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Dorsiventral Structure of Unifacial Leaves in Several *Iris* Species

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今村駿一郎*・肥田美知子**：二三のアイリス属植物における単面葉の背腹構造

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In some plants the dorsiventrality of leaves, expressed in the distribution of stomata and assimilating parenchyma, is determined by their situation with respect to the direction of external factors. Some conifers, *Thujaopsis dolabrata*, *Chamaecyparis obtusa*, *Podocarpus imbricata*, etc. (3, 4, 5, 6, 7, 9, 11), have assimilating organs, whose dorsiventrality is determined by the direction of incident light. Gravity as a determining factor was reported previously by the senior writer in *Iris japonica* (8). Dorsiventrality can be readily recognized from the different color of the upper and lower leaf surfaces. It is rarely found in *Iris* species having equitant leaves, which usually have an isobilateral structure. But such cases of dorsiventrality were later found in 5 species; they are described in the present paper.

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I. Anatomical observations

1) *Iris formosana* Ohwi.

The species was first collected by Dr. Jisaburo Ohwi in Formosa in 1934 (12). It is similar to *Iris japonica* but is larger in size, and it bears fewer flowers on an inflorescence and is fertile.* The upper surface of the leaf is dark green whereas the lower surface is waxy and glaucous. The collateral fibrovascular bundles do not occur in pairs but occur alternately. In the distribution of the conductive strands no dorsiventrality was found. Only a very few stomata are found on the upper surface, but many short cells occur here among the longer epidermal cells (Plate XIX, Fig. 5a). As previously reported (8, 10), the short cells are rudiments of stomata. The cell layer beneath the epidermis of the upper side has relatively small intercellular spaces. On the lower surface many stomata and a small number of short cells are found (Plate XIX, Fig. 5b). The subepidermal cells are cocoon-shaped with large intercellular spaces between them.

2) *Iris Rossii* Baker.

For this material we are indebted to Mr. Taketo Mizoguchi, who collected the plant near Beppu in North Kyushu.

Fig. 1. Transverse sections of *Iris* leaves, anatomical upper surface shaded, × unpaired vascular bundles. A: *Iris Rossii*. B: *Iris uniflora*. C: *Iris Wilsoni*. D: *Iris Bulleyana*. A and B × ca. 50. B and C × ca. 40.

* *Iris japonica* has 54 chromosomes in somatic cells and, being a triploid, does not set seeds. A very similar plant was collected by Yu, Ching-jiang in Central China, which had 36 chromosomes in somatic cells (17). The leaf structure coincided entirely with that of *Iris japonica*.

The leaf is thin and is strongly striated. The species is similar to *Iris japonica* and *formosana* in the arrangement of the conductive strands which do not occur in pairs but occur alternately; no air channels are found in the mesophyll (Fig. 1, A). The epidermis of both sides is very wavy (Plate XIX, Fig. 1a). Stomata are almost entirely restricted to the lower surface while on the upper surface a small number of short cells are found (Plate XIX, Fig. 1b).

3) *Iris uniflora* Pall.*

For this material we are indebted to Mr. Ryuhon Saito who kindly collected the plant upon our request in middle Korea. In a cross section of the leaf, major collateral fibrovascular bundles stand in pairs, between which minor unpaired bundles are found on the upper side (Fig. 1, B). Between the large vascular bundles air channels are formed. The upper leaf surface is dark green and has scarcely any stomata (Plate XIX, Fig. 2a). In contrast to *Iris formosana* and *Iris japonica*, short cells are scarce on the upper surface. The lower leaf surface is glaucous and has many stomata (Plate XIX, Fig. 2b). The subepidermal layer is rich in intercellular spaces.

4) *Iris Wilsoni* Wright and 5) *Iris Bulleyana* Dykes.

These two are species from south-western China. We obtained herbarium material and viable seeds by the courtesy of Dr. M. Reed, Brooklyn Botanic Garden, U.S.A.

The two species are very similar in their anatomical leaf features. The distribution of the conductive strands is similar to that of *Iris uniflora*, having paired vascular bundles and a small number of unpaired ones on the upper side (Fig. 1, C, D). The stomata are restricted almost entirely to the lower surface. Rare short cells are found on both surfaces (Plate XIX, Fig. 3a, b; Fig. 4a, b).

With the exception of *Iris japonica* and *Iris Rossii* all species have papillae on the epidermal cells, which are especially conspicuous on the lower surface (Plate XIX, Fig. 2b, 3a, 3b, 4b and 5c).

II. Physiological observations

Observations and experiments were performed to investigate the factors which induce the dorsiventrality of leaves. Mainly, *Iris japonica*, *Iris formosana* and *Iris uniflora* were used as materials. In the mode of dorsiventrality induction two types could be distinguished.

a) Labile induction

The dorsiventrality of a newly developing leaf can be easily inverted by the reversal of the direction of the controlling external factors. In a vertical position the developing leaf becomes isobilateral with stomata on both surfaces. When the plant is fixed in an inclined position the stomata and spongy parenchyma develop on the surface directed downward whereas on the opposite side many short cells

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* Synonym. *Iris ruthenica* Ker-Gawl. var. *nana* Maxim.

and few stomata are formed. By repeated reversals we can obtain plants, whose leaves have a reversed dorsiventrality. Seen from one side, some leaves appear dark green while others are pale green. A leaf, which has areas with and without stomata side by side on one surface, can also be obtained. *Iris formosana* and *Iris japonica* belong to this type. The inducing factor is gravity, and light, so far as the experiments indicate, has no significant rôle in induction (8). One experiment with *Iris formosana* will be mentioned here.

Vigorous plants with developing leaves were transferred to a dark room and

Fig. 2. Distribution of stomata, short cells and initial cells in a leaf, which was fixed in inverted position for 54 hours while developing in the dark room. *Iris formosana*. Solid line initial upper surface, broken line lower surface.

kept in an inverted position by fixing the basis of the plant with metal forks. After having been left in the dark for 54 hours they were returned to the initial position and grown in day light. When the developing leaves had attained their full size, the distribution of stomata and short cells was observed and the average size of the epidermal cells was established from the tip to the base in intervals of 1 cm. The observations carried out on one plant are represented in Fig. 2. The initial length of the leaf was 13.7 cm and it became 15.8 cm in the dark room. The final length was 44.7 cm and the leaf blade was somewhat constricted in the middle portion, indicating that the expanding growth of the leaf was somewhat inhibited by darkness. As the ultimate density of cells per unit area may be modified by the subsequent expanding growth, the number of stomata and short cells observed were corrected by the size of the surrounding epidermal cells to make clear the mode of differentiation in young epidermal tissue.* The distribution of the stomata shows a completely inversed dorsiventrality in a portion between 10 and 13 cm from the leaf tip. The number of stomata is strikingly reduced on the initial lower surface, which was directed upward in darkness and markedly increased on the opposite surface. On the other hand, the short cells increased on the former surface and decreased on the latter. In the transition zone various types of intermediate structures of stomata and short cells are found, indicating the homologous nature of both cell types (10). An initial cell can differentiate into a short cell or two guard cells, according to its direction in relation to gravity. The initiation of initial cells is influenced by the action of gravity, and they are formed in larger numbers on the surface directed downward.

The inversion in the distribution of initial cells on the experimental leaf reaches nearer the leaf basis than that of stomata (Fig. 2). The behavior is the same as in *Iris japonica* (8).

The seedlings of *Iris formosana* are prostrate and gravity acts only in one direction. Several first leaves are slightly dorsiventral, having more stomata on the lower than on the upper surface. In the following leaves the dorsiventral character is gradually intensified up to the 4th or the 5th leaf, in which final structure is attained.

b) Stable induction

To this second group belong *Iris uniflora* and *Iris Rossii*. In this group, once the dorsiventrality is established, it cannot be influenced by experimental treatment. Not only the developing leaf but all leaves, which later develop, retain the same dorsiventrality as the older leaves, irrespective of the inclination of the plant to the vertical line. Whether the lateral shoots of higher order which may develop

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* The relative size of epidermal cells was determined, taking their average size on the lower side at the leaf tip as a unit. The number of stomata and short cells at each point of Fig. 2 was obtained from the observed number multiplied by the relative dimension of the epidermal cells.

long after the fixation in inverted position, may also retain the same dorsiventrality as the mother axis, could not be decided, as our experiments lasted only about a half year.

Table 1. Number of stomata per mm² in seedling leaves of *Iris uniflora*, average of ten observations.

Plant	Surface	Main axis	Axillary shoot of the		
			first leaf	second leaf	third leaf
1	I	157±4.6	173±11.9	17±3.8	111±8.6
	II	167±9.8	11±3.9	122±6.7	22±3.7
2	I	186±6.0	192±5.8	194±8.2	53±3.8
	II	184±7.0	126±5.6	42±6.2	144±6.5

Leaves on the main axis of a seedling of *Iris uniflora* are erect and do not show any sign of inequality of both surfaces. The lateral branches, however, have distinctly dorsiventral leaves. Individual branches are not dorsiventral in the same sense, as shown in Table 1. The arrangement of leaves and shoots of a seedling is given diagrammatically in Fig. 3. In the early stages of development, the plane

Fig. 3. Diagram of branching in a seedling of *Iris uniflora*, anatomical upper surface solid black, p, prophyll.

of a lateral shoot is almost parallel but slightly oblique to that of the mother axis (13, 16). The leaf side of a lateral shoot which stands nearer to the mother axis, becomes the anatomical upper surface, and the opposite side becomes the lower surface. Induction occurs, even if the basis of the plant is deeply covered by soil at a very early stage of germination. As the determining agency there is no reason to assume any external factor other than gravity. A slight inclination of the initials of the lateral shoots may induce dorsiventrality of the growing point from which the following leaves are developing. Also *Iris Bulleyana* and *Iris Wilsoni* do not show any dorsiventrality in the main axis of the seedlings. In all probability these two species belong to the second type of induction although we could not perform any detailed experiments.

Discussion and Summary

In all *Iris* species with unifacial leaves the rhizome lies in the soil horizontally and is distinctly dorsiventral. In most of them, the foliage shoots with equitant and isobilateral leaves which stand erect, both leaf surfaces being exposed equally to light and gravity. However, some species, for instance, *Iris gracilipes* A. Gray, *Iris tectorum* Maxim., etc. have plagiotropic shoots which do not show any anatomical inequality of the surfaces of their leaves.

In the species described in the present report a structural dorsiventrality was found. In *Iris formosana* the seedling has, except for the first 1 or 2, dorsiventral leaves. The dorsiventrality can be inverted by changing their direction in relation to gravity, as in *Iris japonica*. In *Iris uniflora* and *Iris Rossii* the seedling is not dorsiventral but the lateral shoots are distinctly so. Once established, this dorsiventrality cannot be inverted by changing the direction in respect to external factors. The growing point seems to be determined dorsiventrally and it gives rise to leaves with unequal surfaces. In this case also gravity seems to have the determining rôle. In the mode of induction *Iris Wilsoni* and *Iris Bulleyana* seem to belong to the stable type.

It is noteworthy that all the above species are members of Asiatic flora. They belong to the section *Apogon* except *Iris japonica* and *Iris formosana*, which are representatives of the section *Evansia*. The taxonomic or systematic significance of leaf dorsiventrality cannot be evaluated until more *Iris* species are thoroughly examined. In literature the indications are that many have leaves with unequal surfaces.*

The causal sequence of aitiogenous induction of dorsiventral differentiation of the leaves is yet obscure. The unequal distribution of food materials and some determining hormones may probably be the factors which cause the unequal differentiation, as can be assumed from the eccentric growth in the thickness of the inclined trunks or lateral branches in many plants (14, 15).

For the materials used in the present study we are indebted to Dr. M. Reed, Brooklyn Botanic Garden, U.S.A., Dr. Yu, Ching-jiang, Dr. Shoichi Tanaka, Mr. Ryuhon Saito and Mr. Taketo Mizoguchi. We wish to express to all the above mentioned persons our sincere thanks for kindly supplying the rare plant materials.

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* In "The Genus *Iris*" of Dykes, the remark, "Upper side glossy and lower side glaucous" is found in the description of leaves of *Iris Forrestii*, *I. Clarkei*, *I. bracteata* and *I. graminea* (2). That the assimilating parenchyma differs on both surfaces in *Iris Douglasiana* is shown in Fig. 9 of Plate 80 in "Monocotyledons" of Arber (1).

Plate XIX. Epidermal tissues of *Iris* leaves.

Fig. 1. *Iris Rossii*.

Fig. 2. *Iris uniflora*.

Fig. 3. *Iris Wilsoni*, from herbarium material.

Fig. 4. *Iris Bulleyana*, from herbarium material.

Fig. 5. *Iris formosana*.

a: upper surface, b: lower surface, c: lower surface, mounted with small quantity of water to make the papillae of epidermis clear. $\times$ ca. 150.

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Further Studies on Paper Chromatography of Anthocyanins, Involving an Examination of Glycoside Types by Partial Hydrolysis*

Studies on Anthocyanins XXIX**

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阿部幸頼***・林 孝三****: アントシアン配糖体およびその部分的加水分解産物の
ペーパークロマトグラフィー (アントシアン色素の研究 第29報)

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Since the technique of paper chromatography was first introduced by Bate-Smith (1, 2) into characterization of natural anthocyanins, remarkable progress has been made by succeeding investigations of several workers (3-6). Though modern procedure in these experimentations proved to be an effective tool for prompt determination of sugar-free pigments, yet something seemed to be still required for the correct determination of individual glycosides, especially of their glycoside types. In the course of our studies on paper chromatography of natural anthocyanins, we have found that a diglycoside in general is partially hydrolyzed to give monoglyco-

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