morphological differences of leaves between the two varieties are from the size and orientation of their cells. It remains unclear in the absence of such a comparative study between the two varieties. Besides, *A. hispidus* var. *hispidus* and *A. hispidus* var. *insularis* provide an opportunity to discuss the development of leaf form variation as a specialization to coastal environment. Therefore, we conducted the morphological and anatomical analyses using *A. hispidus* var. *insularis* to divulge such adaptive mechanism and the attendant foliar morphological differentiation.

2. Materials and Methods

Leaves of *Aster hispidus* var. *hispidus* and *A. hispidus* var. *insularis* were collected from the field in 2008 at flowering time (Figure 2, Table 1). For the morphological analysis, we measured the length, width, and thickness of stem leaves at the middle part of plant height for each sample. We analysed 226 leaves from 15 individuals in *A. hispidus* var. *hispidus* and 115 leaves from 26 individuals in *A. hispidus* var. *insularis*. Measurements were made using digital callipers. Leaf measurements were taken from the fully expanded leaves at the middle part of plant height, ensuring the same level of development.
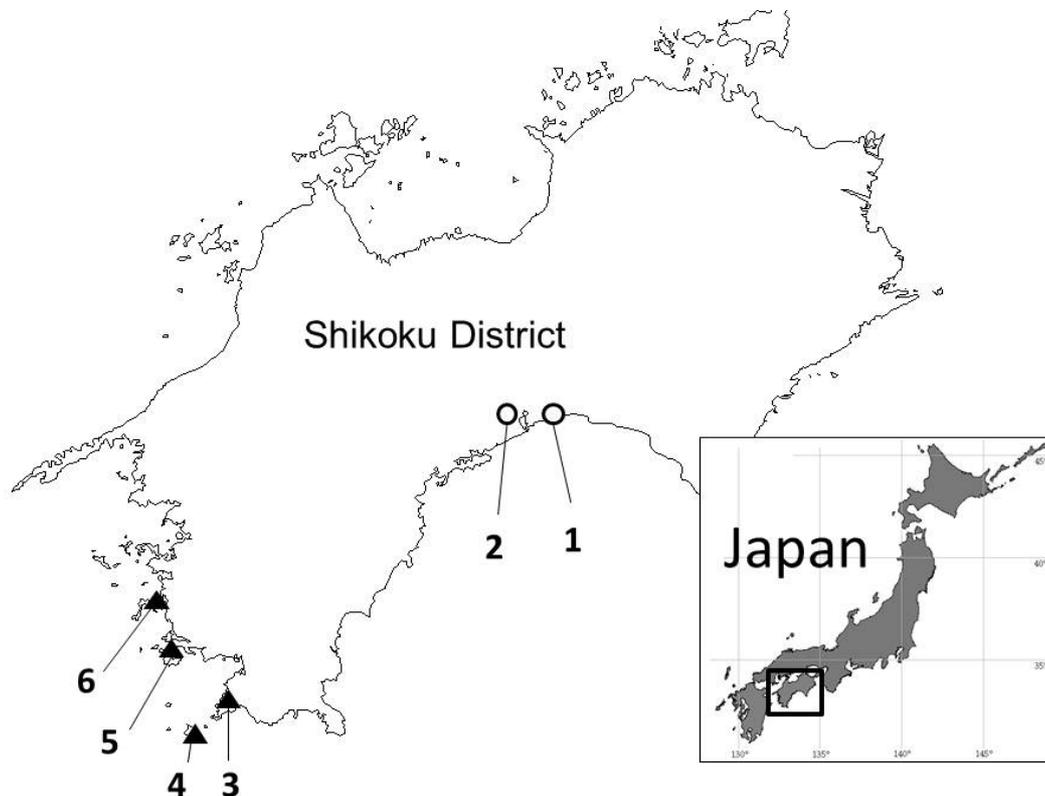
Figure 2. Sampling areas in this study

○, *Aster hispidus* var. *hispidus*; ▲, *A. hispidus* var. *insularis*. See Table 1 for other abbreviations.

Table 1. Sampling localities used in this study

Species	No.	Locality	Latitude	Longitude
Aster hispidus var. *hispidus*	1	Monobe, Nankoku City, Kochi Prefecture	N33°33'	E133°40'
	2	Mt. Hitsuzan, Kochi City, Kochi Prefecture	N33°33'	E133°32'
A. hispidus var. *insularis*	3	Kashiratsudoi, Hata-Gun Ootuki-Cho, Kochi Prefecture	N32°48'	E132°41'
	4	Okinoshima Island, Sukumo City, Kochi Prefecture	N32°43'	E132°32'
	5	Uchidomari, Minamiuwa-Gun Ainan-Cho, Ehime Prefecture	N32°56'	E132°29'
	6	Matsugahana, Uwajima City, Ehime Prefecture	N33° 3'	E132°28'

For the anatomical analysis, the leaves were fixed in a solution of formaldehyde, ethanol and acetic acid (FAA). Leaf index (the ratio of length and width) was calculated in order to determine the leaf shape. To count the number of cells on the blade, the surfaces of leaves were replicated using Suzuki's Universal Micro-Printing (SUMP) method. The middle part of the blade along the midrib was analyzed to determine the number of cells on the blade. The same SUMP image was analysed 10 times for each trait using a light microscope (Figure 3). To observe the palisade cells, the fixed leaves were dehydrated by immersion in a graded ethanol series and then embedded in Histparaffin to section at a thickness of eight μm. The widest part of the blade was analyzed to determine the height of epidermal cells (Figure 4). Slides were examined using a light microscope. Statistical analyses were performed using a *t*-test to compare the characteristics of the two varieties. Since the leaf index in

either population was not normally distributed, nonparametric pairwise comparison was made (Mann-Whitney *U* test).
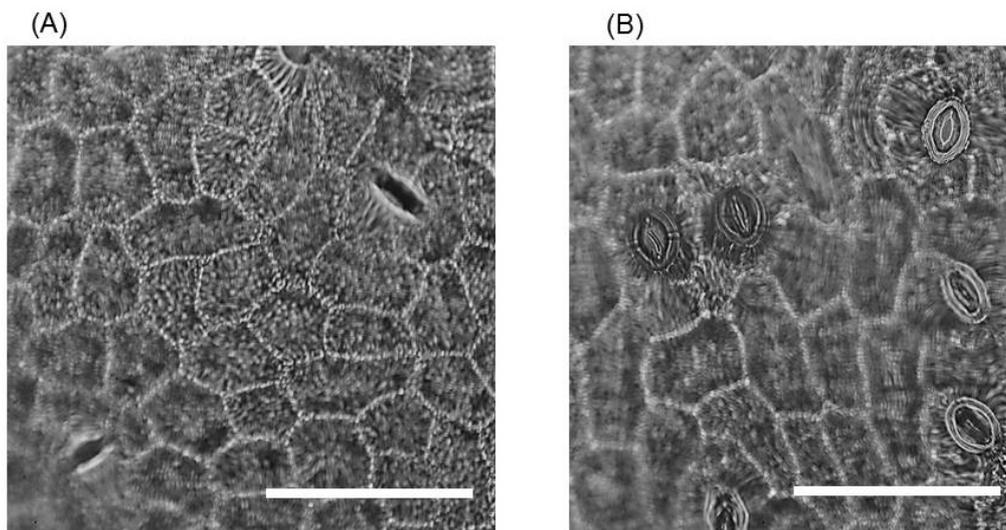

Figure 3. Comparison of the size of epidermal cells of (A) *Aster hispidus* var. *hispidus* and (B) *A. hispidus* var. *insularis*. Bar = 100 µm.
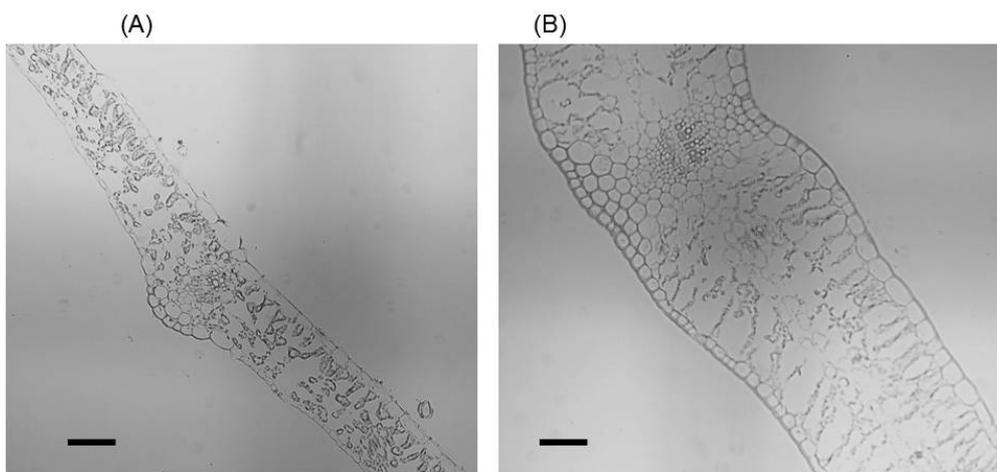

Figure 4. Transverse sections of a leaf (A) *Aster hispidus* var. *hispidus* and (B) *A. hispidus* var. *insularis*. Bar = 100 µm.

3. Results

Leaves of *Aster hispidus* var. *insularis* were significantly longer and wider than those of *A. hispidus* var. *hispidus* ($P < 0.01$). The leaf size was 30.65 ± 6.49 x 4.69 ± 1.35 mm^2 and 45.59 ± 17.83 x 8.17 ± 1.71 mm^2 for *A. hispidus* var. *hispidus* and *A. hispidus* var. *insularis*, respectively (Table 2). The leaf indices were 6.9 ± 1.8 and 4.6 ± 0.9 for *A. hispidus* var. *hispidus* and *A. hispidus* var. *insularis*, respectively, and the difference between the two varieties was significant based on the Mann–Whitney *U* test ($P < 0.01$). The leaves of *A. hispidus* var. *insularis* (0.45 ± 0.10 mm) were twice thicker *A. hispidus* var. *hispidus* (0.18 ± 0.02 mm).

Table 2. Morphological and anatomical measurements (average ± standard deviation) of *Aster hispidus* var. *hispidus* and var. *insularis*

Trait	var. *hispidus*	var. *insularis*	Significance
Morphological measurement			
Leaf length (mm)	30.65 ± 6.49	45.59 ± 17.83	**
Leaf width (mm)	4.69 ± 1.35	8.17 ± 1.71	**
Leaf index	6.9 ± 1.8	4.6 ± 0.9	**
Leaf thickness (mm)	0.18 ± 0.02	0.45 ± 0.10	**
Anatomical measurement			
Epidermal cell length (μm)	38.83 ± 6.63	46.32 ± 6.60	**
Epidermal cell width (μm)	37.68 ± 6.21	40.30 ± 4.73	*
Epidermal cell numbers in longitudinal direction	1240 ± 393	779 ± 249	**
Epidermal cell numbers in latitudinal direction	266 ± 117	180 ± 60	**
Epidermal cell height at adaxial side (μm)	16.83 ± 4.59	31.78 ± 5.64	**

** and * indicate $p < 0.01$ and $p < 0.05$, respectively, according to the *t*-test.

The data of *Aster hispidus* var. *hispidus* are after Hayakawa et al., 2012 and present study.

Epidermal cells at adaxial side of *Aster hispidus* var. *insularis* were significantly longer and wider than those of *A. hispidus* var. *hispidus* ($P = 0.01$ and $P = 0.05$, respectively). The length and width of the cells were 38.83 ± 6.63 and 37.68 ± 6.21 μm for *A. hispidus* var. *hispidus* and 46.32 ± 6.60 and 40.30 ± 4.73 μm for *A. hispidus* var. *insularis*. The epidermal cell numbers in longitudinal and latitudinal directions of leaves were found to be 1240 ± 393 and 266 ± 177 for *A. hispidus* var. *hispidus* and 779 ± 249 and 180 ± 60 for *A. hispidus* var. *insularis* (Table 2). The cell numbers of *A. hispidus* var. *insularis* were significantly lower than those of *A. hispidus* var. *hispidus* in both directions ($P = 0.01$).

The cross sections in a sample of leaves revealed the adaxial epidermal cell height to be 31.78 ± 5.64 μm for *Aster hispidus* var. *insularis* and 16.83 ± 4.59 μm for *A. hispidus* var. *hispidus,* and the difference is significant ($P = 0.01$).

4. Discussion

Our results revealed that the leaves at the middle part of plant height of the coastal ecotype *Aster hispidus* var. *insularis* are not only larger but also thicker than those of its inland variety, *A. hispidus* var. *hispidus* (Table 2). Changes in features of leaves are commonly observed in plants subjected to increased soil salinity, as in the case of mangal plants such as *Laguncularia racemosa* (L.) C.F. Gaertn. (Biebl & Kinzel, 1965) and *Avicennia germinans* L. (Suárez & Sobrado, 2000). In the latter, leaf succulence was caused by increased leaf water content and subsequent accumulate large amount of solute, without an increase in the osmotic pressure of cells (Suárez & Sobrado, 2000). Tadano (1993) also reported that thicker and more succulent leaves maintain low salinity levels in the leaves. Therefore, the thicker leaves of *A. hispidus* var. *insularis* could be considered an adaptive feature to growth in coastal environments where droughts and salinity stress are common. Leaf thickening was correlated with cell size. As expected, the leaf cells of *A. hispidus* var. *insularis* were larger in size but fewer in number than those of *A. hispidus* var. *hispidus*. A decrease in leaf cell number may reflect a suppression of cell division under strong drought stress. Li et al. (2011) reported obvious reductions in mesophyll thickness in *Campylotropis polyantha* (Franch.) Schindl. cultivated under conditions of water deficiency. On the other hand, larger cell size in drought-tolerant genotypes was also reported in vascular tissues of *Boehmeria nivea* (L.) Gaud. (Liu et al., 2005). One of the causes of the difference in cell size between the coastal and inland varieties is that the large cell volume of *A. hispidus* var. *insularis* offers the plant the ability to store a greater amount of water in the leaf cells and compensate for the decrease in cell numbers. In fact, artificial tetraploid plants of *Dendranthema nankingense* (Nakai) Tzvel. with cells larger than those in normal diploid plants showed higher levels of abiotic stress tolerance, including drought and salinity stresses. Whether the tolerance was induced by increasing cell size was unclear though (Liu et al., 2011). *Aster hispidus* var. *insularis* may be able to grow along

the coast by overcoming increased drought stress and soil salinity. For future studies, it will be necessary to investigate the correlation between cell size and water content in leaves of these species. In Japan, some other succulent taxa that evolved from related inland species and adapted to coastal environments are *Cirsium maritimum* Makino (Asteraceae), *Dianthus japonicus* Thunb. (Caryophyllaceae), and *Arabis stelleri* DC. var. *japonica* (A. Gray) Fr. Schm. (Cruciferae). Studies on these species are also necessary to confirm whether the anatomical differences found in this study represent a general tendency in the evolution of coastal plants from inland relatives.

Recent studies have provided some evidence for the coordination of cell proliferation and expansion of the leaf. For example, when cell proliferation in a leaf is reduced because of certain mutations, the reduction in the final leaf area is compensated for by an increase in the size of individual leaf cells. This phenomenon of compensation could aid in understanding the regulation of cell proliferation and expansion at the organ level (Tsukaya, 2003; 2008). In fact, Kim and Kende (2004) reported that a loss-of-function mutation in the *AN3*/*GRF-INTERACTING FACTOR1* gene of *Arabidopsis thaliana* (L.) Heynh., which positively regulates cell proliferation in leaf primordia, resulted in a typical compensation syndrome (Horiguchi et al., 2005). In addition, several other genes that affect leaf cell proliferation have been described as causes of the compensation syndrome (Ali et al., 2007; Fujikura et al., 2007). The genetic background of cell proliferation and leaf expansion are very useful from the viewpoint of evolutionary developmental (evo-devo) studies of leaf differentiation between *A. hispidus* var. *hispidus* and var. *insularis*. Therefore, it would be very interesting to compare leaf morphologies of transgenic model plants by introducing these candidate homologues isolated from them.

Acknowledgements

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