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Morphological Character of Crystalline Inorganic Components Present in Plants: Calcium Oxalate Crystals in the Leaves of Sweet Potato grown in Natural Light and in Darkness

Kouichiro Umemoto

Kyoto College of Pharmacy[1)]

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A series of experiments was carried out to prove the possibility that the morphological character of crystalline inorganic components in a plant might become a valuable index in elucidating the kinship of plants, and detailed examinations were made on the crystalline pattern of calcium oxalate present in the leaves of sweet potato plants (*Ipomoea batatas* Lam. var. *edulis* Makino) grown in vastly different conditions; presence and absence of light (natural light and darkness). It was thereby found that the crystalline pattern was identical in two samples, indicating that the morphological character of crystalline inorganic components formed in a plant is independent of its photochemical reaction system. These results, together with results obtained from my past studies, indicate that "the morphological character of crystalline inorganic components present in a plant is one of the important characters inherent in and is a specific character reflecting the properties of the protoplasmic character and a genetic character."

In connection with a series of studies on inorganic components, especially crystalline inorganic components, present in animals[2)] and plants[3,4–14)] by the use of the "low-temperature plasma ashing method for biological materials" devised by the author, it seemed of interst to prove the possibility that the morphological character of the crystalline inorganic components present in plants would be an important index in elucidating the interrelationship of plant species (genuses and/or families).

The present author has hitherto been able to clarify that the morphological character of crystalline inorganic components present in plants has the characteristics of (1) being specific to each plant, (2) essentially no changes occur under vastly different growth conditions, and (3) being inherited under some genetic rule. In connection with (3), some examinations have been made on the possibility of morphological mutation of crystalline inorganic components present in natural and artificially produced mutant strains of rice and other plants, and it was found that such mutations are not easily produced. As a result of these studies, it has become increasingly evident that there is a great possibility that the morphological

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character of these crystalline inorganic components would give valuable informations for various fields of botanical studies.

In the present series, detailed examinations were made on the morphological character of calcium oxalate crystals in the leaves of sweet potato (*Ipomoea batatas* Lam. var. *edulis* Makino) grown in natural light and in darkness, and it was thereby found that the pattern of crystals in both sample leaves was the same and that the morphological character of crystalline inorganic components formed in plants is independent of the photochemical reaction system. This fact endorses directly and indirectly the prediction that the morphological character of the crystalline inorganic components has characteristics outlined above as (1), (2), and (3). Consequently, this leaves no doubt for the author's claim that "the morphological character of crystalline inorganic components present in plants is one of the important characters inherent in plants."

Experimental Material

The tuberous root of sweet potato plant of a suitable size was allowed to germinate in a room with natural light, at a temperature of $25° \pm 2°$ and relative humidity of 60—70%. When some bud started to germinate, the potatoes were divided approximately into three parts (each piece to contain one germinating bud). Each of these three divided pieces was grown in darkness, in the shade, and in natural light direct sunlight), and the leaves that eventually developed were submitted as samples. Growth conditions were the same for all three kinds, except for the marked difference in the amount of light.

Result

A) Growth in Natural Light (Direct Sunlight)

Leaves were collected shortly after budding, when the long axis (minus the petiole) became about 3 mm, to the maximum length of *ca.* 90 mm, various stages of growth, and the pattern of calcium oxalate crystals was followed. The results of observations were as follows:

(1) Veins——Throughout all the stage of leaf growth, minute granules which glistened under the Nicol prism (but without interference fringe) were observed. At the tip of the 3-mm leaf, near the costa, one or two clustered crystals, with fairly distinct ridges, were observed (Fig. 1-A, B, C). These clustered crystals attained a distinct shape and became numerous when the leaf reached around 9 mm in length, and its size became about 20 μ (Fig. 1-D, E). The number and size of these clustered crystals failed to change after the leaf became *ca.* 20 mm or more in length. At the center of the costa, clustered crystals are hardly observed in leaves of 3 mm in length but clustered crystals of fairly distinctive form began to appear as the leaves attained more than 9 mm in length (Fig. 1-F). When the leaves became around 70 mm in length, clustered crystals of irregular shape began to appear in a line and this line increased in length and number as the leaves grew (Fig. 1-G, H, I).

In the branching veins, excluding a relatively thick vein where the crystal pattern was approximately the same as that in the central part of the costa, irregularly shaped clustered crystals of 5—10 μ were observed in the leaves of 3 mm in length (Fig. 2-A, B, C), the crystals being slightly smaller than those (around 15 μ) at the tip of such leaves. These crystal aggregates increased in number and size in proportion to the growth of leaves up to around 70 mm in length, growing to clustered crystals with regular shape (Fig. 2-E, F, G, H). This crystal pattern does not change much further when the leaves grow to more than 70 mm in length.

(2) Mesophyll——Some minute crystals are present but clustered crystals were not observed, differing from the veins.

B) Growth in Darkness

Leaves withered after reaching about 30 mm in longer length and, therefore, these leaves of longest length were collected just before withering to be used as samples. All the leaves collected were white to pale yellowish white.

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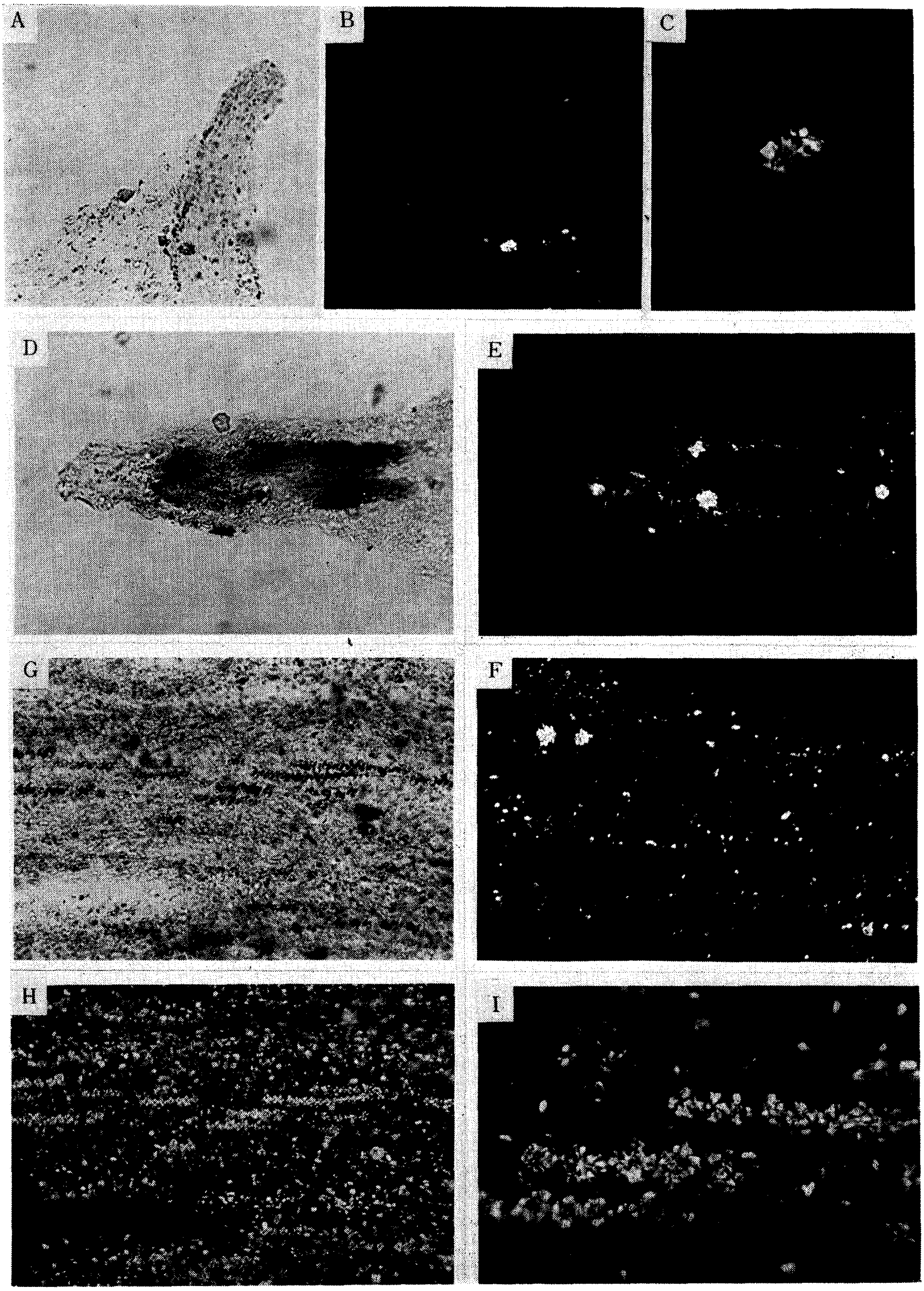

Fig. 1. Low-temperature Ashed Image of the Leaves of Sweet Potato Grown in Direct Sunlight

leaf length: about 3 mm (minus the petiole)
apex; A: ×100 normal light, B: ×100 crossed Nicols, C: ×400 crossed Nicols
leaf length: about 9 mm
apex; D: ×100 normal light, E: ×100 crossed Nicols costa; F : ×100 crossed Nicols
leaf length: about 90 mm
costa; G : ×100 normal light, H: ×100 crossed Nicols, I: ×400 crossed Nicols

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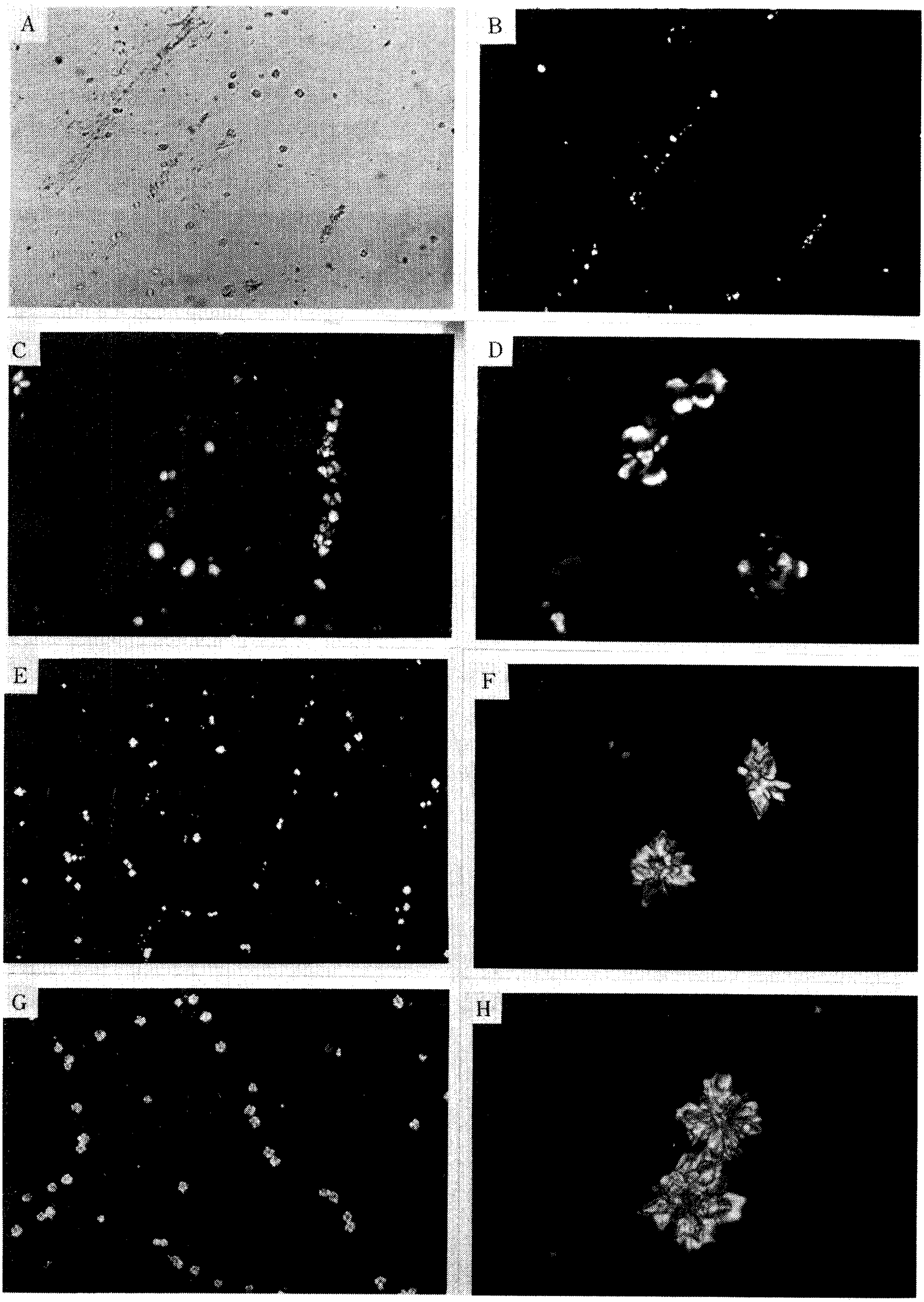

Fig. 2. Low-temperature Ashed Image of the Leaves of Sweet Potato Grown in Direct Sunlight (branching veins)

leaf length: about 3 mm
A: ×100 normal light, B: ×100 crossed Nicols, C: ×200 crossed Nicols, D: ×400 crossed Nicols
leaf length: about 9 mm
E: ×40 crossed Nicols, F: ×400 crossed Nicols
leaf length: about 90 mm
G: ×40 crossed Nicols, H: ×400 crossed Nicols

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Crystal forms observed in these leaves were identical with crystal forms present in the leaves collected from plants grown in natural light, as described above, and the pattern was the same as that in the leaves of around 9 mm in length grown under a natural light, these being no clustered crystals in the mesophyll and minute granules that glistened under the Nicol prism, though without interference fringe. At the tip of the leaves, on the costa, were clustered crystals of distinct form (Fig. 3-A, B, C), agreeing with those observed in the leaves of more than 9 mm in length from plants grown in natural light. At the center, on the costa,

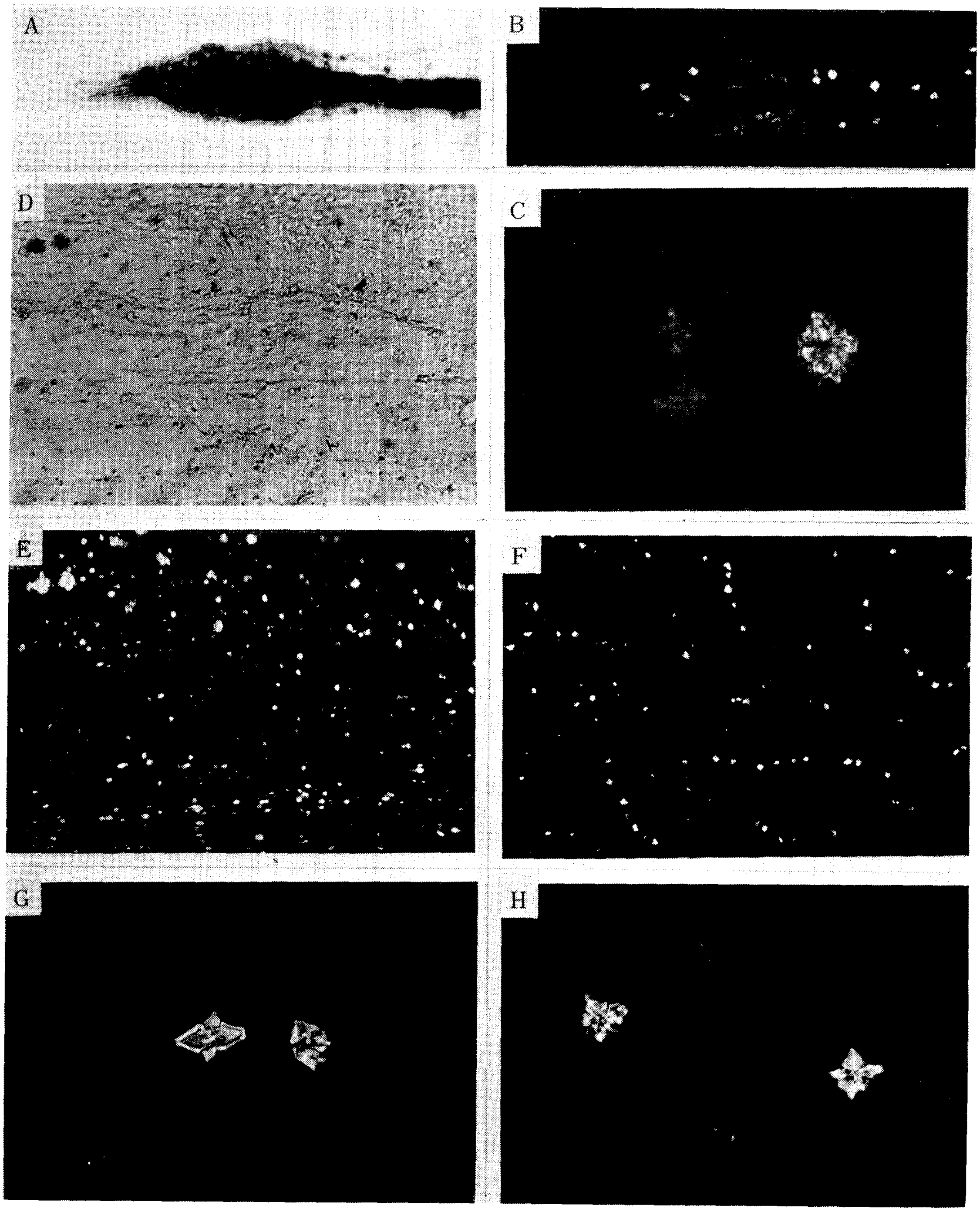

Fig. 3. Low-temperature Ashed Image of the Leaves of Sweet Potato Grown in Darkness

leaf length: about 30 mm
apex; A: ×40 normal light, B: ×40 crossed Nicols, C: ×400 crossed Nicols
costa; D: ×100 normal light, E: ×100 crossed Nicols
branching veins; F: ×40 crossed Nicols, G,H: ×400 crossed Nicols

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were smaller clustered crystals (Fig. 3-D, E), and small (5—10 μ) clustered crystals of irregular form were observed on branching veins (Fig. 3-F, G, H).

C) Growth in Shade

Crystalline pattern in the leaves was not greatly different from that found in the leaves from plant grown in direct sunlight (Fig. 4-A, B).

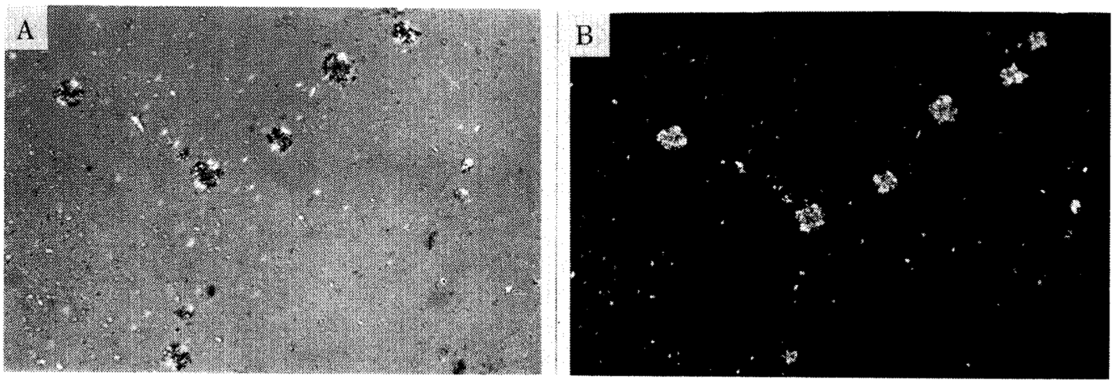

Fig. 4. Low-temperature Ashed Image of the Leaves of Sweet Potato Grown in the Shade

leaf length: about 70 mm
A: ×100 normal light, B: ×100 crossed Nicols

Discussion

There is no doubt that the crystalline inorganic components of a plant reflect the properties of the protoplasmic character of that plant. On the other hand, as in the present series of experiments, it would be natural to assume that there may be a considerable qualitative and quantitative difference in plant components, including the presence and absence of chlorophyll, in the leaves from plants grown under extremely different conditions, such as the presence or absence of light. It may be appropriate, therefore, to assume that such difference in the properties of the protoplasmic characters would be reflected in the inorganic crystal nucleoids and formation of inorganic crystalline nucleus which, in turn, might produce different crystalline pattern of calcium oxalate in these two samples.

However, as was explained in the foregoing section, pattern of calcium oxalate crystals in these two sample leaves was entirely identical. In addition, as was revealed earlier, essentially the morphological character of crystalline inorganic components in a plant does not change according to geographical or seasonal difference during the growth of plants, and this morphological character is undeniably inherited in offsprings. Taking all these into consideration, it is clear that crystalline inorganic components in plants have specific characteristics which cannot be understood from the theory of crystallization in solution alone. Therefore, the author is of the opinion that crystalline inorganic components should be discussed separately as *in vitro* and *in vivo*.

Conclusion

The result of present experiment indicated that morphological character of calcium oxalate crystals formed in the leaves of sweet potato is entirely independent of its photochemical reaction system, and this fact is considered to be applicable, with high probability, to many of other crystalline inorganic components of various shapes present in numerous plants. The present conclusion, considered together with past experimental results, leaves no room for doubt that "the morphological character of crystalline inorganic components present in plants is one of the important characters essentially inherent in a plant and is a specific charac-

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ter reflecting the properties of the protoplasmic character and also indicating a genetic character at the same time." Consequently, the author believes that it would be highly significant to establish a "systematic tree of plants reflecting the morphological characteristic feature of crystalline inorganic components," and that this systematic tree might in future offer the possibility for systematic discovery of plants or plant groups that can be used as pharmaceutics, *etc.*

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