Carpobiological Studies of Crinum asiaticum L. var. japonicum BAK.

By

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With Plates XV & XVI and 42 Text-figures

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INTRODUCTION

Differing from seeds in general, the seed of *Crimum asiaticum* (Baker 1888) has a peculiar character. It is soft and succulent just like a tuber. Notwithstanding that it has no other special floating tissue than the parenchymatous endosperm with an air-containing intercellular space, it is widely distributed on the Pacific coast, and generally accepted to be dispersed by the ocean currents (Hehnsley 1873–1878; Moseley 1875; Goebel 1889; Schimper 1891; Warming and Graebner 1918). The occurrence of it in Cracatau (Docters van Leeuwen 1921) seems to prove this idea. In Japan, a variety of this species named *japonicum*¹⁾ shows a wide distribution on the southern coast.

Various experiments have been made by several authors (C.

¹⁾ Crinum asiaticum, var. japonicum (Baker 1888) is in its general features nearly the same as C. asiaticum, only with a slight difference in the following characters, namely: leaves firmer in texture; pedicels longer; limb a little shorter.

Darwin 1857, Salter 1857, Birger 1907) to discover vitality of the seeds in sea-water. According to these authors, the powers of resistances of the seeds to sea-water differ greatly, even in the varieties of the same species. Some kinds of seeds are killed by a short immersion, but others survive several months.¹⁾

As to the seed of *Crimum* here dealt with, there is also an experimental fact discovered by Guppy (1906), that the seed of *Crimum* (species not described) put into sea-water can float without losing its germinating power for a week or two. But the ability to float for two weeks seems not sufficient for the wide dispersal, and moreover, the behaviour in question was not observed in detail.

The writer examined, therefore, the seed of *Crinum assaticum* var. *japonicum* carpobiologically:²⁾ that is, he endeavoured to learn what structure and development the seed has, how long it can float and retain the power of germination in sea-water and various other media, and what mode of adaptation it possesses against the environmental factors, especially those of the sandy shore.

The materials used for the experiments were collected chiefly at the beach of the Seto Marine Experimental Station, Wakayama Prefecture.

I. MORPHOLOGICAL STUDIES OF THE SEED

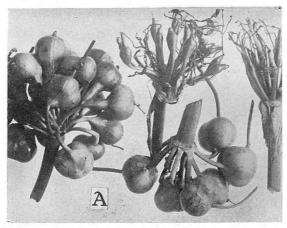
1) Morphology of the Seed

The seed of "Crinum" has a naked ovule without integument (Goebel 1889, Schlimbach 1924), and is bulbiform, its outside being protected by a cork-layer. At first the embryo is in the embryo-sack which is imbedded in the nucellus attached to the placenta as usual, but the endosperm develops then enormously, growing out of the

¹⁾ For example some species of Cruciferae, Cyperaceae, Chenopodiaceae, Compositae, Umbelliferae, Leguminosae and Caprifoliaceae etc.

^{2) &}quot;Carpobiology," a new term introduced by Ulbrich (Biologie der Früchte und Samen, "Karpobiologie." 1928).

nucellus and forming a large fleshy mass, completely surrounding the small cylindrical embryo (Fig. 1). This growth of the endosperm in thickness takes place chiefly on the outside where we find then



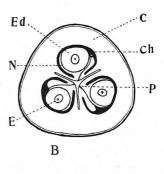


Fig. 1—A, fruits of *Crinum asiaticum* L. var. *japonicum* Bak. ca. 1/5; B, diagrammatic cross-section of the young fruit; C, carpel; Ch, chamber; P, placenta; Ed, endosperm; N, nucellus; E, embryo-sack. ca. 8/3.

chlorenchym-layers (Goebel 1923). The nucellus reduces on the contrary little by little, remains only in the part attached to the placenta, and at last when the endosperm becomes more corpulent, no trace of it, neither nucellus nor microphyle, can be seen. Ultimately a thin protective coating of cork is formed on the surface of the seed (Plate XVI. 6). Thus the ripe seed is simply a mass of the endosperm with the embryo therein, consisting of a soft fleshy mass with an air-containing intercellular space, protected by the cork-layer.

The embryo may be divided into four parts: the sucker, the cotyledonary sheath (Rendle 1901), the base of the plumule (including the short axis and the basal sheath) and the radicle (Fig. 2).

When the seed is allowed to germinate at the edge of a desk, the radicle is pushed outwards and downwards chiefly by the growth of the cotyledonary sheath. Molisch, (1926) saw the radicle always

appearing through the flat side of the seed. The writer observed,

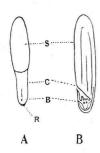


Fig. 2—A, embryo of *Crinum* in surface view. S, sucker; C, cotyledonary sheath; B, base of plumule; R, radicle; B, longitudinal section of the embryo. ca. 2/1.

however, that the cotyled on ary sheath (in cluding the radicle) acts positively towards gravitation from an early stage within the end osperm, and breaking through the endosperm and cork-layer, it makes its way out and grows downwards, so that we can see the radicle emerging from various sides of the seeds, according to their previous position (Fig. 3). The elongation of the cotyledonary sheath carries the radicle downwards, while the upper part of the cotyledon remains in the seed to form a swollen sucker by means of which the nourishment of the endosperm is gradually absorbed. After the elongation of the coty-

ledonary sheath the lateral roots grow out with ca. 2/5 divergence successively upwards. When the seedling is exposed in the air, the

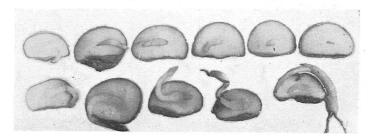


Fig. 3—Showing embryos emerging from various sides of seeds. ca. 1/3.

base of the plumule thickens to assume a bulbous form¹⁾ (Fig. 4, 5), and when the leaf-sheath has been withered by dryness, the leaf-sheath covers tightly the inner part of the seedling, protecting it from withering (Fig. 6; Plate XV. 2).

¹⁾ Strictly speaking, the thickening of the base of the plumule corresponds to a form intermediate between a corm and a bulb, as both the leaf-bases and the stem partake of it.

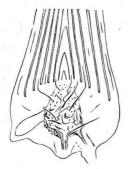


Fig. 4—Longitudinal section of the bulb. ca. 10/3.



Fig. 5—Seedling showing the base of the cotyledonary sheath thickened bulbiformly. ca. 1/1.

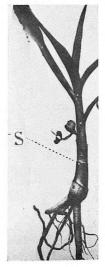


Fig. 6—The leaf-sheath which has been withered and covers tightly the outer part of the seedling. S, separation layer. ca. 1/2.

2) Weight and Size of the Seed

The weight and the diameters of the seed were measured with seeds in the resting stage, on the 2nd of

Dec., 1925. The average weight of 365 seeds was 8.54 ± 0.397 gms., the range being 1 to 24 gms. (Fig. 7). The long and the short

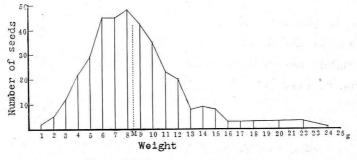


Fig. 7—Weight of the seed. M, mean.

diameters of 339 seeds were 29.73 ± 0.128 mm. and 17.66 ± 0.094 mm. respectively. Before the opening of the capsule, the seeds are found in a compressed condition so that after the opening of the former the dimensions of the seeds may increase 1 mm. or so.

3) Formation of the Cork-layer of the Seed

The cork-layer of the seed is formed at so early a stage that the seed is small (5–10 mm. in long diameter) and still covered with the green carpel. At first the cell division is very rapid at the peripheral portion of the seed (Plate XVI. 3), and then a cambial layer is formed on the inner side of the 6th–7th layer from the periphery (Plate XVI.

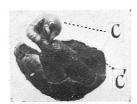


Fig. 8—Seed in which a part of the endosperm and embryo was removed and a callus was formed in a part of the cotyledonary sheath (C), and in the endosperm (C'). ca. 2/3.

4, 5, 6). And the cells outside of the cambium thicken gradually and change to a cork-layer (Plate XVI. 7, 8).

When a part of the endosperm is cut off, and exposed in the air, there is formed also a cork-layer from the wound-curing cambium and the seed is protected from desiccation and infection by microbes (Fig. 8; Plate XV. 5), but the seeds, wounded in a part of the endosperm in sea-water or N/2 NaCl solution, decay

before callusing.

4) Water-content and specific Gravity of the Seeds

a) Water-content of the Seed

The seed is a soft fleshy mass with an air-containing intercellular space. Schlimbach (1924) measured the water-content of the seed of *Crinum* (species not described) and found it to be more than 92%. The author examined it also on the 25th of Dec., 1926, with seeds of var. *japonicum* collected 43 days before.

The seeds were divided into three groups according to size (large, medium and small), and each was chopped up and put into the

weighing glass tubes, and desiccated in the electric drying oven for 40 hours at 98°C. The result of this observation is recorded in the following table.

TABLE I

	Average weight before desiccation (In grams)	Average weight after desiccation (In grams)	Water-content (In %)
	18.370	1.677	90.8
	10.593	1.046	90.1
Average	4.439	0.541	87.8
	11.134	1.088	89.6

The water-content of large seeds is a little greater than that of smaller ones.

Further test was made upon different parts of the seed, namely, the cork-layer, chlorenchym-layer (endosperm with chlorophyll), white-layer (endosperm without chlorophyll), embryo, sucker and cotyledonary sheath. The materials used were in the following conditions: (1) seeds a little unripe, collected in Sept., 1927, and placed in the storeroom for 20 days, (2) seeds well ripe, collected in Oct., 1927, and placed in the store-room for one year (Oct., 1927–Oct., 1928), (4) seedlings stored in the store-room for one year (Oct., 1927–Oct., 1928).

TABLE 2

Part of the seed		Materia	l No.	
	(1)	(2) (Water-con	(3) tent in %)	(4)
Cork-layer	0.01	10.0	0.08	0.008
Chlorenchym-layer	93	94	84	88
White-layer	92	93	82	87
Embryo	87	86	86	
Sucker	_		_	78
Sheath of cotyledon	*****	-		87

From this data it is seen that the cork-layer contains practically no water, and the outer part of the endosperm contains more water or less of the intercellular space than the inner layer, the order being as follows: chlorenchym-layer > white-layer > embryo. The water-content of embryo does not change even if the seed is exposed in dry air for one year.

b) The specific Gravity of the Seed

The specific gravity of the seed was measured by the aid of the hydrostatic balance, and the result is shown in the following table.

TABLE 3

Materials	Specific gravity of the fresh seeds ¹⁾
Seeds stored in damp cellar (temp. 6°-18°C.) from December 1st 1925 to July 5th 1926 (243 days)	0.832
Seeds desiccated in $\rm H_2SO_4$ -desiccator from December 1st 1925 to July 5th 1926 (243 days)	0.823
Seeds floated in N/2 NaCl solution from December 1st 1925 to July 5th 1926 (243 days)	0.795
Seeds floated in well-water from December 1st 1925 to July 5th 1926 (243 days)	0.932
Seedling floated in sea-water from December 1st 1925 to July 5th 1926 (243 days)	0.740
Seeds immersed in sea-water from March 25th 1926 to July 5th 1926 (100 days)	0.805

From this table it may be seen that (τ) the difference between the specific gravity of the seeds stored in the damp cellar and that of those in the H_2SO_4 -desiccator is only 0.009, (2) the seed floated in well-water for 243 days shows the greatest specific gravity, and (3) the decrease of the specific gravity in sea-water is remarkable, suggesting that it may be convenient for the dispersal by the aid of the ocean currents.

¹⁾ Though the specific gravity of the fresh seeds has not yet been measured, all of them float very well without exception.

II. CHEMICAL STUDIES OF THE SEED

1) Microchemical Investigations of the Seed

Starch grains are not detected at all in the endosperm even at the dormant stage of the seed, as was reported by Schlimbach (1924). Even in the chlorenchym-layer, which lies at the inner side of the cork-layer, the assimilation starch is not detected in the chloroplast, but in the embryo, especially in the sucker of the cotyledon there

are many large reserve starch grains (Fig. 9). It is very interesting that starch is not reserved in the endosperm but only in the embryo (Fig. 10).

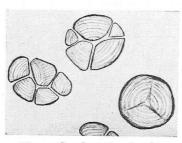


Fig. 9—Starch grains in the embryo. ca. 850/1.

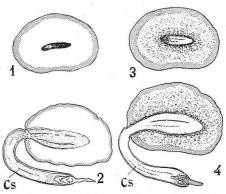


Fig. 10-—Localization of the starch grains and reducing sugar in the seed and seedling. 1-2, starch grains (after Iodinepotassium iodide); 3-4, reducing sugar (after α-naphthole and MAYER's method); Cs, cotyledonary sheath. ca. 2/3.

Before germination the starch grains distribute themselves uniformly in the tissues of the embryo except in the vascular system, the radicle and the plumule. After germination they become smaller and crowd together around the vessels, and may be detected as long as the plumule is living, but soon after the plumule gets a supply of water, the starch grains can no longer be detected in the sucker.

Reducing sugar was detected everywhere in the cells of the endosperm and embryo (Fig. 10), even after the seedling ceased to grow any more in the seed, but when the seed in this condition got a supply of water, sugar disappeared in a week or so from the cells of the seed except in the vessels in the sucker.

Other substances were examined also with materials in the dormant and germinated condition, and the results are shown in the following table.

TABLE 4

	Seed in the resting stage				Seedling			
Substances	Endo	sperm	Embryo		Endosperm		Embryo	
:	Green layer	White layer	Sucker	Radicle	Green layer	White layer	Sucker	Radicle
Starch	+	0	+++++	+++	o	o	++++	++
Reducing sugar	+++	+++++	++	+++	++	+++++	++	++
Dextrin ¹⁾	+++++	+++++	+++++	+++	++	+	++	+
Albuminous subst.	+	+	+++	++	+	++	++	++
Amino acids	+	+	++	++	+	++	++	++
Peroxydase ²⁾	++	+++	++++	++	+	+	++	0

It may be mentioned further, that the seed contains no nitrate or nitrite, instead of which amino acids are detected.

2) Catalase Activity of the Seed

For the detection of catalase activity, a modified form of Overholser's (1928) modification of Heincke's (1927) apparatus was used. The samples usually weighing about one gram were divided into three parts: the chlorenchym-layer, the white-layer and the embryo. The activity was measured by the time required to liberate from 0 to 30 cubic centimeters of oxygen at 20°C.

Description of the sap gave a reddish brown colour with iodine. The extract of the sap with water and alcohol of various concentrations, after precipitation of the reducing sugar by Benedict's reagent, and hydrolysis by hydrochloric acid, is also positive to Benedict's reaction, which indicates therefore that the sap contains dextrin. As the extracts with 55% and 70% alcohol show a greater amount of precipitation, it may be concluded that crythro- and achroodextrin form the chief constituents of the dextrin.

²⁾ For the detection of peroxydase, guaiac-alcohol, α -naphthole and benzidine were used, and the benzidine reaction was the most conspicuous.

Table 5

Catalase activity of the seed of Crinum

	Average time required to liberate from 0 to 30 cubic centimeters of oxygen at 20°C.				
Material	Endos	Embryo			
	Chlorenchym-layer	. White-layer			
New seed	13'10"	26'27"	2'57"		
Old seed (Stored one year) and germinated)	11' 5"	1 ^h 25"	2'20"		
Old seed . (Stored one year and) (not yet germinated)	17'10"	many hours	4′20″		

As shown in this table, the catalase activity of the embryo is much higher than that of the endosperm, and that of the chlorenchymlayer is greater than that of the white-layer.

As the seed has the chlorenchym-layer^D in its outer portion just beneath the cork-layer, it seems probable that assimilation may occur, but even after a long exposure in the sunlight starch is not formed, though it is not clear whether the chloroplast in question has a tendency to form starch or not.

3) Respiration Rate of the Seed

When the seeds of *Crimum* are covered by vaseline on their surface, or they are laid in boiled airless water covered with liquid paraffin, they do not germinate even after a long time. The writer examined therefore the respiration rate of the seed.

To determine this a modification of Nishi's (1924) method was used; a current of carbon dioxide-free air was drawn through a vessel containing the seed and then through an absorption tube containing N/20 NaOH solution, and the remaining alkali was titrated with N/20

D The chlorenchym-layer is formed in the seed within the capsule long before it is ripe (see p. 186).

H₂SO₄ solution. For the indicator thymol phthalein was used. As the materials, both germinated and not yet germinated seeds were used, twenty in each set at 20°C. The results obtained are given in the following table.

Table 6
Respiration rate of the seed^D

Material	Fresh weight	Dry weight	CO ₂ per hour per gm. of original dry weight			
Materiai	(in gms.)	(in gms.)	In the dark (in mgms.)	In the light ²⁾ in mgms.)		
Seeds, already germinated	6.94	1,22	0.18	0.40		
Seeds, not yet germinated	9.78	1.35	0.10	0.15		

III. GERMINATION AND BUOYANCY OF THE SEED IN VARIOUS CONDITIONS

It is very interesting to know how long the seeds can float in a living condition in sea-water, fresh water and some artifical media such as Brenner's-, N/2 NaCl-, N/2 NaCl-+0.3% CaCl₂ solutions³. For this purpose, every 50 seeds were put into a wide glass vessel of

³⁾ Depression of the freezing-point and pH-value of the media determined by the colorimetric method of CLARK and LUBUS (1923) were as follows:

Media	Depression of the freezing point	pH-value
Sea-water	2.000	8.3
Well-water	0.015	7.I
N/2 NaCl soln.		7.2
3.57% NaCl soln.	2.060	
Cell-sap of endosperm	0.560	
N/2 NaCl soln.+0.3% CaCl ₂ soln.	1.390	7.3
Brenner's soln.	1.360	8.3

The pH-value however so far as their range is concerned, seem to have no significance with regard to germination, other than the chemical composition itself.

D The respiration rate of the seed is comparatively marked, but in the field the rate will be decreased by the winter cold, sea-water or burying.

²⁾ Diffused light+Nitra-lamp of 220 V. 120 W. at 0.5 M. distance. Why the respiration rate in the light is greater than in the dark, is not yet understood.

10 l. capacity, each filled with 5 l. of various liquids, and covered with glass-plates to prevent change in the concentration of the media. Each medium was replaced every two weeks during the first two months, by a fresh supply of the same one to prevent the propagation of microbes. But thereafter the media, often filtered by cotton, were not renewed.

Other groups of the seeds were stored in the CaCl₂-, H₂SO₄-desiccator, store-room, damp cellar and a water-saturated atmosphere, and the mode of germination was observed. The results of these examinations are given in the following paragraphs.

1) Germination in Sea-water

The seeds put into the vessel with the sea-water¹⁾ were all floating and germinated during 73 days, developing a few leaves. In sea-water, however, the leaves become thick with short blades curved in dorsoconvex mode and the roots could not grow more than two mm. When the leaves of the plumule begin to be injured from the tip downwards by sea-water, there is formed a separation layer in the upper part of leaf-sheaths and prevents the extension of the injury



Fig. 11—Seeds germinated in sea-water (after 200 days). ca. 1/2.

further into the sheathbase. Thus a number of leaf-sheaths come to fold round the young part of the seedling and the latter floats safely in the sea-water more

¹⁾ The sea-water used during the experiment, had been collected at the Akashi Straits in the Seto-inland-sea, on the 28th of November, 1925, and filtered twice through a cotton plug before use. The specific gravity of the sea-water was found to be 1.025 (at 15°C), and the depression of the freezing-point, 2.000 (3.12 atm. p.) at 15°C.

According to Yendo, the composition of the sea-water at the Bay of Shin-Wakanoura, near the Seto-inland-sea, is as follows. NaCl 3.20–2.12 gms., KCl 0.13–0.08 gms., MgCl₂ 0.33–0.38 gms., CaSO₄ 0.04–0.07 gms, MgSO₄ 0.23–0.13 gms., specific gravity 1.025 (15°C), Temperature 10°–27.°5C.

than two years without being injured further inside, and with fresh young leaves within (Fig. 11). When the seedlings in such a condition are removed into fresh water or sand, they are able to grow further, putting forth again new roots and leaves (Fig. 12).

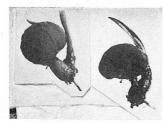


Fig. 12—Seedlings, which, after 200 days floating in sea-water, were transferred into fresh water and grew anew. ca. 1/3.

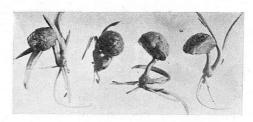


Fig. 13—Seedlings germinated in sea-water diluted to double volume (after 200 days). ca. 1/4.

When the sea-water is diluted to double its volume with distilled water, the roots grow anew and the leaves show a fresh appearance, so that the degree of injury seems much slighter (Fig. 13). The

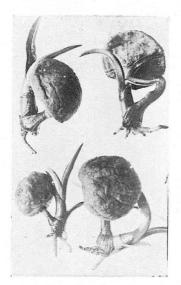


Fig. 14—Seeds germinated in Brenner's solution (after 200 days). ca. 1/2.

weight of seeds floating in sea-water increases during the first one week but thereafter it decreases very gradually (see Fig. 19).

2) Germination in Brenner's Solution

The seeds in Brenner's solution¹⁾, were all floating as long as they were living, and during 64 days 100% of them germinated. The roots attained the length of ten to fifteen mm., but then their tips all became a little brown, and ceased to grow further. The leaves were a little thinner and longer than in the sea-water, indicating that the seed-

¹⁾ Brenner's solution: NaCl 1.82 gm., KCl 0.06 gm., MgCl₂+6aq. 0.47 gm., MgSO₄+7aq. 0.28 gm., CaCl₂+6aq. 0.16 gm., water 100 gm.

lings are less injured in Brenner's solution. The change of weight of the seeds in this solution was almost the same as in the case of sea-water as shown in Fig. 14.

3) Germination in N/2 NaCl Solution

The seeds in N/2 NaCl solution were all floating as long as they were living, and 100% of them germinated during 79 days. But when the cotyledonary sheath grew to seven mm. or so, the

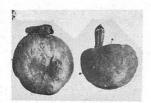


Fig. 15—Germination of seed in N/2 NaCl solution (floated 20 days). ca. 1/2.

base of the plumule began to be somewhat transparent, and about a fortnight after became black and decayed by degrees (Fig. 15). But then a callusforming cork-cambium, arose in the cotyledonary sheath, and

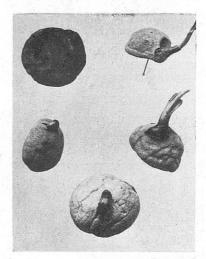
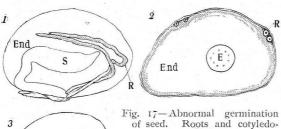


Fig. 16—Seedlings germinated abnormally in N/2 NaCl solution. ca. 1/2.



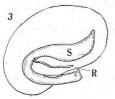


Fig. 17—Abnormal germination of seed. Roots and cotyledonary sheath growing in the endosperm or under the corklayer of the seed. I and 3, diagrammatic longitudinal section; 2, cross-section; S, sucker; End, endosperm; R, root. ca. 3/2.

prevented decay. (Plate XV. 4). In this condition the endosperm and sucker remained still alive more than one year, but the plumule does not regenerate under whatever conditions they may be removed. The weights of the seeds

increased during the first week by about 5%, then kept nearly constant until they germinated, and after that decreased gradually (see Fig. 19).

There were, however, a few seeds which germinated abnormally, namely, only exposing the leaf from the seed, whereas the root and cotyledonary sheath grew in the endosperm or under the corklayer of the seed. In such a condition they were able to exist more than 200 days after germination (Fig. 16 and 17).

4) Germination in a Solution of N/2 NaCl+0.3% CaCl2

The seeds in the N/2 NaCl+0.3% CaCl₂ solution were all floating as long as they were living, and 100% of them germinated during 75 days (Fig. 18). After germination, their roots hardly grow,

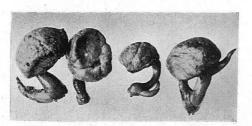


Fig. 18—Germination of the seed in N/2 NaCl+0.3% CaCl2 solution (after 200 days). ca. I/2.

and the cork-layer is formed on their outside.

The roots are not injured as in the N/2 NaCl solution, the cotyledonary sheaths exist long and a few succulent leaves with short blades like those produced in the sea-water,

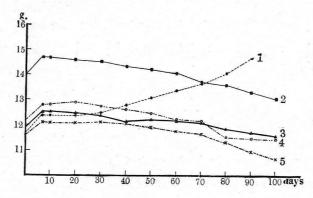


Fig. 19—Change of average weight of the normal seeds in various media. I, well-water; 2, sea-water; 3, Brenner's solution; 4, N/2 NaCl+0.3% CaCl2 solution, 5, N/2 NaCl solution.

were put forth, and they were able to exist more than two years in this condition without growing. The change of weight of the seeds in this solution was quite the same as in the N/2 NaCl solution (Fig. 19).

5) Germination in Well-water

In well-water the seeds remain floating even after they have grown to seedlings. The seeds all germinated during 49 days after they were put in the vessel and they grew vigorously with normal roots and leaves (Fig. 20). The weight of the seeds increased during

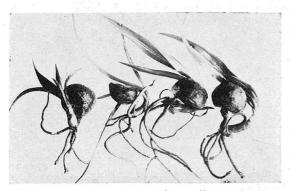


Fig. 20—Seedlings germinated in well-water (after 200 days). ca. 1/4.

the first one week, and even thereafter the weight of these seeds increased continually.

As shown in these paragraphs the weight of all the seeds immersed in liquid media all increases about 5% over that of the fresh

seeds during the first week. This is probably due to the penetration of the solvent into the cork-layers. After that time the change of the weight during 30 days is very little in all the media. This indicates that the time necessary for the media to penetrate into the endosperm through the cork-layer is comparatively long. Thereafter the seeds show a different mode of change in weight according to the medium. The seeds in sea-water, N/2 NaCl solution, Brenner's solution and N/2 NaCl solution +0.3% CaCl₂ solution gradually decrease in weight, while those in the well-water increase on the contrary, indicating that the growth is going on normally.

6) Germination of Seeds laid on Sand and buried in Sand respectively

The seeds were divided into two groupes, those of the first group were laid on washed sand and the others were buried in sand at a depth of two cms. All these seeds germinated at the same time, and the seedlings of the latter group developed very well, while those of the former grew slowly after germination. This is attributed to the fact that neither the sheath of the cotyledon nor the roots can penet-

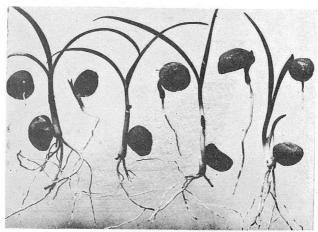


Fig. 21—Seedlings germinated on sand (upper row), and in sand (lower row). They were sowed at the same time but the growth of the former was delayed because their radicles took much time to penetrate into the sand. ca. 1/4.

rate into the sand easily (Fig. 21). It is conceivable therefore that it is hard for seeds brought to a dune or sandy place to grow after germination unless they are buried in the sand by wind or other agencies. But if they are covered to a depth of over ten times their diameters, they lose the ability to expose the plumules on the sand.

7) Germination of Seeds in a Watersaturated Atmosphere

The seeds, which were suspended with twine in a glass cylinder wrapped inside with blotting paper wetted with a constant supply of

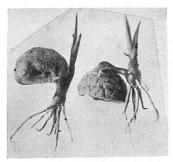


Fig. 22—Germination of the seed in the saturated atmosphere. ca. 1/3.

water and covered up with a glass plate, germinated in the usual mode, but after the roots attained a length of five cms., their tips were seen gradually withering. (Fig. 22).

But if the seedlings are cultivated in distilled water or Knop's solution, their roots grew rapidly again, especially markedly in the distilled water (Fig. 23).

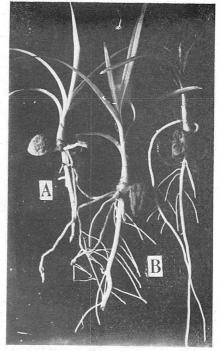


Fig. 23—Seedlings cultivated in water. A...seedling cultivated in Knor's soln., B...seedlings cultivated in distilled water. ca. 1/4.

8) Germination in the Desiccator

97% of the seeds which were stored in the H₂SO₄-desiccator in the greenhouse lived for more than one year, and about 40% of them germinated in this desiccator during 275 days. Even the seeds which were in the non-germinated condition in the desiccator during two years, were still living and when they were removed into freshwater they were able to germinate. The leaves in the desiccator are succulent with short blades rolled inwards and the sheath of the cotyledon is short and protected by a thick epidermis. (Fig. 24, 25). Roots come out but they can not grow more than one or two mm., and their surface is covered with a thick cork-layer just

like a velamen.

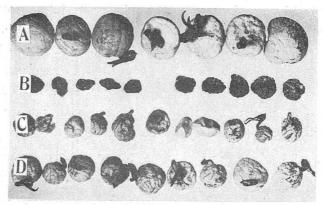


Fig. 24—Germination of the seed in the desiccator. A), large-sized seed; B), left half without cork-layer and cork-cambium; right half without cork-layer. C), small-sized seed; D), middle-sized seed. ca. 1/4.

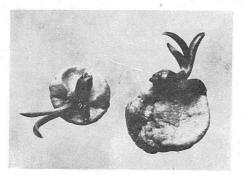


Fig. 25—Germination of the seed in the desiccator. ca. 2/3.

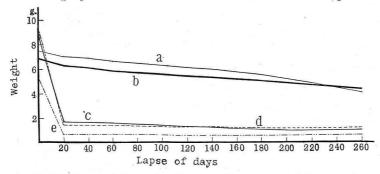


Fig. 26—Weight change of the seeds with or without cork-layer under various conditions. α, H₂SO₄-desiccator; δ, store-room; c, CaCl₂-desiccator (without cork-layer); d, H₂SO₄-desiccator (without cork-layer); e, CaCl₂-desiccator (without cork-layer and cork-cambium).

The seeds in the desiccator decrease in weight very gradually, as shown in Fig. 26, but if the cork-layer is removed, the decrease of weight is very rapid, so that the protective action of the cork-layer is well shown.

9) Germination in the Store-room

The seeds laid in the store-room, decreased in weight very gradually just like the seeds placed in the $\rm H_2SO_4$ -desiccator (Fig. 27).



Fig. 27—Delayed germination of seed in the store-room. ca. 1/2.

From this fact, we know that the cork-layer works very effectively for the retention of water. The germination of seeds both in the H₂SO₄-desiccator and the store-room was delayed, probably by the want of water, much more than that of the ones in sea-water,

well-water, 3.57% NaCl solution and saturated atmosphere, even though they were placed in the same green house (Fig. 28 and Supplementary

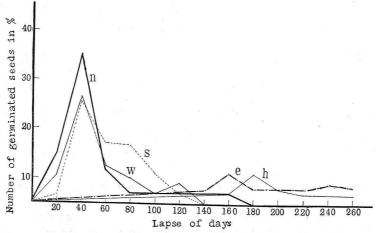


Fig. 28—Number of germinated seeds in various media and conditions. n, 3.75% NaCl soln.; s, sea-water; w, well-water; e, exposed in air; h, H₂SO₄-desiccator.

table 3). The percentage of seeds that germinated during one year in the store-room was about 45%, but the others in the non-germinated condition were still all alive, though they had decreased more in weight than the germinated ones.

10) Rôle played by the Cork-layer in the Durability of the Seeds

a) Seeds deprived of their Cork-layer in various Media

When seeds deprived of their cork-layer are immersed in liquid media, an increase of weight occurs in well-water, while in sea-water

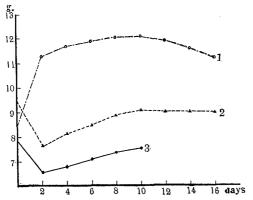


Fig. 29—Weight-changes of the seeds without cork-layer in three media. I, well-water; 2, sea-water; 3, N/2 NaCl solution.

or NaCl-solution the weight decreases markedly during the first two days, then increases gradually (Fig. 29). But in all these cases the seeds decay sooner or later, probably owing to the exosmosis of nutrient substances and to infection by microbes (Supplementary table 1). From this fact we know too, that the

cork-layer serves as a good protective covering for the seed in the liquid media against exosmosis and infection.

That the seeds in the sea-water and NaCl solution at first markedly decrease in weight and then increase gradually, may be attributed to the fact that the liquid substances of the seeds ooze out osmotically into the media and then seeds begin to decay.

b) The Relation between the Germination-power and Water-content of the Seeds

The seeds were stored during two years in the H₂SO₄-desiccator and store-room to observe the relation between their germination-power

and water-content. After two years most of the seeds germinated, and even those which germinated in the desiccator could live for a long time, and their weight decreased relatively little. The non-germinated seeds also germinated after removal into fresh water and there were only a few which desiccated to death.

Conditions of seeds and seedlings	Total number (In %) Weight of the seed before ex (In grams)		Weight of the seed after exp. (In grams)	Average decrease of the weight (In %)
Germinated and still alive	28	3.02	1.51	49
Germinated but withered afterwards	32	2.69	0.90	66
Non-germinated but still alive	34	3.52	1.88	46
Non-germinated and died	6	1.34	0.48	64

TABLE 8

The results in the store-room

Conditions of seeds and seedlings.	Total number (In %)	good before over		Average decrease of the weight (In %)
Germinated and still alive	26	4.28	2.31	46
Germinated but withered afterwards	19	3.01	0.97	71
Non-germinated but still alive	40	4.43	3.17	28
Non-germinated and died	15	2.01	1.02	49

As shown in the above tables, the average decreases in the weight of the seeds in the H₂SO₄-desiccator are not very different from those in the store-room, and the number of the seeds that did not germinate or died in the former is less than that in the latter.

But when the seeds, deprived of the cork-layer, are laid in the $\rm H_2SO_4$ -desiccators, the water-content decreases rapidly. They decrease during 20 days to 85% of the fresh weight, while the intact ones lose only 44% during 266 days in the same desiccator (Eig. 26). The efficiency of the cork-layer against the loss of water is therefore clearly shown. The results of the experiment are shown in the following table.

Table 9

Decrease in the average weight of the seeds, without cork-layre in the desiccator

(In grams)

Lapse of days Desiccation	o	I	3	17	20	117	230
In CaCl ₂ -desiceator	9.29	8.60	7.48	2.06	1,63	1.16	1.15
In H ₂ SO ₄ -desiccator	9.52	7.87	6,69	2.03	1.56	1.27	1,26

Table 10

Decrease in the average weight of the normal seeds in the desiccator and the store-room

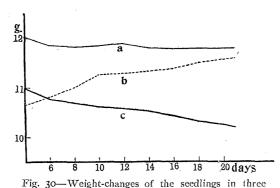
(In grams)

Lapse of days Condition	O	3	5	6	12	18	24	43	152	203	266
In store-room	6.84	6.55	6.52	6.51	6.45	6.37	6.30	6.09	5.12	4.74	4.15
In H ₂ SO ₄ –desiccator	7.51	7.3 9	7.34	7.31	7.21	7.12	7.04	6.50	5.79	5.07	4.14

IV. SEEDLINGS IN VARIOUS CONDITIONS

1) Seedlings in various Media

The influence of various liquid media on the seedling is nearly the same as that on the seed itself (Fig. 30). In well-water, the roots and leaves grow very rapidly, the seedlings gradually increase in weight. But in sea-water they at first decrease in weight, then



media. a, sea-water; b, well-water; c, N/2 NaCl solution.

remain constant in weight, floating comparatively free from harm more than one year, and their roots or leaves can not grow as in the case of well-water. As soon as the roots appear they will be covered by the thick epidermis and can not grow more. The leaves,

too, become thick and grow very slowly.

In the N/2 NaCl solution the weight of the seedlings gradually decreases, and their radicles are injured (Fig. 30, 31, and Supple-

mentary Table 4). The injury never penetrates however into the sucker which is buried in the endosperms, forming a callus in the cotyledonary sheath.

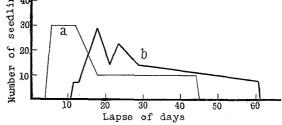


Fig. 3t—Number of the seedlings injured in N/2 NaCl solution. a, slightly injured; b, completely injured.

Another experiment was carried out in con-

nection with plumules planted in pots, and soaked with well-water, N/2 NaCl solution or sea-water twice a day. The plumules soaked with well-water grew very well, but the ones soaked with N/2 NaCl solution were completely injured after 20 days, and the ones soaked with sea-water were injured in their root-tips and their leaves become brown gradually (Fig. 32). This experiment elucidates clearly the fact that Crinum grows usually on the sandy soil at some distance from the beach.

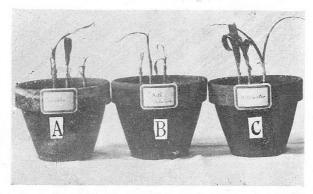


Fig. 32—Plumules soaked with sea-water (A): N/2 NaCl solution (B); well-water (C). ca. 1/6.

2) Scedlings in a damp Cellar

The seedlings were laid in a damp cellar for two years to observe how much they were able to grow with only their own nutriment, preserved in their seeds. During 18 months they grew about 8 cms. in length, and had three or four leaves and three or five very short roots. From that time they became weak and stopped growing, as if they lacked nutriment (Fig. 33).

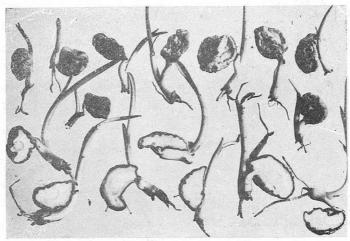


Fig. 33 - Germination of seed in damp cellar. ca. 1/3.

The small-sized seeds became weak rather earlier than the largesized ones. Many creases appeared on the surface of the seeds, as if their preserved nutriment were all absorbed by their plumules. Then the seeds were examined microchemically, and quite unexpectedly, it was found there were still great quantities of sugar and starch. When the seedlings in such a condition were put into water, the residua of sugar and starch disappeared in a few days and the seedlings began to grow again. From this fact it is clear that a supply of water is indispensable to enable the seedling to make adequate use of its nutriment at the time of germination.

V. SOME CHARACTERISTICS OF THE ROOT AND PEDUNCLE

1) Passage Cell and Velamen-like corky Layer

The root of *Crinum* has no root hair, but in place of it there is a passage cell which is known to fulfil the same function as the root

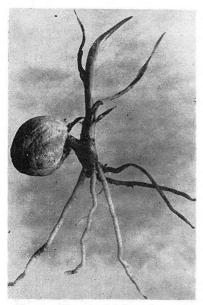


Fig. 34—Plumule dried on dry sand, but after removal into humid sand it produced new roots and leaves. ca, 1/2.

hair (Plate XV. 3). In addition to this, when the plumule is laid on sand or removed into dry sand, there is formed on the surface of the roots, a corky layer which resembles in appearance the velamen of Orchidaceae, though with some histochemical difference (Fig. 34). The corky layer of the root of Crinum is coloured vellow with potassium hydroxide solution, and red with Sudan III while that of the orchid (Dendrobium) is not dyed with them. To find out the efficiency of this corky layer against the loss of water, a root with the corky layer was compared with a usual one, and it was found

that the corky layer is very effective against desiccation, as shown

in the following table (Fig. 35).

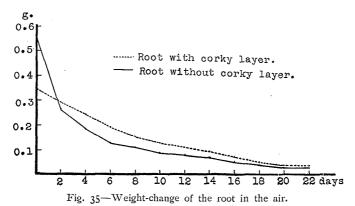


TABLE ΙI Weight-change of roots with and without the corky layer in the air (In grams)

Lapse of days Material	0	2	4	6	8	10	12	14	16	18
The root with corky layer	0.35	0.29	0.24	0.19	0.15	0.13	0.11	0.09	0.07	0,06
The usual root	0.54	0,26	81.0	0.13	0.10	0.09		0.06	0.05	0,04

2) Contractility of the Root

It has been already mentioned that the cotyledonary sheath and the root of Crinum can hardly enter into sand by their own power, unless they are buried passively in the sand by wind or other agencies. The fact that the oriental Crinum is distributed only on sandy shores, may be explained by this.

As an instructive case a Crimum consociation on a sand dune at Tôji, Izu Peninsula may be mentioned. The sand dune is situated alongside of a loam hill, and though the conditions along their boundary are the same except the soil texture, Crinum stocks are found only on the sandy soil even close to the loam (Fig. 36).

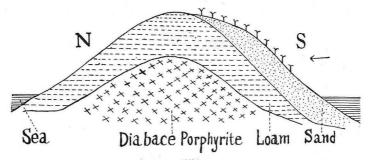


Fig. 36—Localization of *Crinum* on a sand dune at Tôji, Izu Peninsula. No stock is found on the loam.

This may be attributed to the fact that the loam is not only too hard for the penetration of the root but also that there is almost no chance of the seed's being buried by external agencies.

After entering into the sand, the root shows transversal wrinklings on its outer cortex, whereby shortening follows (Fig. 37 Plate XV. 3).

With this shortening the plant is drawn into the sand

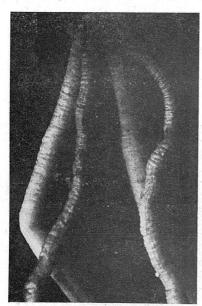


Fig. 37—Contractile root of *Crinum*. ca. 3/1.

more or less, and the exposure of its root on the sand by rain or wind is avoided, just as in the case of

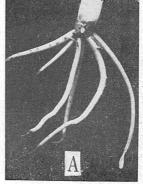




Fig. 38—Pneumathode of the root. A, 1/1; B, 5/1.

Phaedranassa chloracra, Herb. (Amaryllidaceae), which was studied by Rimbach (1898) in the tropical Andes.

3) Pneumathode

When the plumule of *Crinum* is cultivated in water, pneumathodes are formed on the surface of their roots along two vertical lines, and as a rule the lateral roots are produced through these pneumathodes

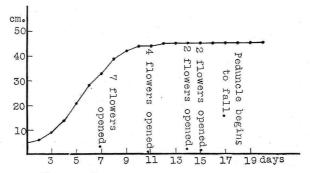


Fig. 39- The mode of growth of the peduncle.



Fig. 40—Peduncle which has fallen on the ground.

(Fig. 38, Plate XV 1).

4) The Growth of the Peduncle

The growth of the peduncle is basipetal and at first very rapid but after flowering it becomes gradually slow, and ceases in a few days (Fig. 39). Three or five days after the blooming of the last flower the peduncle begins to fall on the ground, and the capsule and seeds ripen in this condition (Fig. 40). The fall of the peduncle makes it just like a stolon and is very convenient for propagation of the seeds around the stock at some distance from it.

VI. CONSIDERATION ON THE DISTRIBUTION OF CRINUM ASIATICUM

It is an interesting fact that the genus *Crimum* includes both inland and littoral species, and the former seems, as in the case of Amaryllidaceae in general, to be the older habitat of the genus. If the distribution of *Crimum* throughout the world is considered, the following data are to be seen from the works by Baker (1877, 1888), Bentham (1861), Boldingh (1909). Castillo (1886), Botanical nomenclature (China) (1917), Cooke (1908), Dunn and Tutcher (1912), Durand (1901), Engler-Gilg (1920), Hayata (1906), Hillebrand (1888), Index Kewensis (1893–1915), Koorders (1911, 1923), Lorentz (1909), Makino and Nemoto (1925), Martius (1871), Merrill (1912, 1917), Miquel (1862), Ridley (1907), Robert (1853), Schumann (1901–1905), Small (1913), Thiselton-Dyer (1818). Wight (1853) etc.

The total number of enumerated species is 161, 16 among them being inland plants, 4 marsh or stream-side, and the others all littoral ones, distributed from the tropical to the temperate regions (see supplementary table 2).

As shown in the table, more than a half of these species, both inland and littoral, occur in Africa, like the majority of other closely related genera. It is highly probable, therefore, that the birthplace of *Crinum* was the inland parts of tropical Africa, and that from there it has been distributed to other regions.

Whether all species of *Crinum* have the seeds with bulbiform endosperm containing much water and air, and whether they are protected by the thick cork-layer, are not yet known, but these characters seem to be very useful in regions with a dry season. As to the root, so far as *Crinum asiaticum* is concerned, on account of the light weight of the seed and the short cotyledonary sheath, it is not able to penetrate into hard soil at the germination. Even on the

sandy shore it is difficult for it to settle without the aid of the wind, by which the seed is buried in the sand. Most of the littoral species, inasmuch as their seed is light, seem also to prefer sandy soil, such as the dry beds of rivers or the seashore. There remains, then, only the durability in sea-water, by which the seed could be distributed across the sea.

From these features just mentioned, it may be considered, that the ancestor of *Crimum* was at first an inland plant¹⁾, probably growing on sandy soil. Various species derived from it have been carried to the marshes at stream-sides and to littoral regions suited to its gradual growth and further on, by the aid of ocean currents they have been distributed as littoral plants widely throughout the world (Fig. 41).

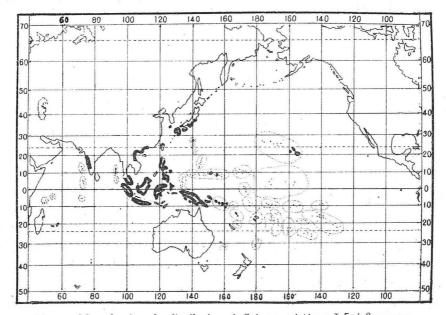


Fig. 41-Map showing the distribution of Crinum asiaticum L. 1/180.000.000

¹⁾ C. longifolium Thune. and C. giganteum Andr., inland plants, are distributed in very distant places: the former in India, Africa and Jamaica., and the latter in Africa and the Hawaiian Is. It is not clear whether this fact is attributed to transplantation, different species, Ocean currents or partly to Wegner's "Verschiebungstheorie."

In Japan, *Crimum asiaticum* L. var. *japonicum* Bak. spreads on sandy shores swept directly or indirectly by the warm Pacific Black Current from Okinawa to Hata Island off the Bôsô Peninsula (about lat. 35°21′ N. and long. 140°8′ E.), while it is never found on the coast facing the Japan Sea, except in the southern parts of Nagato (Fig. 42). Also it has not yet been found in the peninsula of Corea.

In Formosa there is no *C. asiaticum* L. var. *japonicum* Baker. but only *C. asiaticum* L. var. *sunicum* Roxb. which spreads widely in China. This fact is considered to be due to the fact, that in Formosa most of the eastern coast consists of precipitous cliffs, and there is no place suitable for the distribution of this plant.

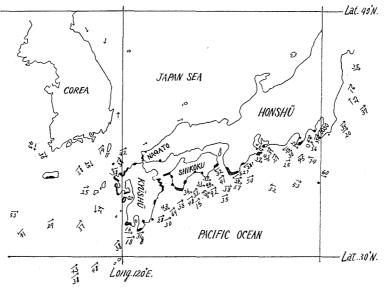


Fig. 42—Map showing the localization of *Crimum asiaticum* L. var. *japonicum* Bak. in Japan. 1/20.000.000.

·Place of occurrence.

.....Directions and speeds of current (in knots per day).

From all these evidences it is fairly certain that *Crinum* in Japan has been brought in recent geological times by the ocean current, probably after the cold period, if such a period really existed in the Pleistocene of Japan. The migration of

this plant may take place in future more widely to sandy shores having suitable factors both edaphic and climatic.

VII. SUMMARY

A. Seed Characters

- 1. The seed of *Crimum* is simply a mass composed of the endosperm with the embryo therein, consisting of a soft fleshy mass with an air-containing intercellular space, protected by the cork-layer. The cork-layer is formed at an early stage still covered with the green carpel.
- 2. The average weight of the seeds is 8.54 ± 0.397 gms., the range being 1 to 24 gms. The long and short diameters are 29.73 ±0.128 mm. and 17.66 ± 0.094 mm. respectively.
- 3. The cotyledonary sheath (including the radicle) acts positively towards gravitation, from an early stage within the endosperm.
- 4. The outer part of the endosperm contains more water or less intercellular space than the inner, and the water-content of the embryo does not change even if the seed is exposed in the dry air for a year.
- 5. The specific gravity of the seed is always less than one, in whatever medium the seed may be immersed. And the specific gravity of seeds stored in a damp cellar and that of those in an H₂SO₄-desiccator show no remarkable difference.
- 6. 97% of the seeds stored in the H_2SO_4 -desiccator lived more than one year, their weight decreasing very slowly.
- 7. The germination of the seeds in desiccator or store-room is delayed very much more than that of those in liquid media.

B. Chemical Features

- 8. The endosperm contains as its main carbohydrate source dextrin, and starch is found only in the embryo.
 - 9. Reducing sugar was detected everywhere in the cells of the

endosperm and embryo, even after the seedling ceased to grow in air, but when the seedling in this condition got a supply of water, sugar disappeared in a few days from the cells of the seed, except in the vascular bundles of the sucker of the cotyledon.

- 10. The catalase activity of the embryo is much higher than that of the endosperm, and that of the chlorenchym-layer is greater than that of the white-layer.
- 11. Even after a long exposure in the sunlight starch is not formed in the chlorenchym-layer of the seed.
- 12. The respiration rate of the seed is comparatively remarkable especially when the seed is in the light.

C. Buoyancy and Viability of the Seeds in various Media

- 13. The seeds in sea-water, Brenner's solution and N/2 NaCl +0.3% CaCl₂ solution all floated and germinated, and when the seedlings under such conditions were removed into fresh water or sea-water diluted to double its volume, they were able to grow further even after more than two years. The seeds in N/2 NaCl solution all germinated, but as soon as the cotyledonary sheath grew, the base of the plumule was injured.
- 14. The order of the injurious actions of the media on the seedlings was 1/2 diluted sea-water < Brenner's solution < sea-water < N/2 NaCl soln. + 0.3% CaCl₂ soln. < N/2 NaCl solution.
- 15. The seeds immersed in the liquid media all become heavier than the fresh seeds, during the first one week, but after that time the change in the weight during 30 days is very little in all the media. Thereafter the seeds show differences in the change of weight according to the media.

D. Rôle played by the Cork-layer in the Durability of the Seeds

16. The cork-layer serves not only as a protection against the

loss of water in the air, but also acts as a good protective covering against the exosmosis and infection in the water. When the seeds deprived of the cork-layer are immersed in liquid media, they decay sooner or later.

E. Some Characteristics of the Scedlings

- 17. When the seedling is exposed in the air without substratum, the basal portion of the plumule thickens to assume a bulbous form, and the withered leaf-sheaths act as a protection for the seedling against withering.
- 18. The seeds brought to a dune or sandy place can hardly grow after germination unless they are buried in the sand by other agencies. But if they are covered to a depth of ten times their diameter, they lose the ability to expose the plumules on the sand.
- 19. The root of the seedling grows markedly in distilled water, but in a water-saturated atmosphere its tip is seen gradually withering.
- 20. The root has no root hair, but a passage cell in place of it, and when the plumule is laid on the sand the corky layer is formed on the surface. After entering into the sand, the root shows, transversal wrinklings on its outer cortex.

F. Distribution of Crinum

- 21. The genus *Crimum* includes both inland and littoral species, and the former seems, as in the case of Amaryllidaceae in general, to be the older habitat of the genus. And it is highly probable, that the birthplace of *Crimum* was the inland parts of tropical Africa, and that from there it has been distributed to other regions.
- 22. Crimum in Japan seems to have been brought in recent geological times by the ocean current, probably after the cold period of the Pleistocene.

In conclusion the writer wishes to express his sincere thanks for many helpful suggestions to Professor K. Koriba, under whose direction this study was undertaken. His thanks are also due to Mr. Z. Tashiro for his helpful advice on the localization, and to Mr. J. Ikari. who kindly sent materials for the writer's use.

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APPENDIX

SUPPLEMENTARY TABLE I
Weight-change of the seeds deprived of their cork-layer
in various media (in gms.)

						, 0	,				
Lapse of days in the media Media		2	4	9	11	16	23	36	55	71	81
	8.18	6.81	6.90	Dec	ayed						
	6.38	5.74	5.97	6.86	6.73	De	cayed				
N/2 NaCl soln.	7.50	5.50	5.84	6.66	6,65	De	cayed				
	8.86	7.54	7.70	8.43	8.60		Dec	cayed			
	8.31	7.23	7.58	8.22	8.27	8.60	Dec	cayed			
Average	7.84	6.56	6.81	7.52	7.56						
	10.71	8.67	9.35	10.59	10.56	10.35	Decay	ed			
	10.27		, 0-		-	9.96	-	Decayed			
		-			ed aga			•			
Sea-water	7.27	6.09	6.61	7.13	7.13	7.11		Decayed			
			Sunk	Float	ed aga	nin					
	11.10	8.84	9.39	10.39	10.58	10.43		Decayed			
	7.74	6.15	6.56	7.42	7.47	7.43		Decayed			
Average	9.42	6.60	8.13	9.07	9.05	9.06					
	10.64	15.95	16.70	17.30	16.77		Decay	ed			
	1 .	12.93					-		14.57		Decaye
Well-water	7.59	9.29	9.54	9.92	9.92	10.22			10.21	Dec	ayed
	6.50	8.50	8.54	9.02	9.02	9.25		Decayed			
	7.05	9.79	10.27	10.85	10.90	11.45			Decay	/ed	
Average	8.35	11.28	11.71	12.10	11.29	11.29					

SUPPLEMENTARY TABLE 2
Distribution of Crinum throughout the world

T 19		Number	of species	
Locality	Littoral sp.	Inland sp.	Marsh or streamside sp.	Sp. occur- ring in
Tropical Africa	59	7		66
South Africa	24	3	I	28
Madagascar Is	2	3		5
Coast of the Red Sea	10			10
Indian Ocean	8	Ī	ı	10
India	16	4		20
Himalaya Mts		2 .		2
Malay Peninsula	4			4
Burma	4			4
Cochin-China	4			4
China	.2			2
Formosa	I			1
Japan proper	I			I
Sumatra	3			3
Java	1			I
Borneo	3		1	4
Philippine Is	6			6
New-Guinea and surrounding Is.	7			7
Norfolk Is	1			1
Australia	12			12
Hawaii Is	2	1		3
Jamaica Is	3	1 .		4
West Indies	1			1
South America	14	1	I	16

SUPPLEMENTARY TABLE 3 Germination of the seeds in various media

							Numb	er of se	Number of seeds germinated (%)	minatec	(%) 1				
Media and conditions	Lapse of day	of day	1-	OI	13	16	19	22	25	28	31	34	37	40	43
Well-water	30th. Nov. '25-1st. Sept. '26	ept. '26	1	1	1		1	9	1	10	1	1	1	ı	30
Sea-water		*	ſ	ĺ	1	1	J	61	1	I	ı	l	ſ	***************************************	29
3.57% NaCl soln.		•	-	(1)	l	1	J	i	1	17	1			1	38
Store-room)			1	i	i	I	1	1	1	1	1	I	-		
H ₂ SO ₄ -desiccator			1	1	1	1	1	l	1	1	1	1	1	-	
Well-water	26th. Apr. '26-1st. Sept. '26	sept. '26	1	1	1	9	1	1	1	13	13	14	14	- 02	7
Sea-water				1	1	1	1	1	7		20	ı	14	1	7
N/2 NaCl soln.			[ĺ	1	1	1	14	1	14	1	7	14	56	j
BRENNER's soln.			1	1	1	9	J		1	1	56	1	. 7	14	7
$\{N/2 \text{ NaCl+0.3}\%$ $\{\text{CaCl}_2 \text{ soln.}$	"		1	1	1	9	J	1	1	1	6	9	7	14	7

Number of seeds germinated (%)

103	9	l		1	1	1	-	1]
100	2	9	1	1	1		l	ļ	l	1
97		1	63	ı	}	l	1	[1	1
94	4	4	1	[1		1	ı
16	2	1		ı	١	ı	1	I	1	1
88		∞	ı	1	I	1	ı	1		1
85		I	03	1		1	J	1	I	1
82	9	13	1		1	l	1	1	j	I
2.0		[67	- Personal	1	}	1	9]	1
94	4	1	1	I	1	l	1	1	1	1
73	1	4	4	I	1	1	9	i	ľ	9
70	ı	-	C)	1	-	1	1	ĺ	1	i
29	1	61		j	1	ı	1	1	1	9
64.	4	-	9		1	I	9	J	14	
19	1	1	9	ı	-	1	9	9	1	14
58	ı	15	- [ı	1	ļ	1~	l	I	ı
55	IO	1	11		1	J	ı	1	ı	I
525	ı	I	ı	1	ı	1	9	1	9	14
49		-		1		13	1~	9	9	1~
46	1		9		1	1	1~	t~	14	1~

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		1									
	195		[-	1	1	1	-	1	1
	190		-	1	23	-	-		[1	Ì
	185	-	J	I	****]	1	1	1
	180	-	1	1	3	1	1		1	1	1
(%)	175		1	1	1	7	1	{	1	{	١
inated	170	1		1	١	33		1	ı		1
Number of seeds germinated (%)	165	1	[I		[I	1]
of seed	091	1	1		9	61	1.	1	1	1	-
Tumber	155		1.	1	33		-		ſ	1	ſ
Z	150		ļ	1	1		1	I	1	J	İ
	140		1	1	I	J	1	-	İ	1]
	130	63	01	1	}	1	1	ſ	1		[
	120	1	63			l	!	1	I		1
	115		1	1	1	1	[1	ı	1	1
	11.2	9		i	1	1	1	1	ı	1	1

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oer of lings ing)	lmuN bəəz viJ)	100	87	61	36	28	100	100	9	100	9
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eds nated	imrəg	100	100	100	29	31	100	100	100	100	100
	ı fstoT es to	100	100	100	100	100	100	100	100	100	100
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	270		1	-	[ı	1		ĺ	ļ	1
	265	1	I	ĺ		4		[1	1	
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(%) F	255	ı		1		1	l		1	1	1
rminate	250	ı	1		61		-	[[I	1
eds ge	245			1	-	63	ļ	[F	ſ	l
Number of seeds germinated (%)	240	-	1	1	-		- ({	1	1	1
Numbe	235	1	ı	-	ı	ın	1	-	1	ı	1
	230			l	ı	ı	ı	1		ı	I
	225		America	A	ı	33	[[J	I	1
	220	-	ı	-	¢1	1		1	1	1	l
	215	1			3	3	1	-	1	-	1

SUPPLEMENTARY TABLE 4

Injurious action of the media on the seedlings

	30	-	14	1	-	1		1	
	27	ı		1	-	20	[]	
%	24	1	22	-	36	1	١	1	-
jured	21	1	14		57	-			
rs in	18	Io	7 29 14	1	1	20	1	1	1
edling	15	20	^	1	1	40		1	1
es Jc	12	30	^]	1	20	1]	1
Number of seedlings injured (%)	6 9 12 15 18 21 24	1	[1	١	1	1	[1
Num	9	30	1	1	1	1	-	1	1
	3	1	[100	[1		[١
	н	-	-		[[1	1	1
	Lapse of days after germination Degree of injury	Seedlings slightly injured	Seedlings completely injured	Seedlings slightly injured	Seedlings completely injured	Seedlings slightly injured	Seedlings completely injured	Seedlings slightly injured	Seedlings completely injured
	Date	\(\) \(\)	Api. som, somsept. ist, so	7 301 100 Cont rat 106	Nov. 30th, 25-3cpt. 13t, 20	N/2 NaCl+0.3% CaCl2 1 2613, 126 Sout 151 176	Tapi: som, so-och: 13th so	No 1 20.1 25 Sent 164 196	100v. 30.41, 25-3ept. 13t, 20
	Media	150 150 TV 0/18			3.5/ % TAGO SOIII.	N/2 NaCl+0.3% CaCl2	soln.	Co. motor	-

_
%
injured
seedlings
ō
Number

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250	ı	1	[1	1	[1
210		I	-	[1
205	1	1	[1	1		Ì	1
200	1	1	1	1	-		33	1
195	1	I	1	1	[1	1	
190	1	1	1		1	1	17	1
45 48 51 54 57 60 63 66 69 72 75 80 85 150 170 175 180 185 190 195 200 205 210 250	1	ı	1	I	1	l	1	
180	Ī	[1	1	I	1	33	1
175	1	I	1	1	1	I	1	1
0/1		1	İ	1	1	i	17	1
150	1	1	1	1	-	1	1	I
85		1	1	1	1	1		1
80		I	1	1	1	1		Τ
75		١	-	1	1	1	I	1
72		1	1	[1	ľ	1	[
69	1	I	1	1		I	1	1
99		1		1]	Ī	1	I
63		۲.	1	1	-	. [1	1
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57		1	I	[1	1	-1
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3	1	1	1	1	1	ın	1	1

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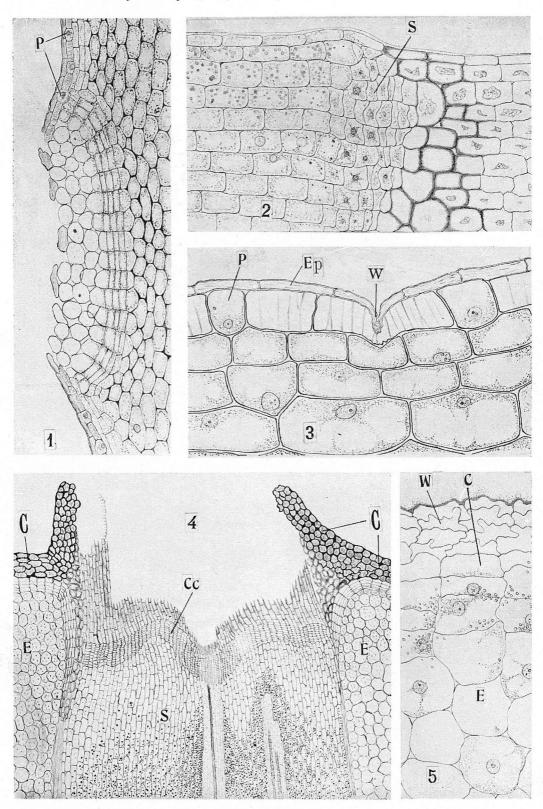
EXPLANATION OF PLATES

Plate XV

- Fig. 1. Pneumatode, formed on the surface of a root of Crinum cultivated in water. P, passage cell. ca. 300/1.
- Fig. 2. Separation layer of the leaf-sheath. The distal portion has been withered and covers tightly the outer part of the seedling. S, separation layer. ca. 300/1.
- Fig. 3. Transversal wrinkling on the outer cortex of the root. P, passage cell; W, wrinkle; Ep, epidermis, already withered. ca. 350/1.
- Fig. 4. Callus-forming cork-cambium, arisen in the cotyledonary sheath of a seed which was in N/2 NaCl solution. C, cork-layer; E, endosperm; S, cotyledonary sheath; Cc, callus-forming cork-cambium. ca. 250/1.
- Fig. 5. Formotion of callus in the injured surface of the endosperm. E, endosperm; IV, withered tissue of the endosperm; C, callus-forming cambium. ca. 350/I.

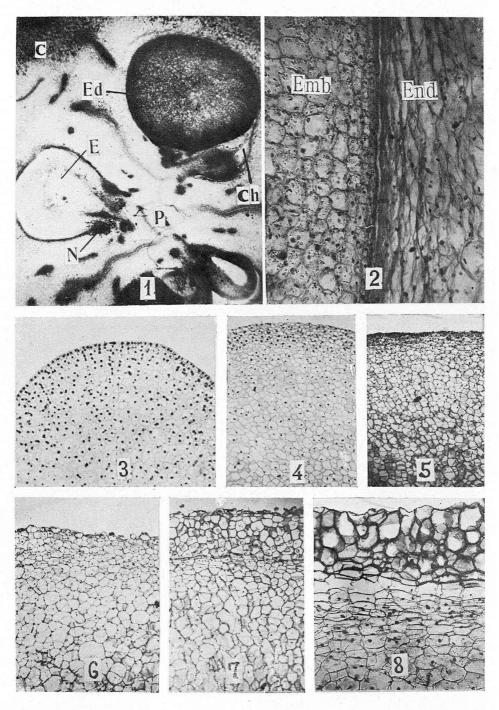
Plate XVI

- Fig. 1. Cross-section of young fruits of *Crinum asiaticum* L. var. *japonicum* BAK. *C*, carpel; *Ch*, chamber; *P*, placenta; *Ed*, endosperm; *N*, nucellus; *E*, embryo-sack. ca. 20/1.
- Eig. 2. A portion of the surface of contact of the endosperm and the embryo. End, endosperm; Emb, embryo. ca. 250/I.
- Fig. 3—8 The development of the cork-layer in the seed. 3, cross-section of the endosperm, showing the cell division at the periphery cells, ca. 200/I; 4, 5, 6, the cambium formation is just in advance of the 6th-7th layer from the periphery, ca. 200/I; 7, the cell wall of the cork-layer is thickened, ca. 200/I; 8, the cork formation is completed. ca. 250/I.



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