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ON THE OCCURRENCE OF AMYLOIDS IN PLANT SEEDS[1])

P. KOOIMAN
Laboratory of General and Technical Biology
Technical University, Delft – Netherlands

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INTRODUCTION

SCHLEIDEN (1838) and VOGEL and SCHLEIDEN (1839) were the first to observe a blue coloration of the cell-walls in the cotyledons of some plant species when the latter were treated with an iodine-potassium iodide solution. The cell-wall substance which coloured blue with this reagent, was called "amyloid" by these authors.

Afterwards several investigators found this amyloid in the cell-walls of cotyledons and of endosperm, viz. TRÉCUL (1858), FRANK (1866), HEINRICHER (1888), REISS (1889), NADELMANN (1890), WINTERSTEIN (1893), VAN WISSELINGH (1898). These authors observed the substance in seeds of the species listed in Table I.

TABLE I
Occurrence of amyloid in seeds according to earlier authors.

Acanthaceae	*Primulaceae*
Schwabea	Anagallis arvensis
Balsaminaceae	Androsace septentrionalis
Impatiens balsamina	Cyclamen europaeum
Leguminosae-Papilionatae	,, neapolitanum
Goodia lotifolia	Glaux maritima
Mucuna urens	Primula officinalis
Leguminosae-Caesalpinioideae	Samolus valerandi
Hymenaea courbaril	*Ranunculaceae*
Schotia latifolia	Paeonia officinalis
,, speciosa	*Sapotaceae*
Tamarindus indica	Dipholis
Linaceae	Mimusops balata
Linum usitatissimum	Sideroxylon
Myrsinaceae	*Thunbergiaceae*
Ardisia	Thunbergia alata
	Tropaeolaceae
	Tropaeolum majus

Some of these authors found amyloid also in seeds of some other plant species, chiefly in those of *Monocotyledones*, but their results could not be confirmed by other investigators. It seems possible that not all these authors applied the same reagent, but this can not be proved directly, because several investigators did not record the composition of their reagent.

MITCHELL (1930) investigated the influence exercised on the colora-

[1]) This work is part of a doctor's thesis (Delft, 1959).

tion of cell-walls by the addition of sulphuric acid to the iodine-potassium iodide solution, and stated that the cell-walls in the seeds of *Tamarindus indica* were already stained blue by the reagent without addition of sulphuric acid. The cell-walls of seeds of *Impatiens balsamina*, of *Primula officinalis* and of *Tropaeolum majus* stained violet when after the iodine-potassium iodide solution a solution of 26 volume percent sulphuric acid was added. After the addition of 50 volume percent sulphuric acid the cell-walls in the endosperm of *Coffea arabica*, of *Diospyros virginiana*, of *Phoenix dactylifera* and of *Strychnos nux-vomica*, and in the cotyledons of *Lupinus hirsutus* also stained blue. Evidently the composition of the reagent is of great influence upon the results.

In the earlier publications where a positive amyloid reaction was postulated for the seeds of a number of *Monocotyledones*, the reagent (apart from differences in the iodine and KJ concentrations) must have contained a fair quantity of sulphuric acid or some other swelling agent. In my own experiments MITCHELL's reagent when applied without any addition, gave no coloration of cell-walls in the seeds of any *Monocotyledon*. In view of the diverging results obtained with reagents of different composition, it is important to specify the reagent used for the amyloid reaction.

In my experiments the cell-walls in the seeds of *Impatiens balsamina*, of *Primula officinalis* and of *Tropaeolum majus* already stained blue with MITCHELL's reagent when the latter was applied without the addition of sulphuric acid. This difference is caused by the fact that MITCHELL soaked the seeds before applying the reagent, while I applied the reagent to sections of untreated seeds. In MITCHELL's experiments the reagent penetrated into the sections in a diluted form.

For this reason I adopted as criterion for a positive reaction on the amyloid in the cell-walls the development of a blue or violet colour when to untreated, dry sections a solution was added which contained 0.3 g iodine and 1.6 g potassium iodide per 100 ml water. This is MITCHELL's reagent without sulphuric acid.

When defined in this way, the amyloids occurring in plant seeds appear to constitute a group of polysaccharides with a closely related structure.

This investigation was undertaken in order to obtain an impression of the occurrence of amyloids in plant seeds, and to see whether some correlation could be detected between the occurrence of these substances and the taxonomic position of the plant species.

This paper gives a full record of a number of experiments on which a short communication appeared some time ago (KOOIMAN, 1957).

METHOD

The amyloid reaction was carried out by adding a drop of the reagent to some sections of a cotyledon or of the endosperm of the plant under investigation. Very small seeds were crushed, and then mixed with a drop of the reagent.

Colouring of the cell-walls was observed microscopically.

RESULTS AND DISCUSSION

A. Leguminosae-Caesalpinioideae

In Table II all the genera of the tribes *Cynometreae*, *Amherstieae* and *Sclerolobieae* of the *Leguminosae-Caesalpinioideae* are listed alphabetically. Behind the name of each genus the number of investigated species is given; a positive sign means a positive amyloid reaction, a negative sign means a negative amyloid reaction.

TABLE II

Results of amyloid reaction in seeds of *Leguminosae – Caesalpinioideae – Tribes Cynometreae – Amherstieae – Sclerolobieae* [1]) (alphabetical)

Afzelia	4+	Eperua	1+	Paramacrolobium	1+
Amherstia	1+	Eurypetalum		Pellegriniodendron	
Amphimas	1—	Gilbertiodendron	1+	Peltogyne	3+
Anthonotha	3+	Gilletiodendron	1+	Phyllocarpus	1+
Apaloxylon		Goniorrhachis		Plagiosiphon	1+
Aphanocalyx	2+	Gossweilerodendron	1—	Poeppigia	1—
Augouardia		Guibourtia	1+	Polystemonanthus	
Baikiaea	2+	Hardwickia	1—	Prioria	
Batesia	1—	Heterostemon		Pterogyne	1—
Bathiaea		Humboldtia	1+	Pseudocopaiva	
Berlinia	3+	Hylodendron	1+	Pseudomacrolobium	
Brachystegia	2+	Hymenaea	4+	Pseudosindora	
Brownea	1+	Hymenostegia	2+	Recordoxylon	
Browneopsis		Intsia	2+	Saraca	1+
Campsiandra	1—	Isoberlinia	3+	Schotia	4+
Cenostigma		Julbernardia	3+	Sclerolobium	3—
Colophospermum	1—	Kingiodendron	1—	Scorodophloeus	1+
Copaifera	8+	Lebruniodendron		Sindora	4+
Crudia	2+	Librevillea		Sindoropsis	
Cryptosepalum	2+	Loesenera		Stahlia	1—
Cymbosepalum		Lysidice		Stemonocoleus	
Cynometra	4+	Macrolobium	2+	Tachigalia	2—
Daniellia	2+	Maniltoa	1+	Talbotiella	
Detarium	1+	Melanoxylon	1—	Tamarindus	1+
Dicymbe	2+	Michelsonia		Tessmannia	1+
Dicymbopsis		Microberlinia		Tetraberlinia	
Diptychandra		Monopetalanthus	1+	Thylacanthus	
Didelotia		Neochevalierodendron		Trachylobium	1+
Elizabetha	1+	Oddoniodendron		Vouacapoua	
Endertia	1+	Oxystigma	2—	Zenkerella	
Englerodendron		Palovea	1+		

Table III records the species with a positive amyloid reaction belonging to the genera listed in Table II.

In Table IV the genera of the other tribes of the *Leguminosae-Caesalpinioideae* are listed. Here the result was always negative.

Whereas previously but four species were known to have amyloid in the cell-walls of the cotyledons (see Table I), this substance now appeared to occur much less exceptionally in this sub-family than could have been anticipated. About 90 species belonging to 43 genera were found to have amyloid.

[1]) The figures indicate the number of species that were investigated; + positive, — negative reaction.

TABLE III

Leguminosae-Caesalpinioideae

Amyloid-containing species

Afzelia africana (Sm.) Pers.
bella Harms
bipindensis Harms
javanica (Miq.) J. Léonard
quanzensis Welw.
Amherstia nobilis Wall.
Anthonotha gilletii (De Wild.) J. Léonard
macrophylla P. Beauv.
pynaertii (De Wild.) Exell et Hillcoat
Aphanocalyx cynometroides Oliv.
spec. (Flora Congo III, 440)
Baikiaea insignis Benth. subspec. insignis J. Léonard
subspec. minor (Oliv.) J. Léonard
eminii Taub.
Berlinia giorgii De Wild.
var. gilletii (De Wild.) Hauman
grandiflora (Vahl) Hutch. et Dalz.
viridicans Bak. f.
Brachystegia stipulata De Wild.
var. lufirensis (De Wild.) Hoyle
spec.
Brownea spec.
Copaifera baumiana Harms
coriacea Mart.
duckei Dwyer
epunctata Amshoff
guianensis Desf.
mildbraedii Harms
officinalis L.
reticulata Ducke
Crudia glaberrima (Steud.) Macbr.
harmsiana De Wild.
Cryptosepalum maraviense Oliv.
spec.
Cynometra alexandri C. H. Wright
ananta Hutch. et Dalz.
leonensis Hutch. et Dalz.
sessiliflora Harms
Daniellia alsteeniana Duvign.
oliveri (Rolfe) Hutch. et Dalz.
Detarium senegalense J. F. Gmel.
Dicymbe altsoni Sandw.
corymbosa Spruce
Elizabetha speciosa Ducke
Endertia spectabilis Van Steenis et De Wit
Eperua bijuga Mart.
Gilbertiodendron demeusei (De Wild.) J.Léonard
Gilletiodendron mildbraedii (Harms) Verm.
Guibourtia coleosperma J. Léonard
Humboldtia laurifolia Vahl
Hylodendron gabunense Taub.
Hymenaea altissima Ducke
courbaril L.
oblongifolia Huber
parvifolia Huber
Hymenostegia afzelii Harms
laxiflora (Benth) Harms
Intsia amboinensis A.D.C.
bijuga O. Kuntze
Isoberlina angolensis (Welw.) Hoyle et Brenan
scheffleri (Harms) Greenway
tomentosa (Harms) Craib et Stapf
Julbernardia baumii (Harms) Troupin
paniculata (Benth.) Troupin
seretii (De Wild.) Troupin
Macrolobium bifolium (Aubl.) Pers.
multijugum Benth.
Maniltoa gemmipara Scheff.
Monopetalanthus pteridophyllus Harms
Palovea guianensis Aubl.
Paramacrolobium coeruleum (Taub.) J. Léonard
Peltogyne densiflora Spruce
pubescens Benth.
venosa (Vahl) Benth.
Phyllocarpus riedelii Tul.
Plagiosiphon emarginatus (Hutch. et Dalz.) J. Léonard
Saraca indica L.
Schotia bequaertii (De Wild.) De Wild.
brachypetala Sond.
latifolia Jacq.
speciosa Jacq.
Scorodophloeus zenkeri Harms
Sindora cochinchinensis Baill.
irpicina De Wit
klaineana Pierre et Pellegr.
sumatrana Miq.
Tamarindus indica L.
Tessmannia africana Harms
Trachylobium verrucosum Oliv.

Amyloid is strictly confined to the three tribes of Table II which are taxonomically nearly related.

The classification of the genera in the tribes *Cynometreae*, *Amherstieae* and *Sclerolobieae* has always presented difficulties. As recent examples of diverging opinions the views of DWYER (1954b) and of LÉONARD

TABLE IV

Results of amyloid reaction in the tribes of the *Leguminosae-Caesalpinioideae* (except *Cynometreae*, *Amherstieae* and *Sclerolobieae*) [1]

4. *Bauhinieae*	
Bandeiraea	2—
Bauhinia	3—
Cercis	2—
Gigasiphon	
5. *Cassieae*	
Apuleia	2— G
Baudouinia	
Cassia	3— G
Ceratonia	1— G
Dansera	
Dialium	4—
Dicorynia	2—
Distemonanthus	1—
Koompassia	2—
Labichea	1—
Martia	1— G
Oligostemon	1— S
Petalostylis	1—
Storckiella	1—
Stuhlmannia	
Uittienia	
6. *Dimorphandreae*	
Brandzeia	
Burkea	1—
Chidlowia	1— S
Dimorphandra	3—
Erythrophloeum	1—
Kaoue	
Mora	
Pachyelasma	1—
Sympetalandra	
7. *Eucaesalpinieae*	
Acrocarpus	2—
Aprevalia	
Bussea	1— S
Caesalpinia	4— G
Cercidium	2— G
Colvillea	1— G
Delonix	1— G
Gleditschia	1— G
Gymnocladus	1— G
Haematoxylon	1— G
Hoffmanseggia	2—
Jaqueshuberia	
Mezoneurum	1—
Moldenhawera	1—
Parkinsonia	1— G
Peltophorum	1—
Pogocybe	
Pterolobium	1—
Schizolobium	1— G
Stachyothyrsus	
Wagatea	1—
Zuccagnia	
8. *Kramerieae*	
Krameria	2—
9. *Swartzieae*	
Aldina	
Baphiopsis	1—
Cordyla	1— S
Exostyles	1— S
Holocalyx	1— S
Lecointea	
Mildbraediodendron	1— S
Swartzia	10— S
Zollernia	2— S

(1959) may be mentioned. DWYER suggests to unite the three tribes, because in his opinion the characters by means of which BENTHAM (1865) had tried to delimit the latter, are not of essential value. However, LÉONARD, in dealing with the *Amherstieae* and *Cynometreae* of Africa, judges it advantageous to keep these tribes apart, but he introduces other characters to define them. BAKER (1926–1930) united these two tribes, but he made a subdivision of the enlarged tribe; LÉONARD's *Amherstieae* fall in the first part while the second and third parts correspond to LÉONARD's *Cynometreae*.

Of BENTHAM's *Sclerolobieae* only *Dicymbe* and *Phyllocarpus* appeared to contain amyloid. DUCKE, the great authority on the Brazilian *Caesalpinioideae*, (1949) classifies *Dicymbopsis* and *Tachigalia* in the *Amherstieae*, but *Dicymbe* and *Phyllocarpus* in the *Sclerolobieae*, at the same time stating that *Dicymbe* and *Dicymbopsis* are nearly related. The

[1]) The figures indicate the number of species that were investigated
— = negative reaction
G = galactomannan present in the seeds
S = starch present in the seeds.

problematical genus *Thylacanthus* would be nearly related to *Dicymbe*. DUCKE too seems to have felt the difficulties caused by the recognition of the three tribes!

As since BENTHAM's days a large number of species have been discovered, and as many species have meanwhile become better known, it seems that a new classification, which is urgently needed, should be attempted. In my opinion, LÉONARD's methods of experimental taxonomy offer a good perspective. Perhaps the occurrence of amyloid in the seeds may serve as a useful character.

The following genera of Table II lack amyloid:

a) *Batesia, Campsiandra, Melanoxylon, Poeppigia, Sclerolobium, Tachigalia.*
b) *Gossweilerodendron, Hardwickia, Kingiodendron, Oxystigma.*
c) *Pterogyne.*
d) *Colophospermum.*
e) *Stahlia.*

The genera referred to under a) constitute the bulk of the *Sclerolobieae* in the sense of BENTHAM: only *Tachigalia* belongs, according to BENTHAM, to the *Amherstieae*. However, DWYER (1954a) adduced convincing arguments for a relationship between *Tachigalia* and *Sclerolobium*.

The genera referred to under b) are nearly related; to this group we should refer *Prioria* (LÉONARD, 1957) and perhaps *Stahlia*. This group of genera might, as well as group a), occupy a special place in the combined tribe *Amherstieae-Cynometreae-Sclerolobieae*. Group b) would occupy also a special place in the *Cynometreae* of LÉONARD.

The genus *Pterogyne* does not seem to belong to this tribe s.l., but to the tribe *Dalbergieae* of the *Leguminosae-Papilionatae*; the anatomy of the wood led COZZO (1951) to this conclusion. The deviating number of chromosomes also points to the desirability of a reclassification of *Pterogyne* (TURNER and FEARING, 1959).

The genus *Colophospermum* occupies an isolated place in the tribe s.l., although it undoubtedly belongs to the latter (LÉONARD, 1957). The number of its chromosomes (36) is exceptional, the most common number in the tribe being 24 (TURNER and FEARING, 1959).

Summarizing the results obtained by means of the amyloid-reaction on members of the *Leguminosae-Caesalpinioideae*, it may be said that amyloid-containing genera are found exclusively in the tribes *Cynometreae-Amherstieae-Sclerolobieae*. In these tribes two groups of genera with amyloid-lacking species occur, while the majority of the genera consists of amyloid-containing species.

Amyloid is a major constituent of the amyloid-containing seeds of the *Leguminosae-Caesalpinioideae*. In *Tamarindus indica* seeds, for instance, it occurs in the cotyledons in quantities of about 60 %. It is not surprising, therefore, that already several of the earlier authors came to the conclusion that amyloid is a reserve substance (FRANK, 1866; GODFRIN, 1884; HEINRICHER, 1888; REISS, 1889; RIEDEL, 1897).

The correlation existing between the occurrence of amyloid in species of the *Leguminosae-Caesalpinioideae* and the position of these species in the taxonomic system is therefore an example of a correlation existing between physiological and morphological characteristics in the sub-family.

It is interesting to note that similar correlations between physiological and morphological characters are to be found in some other tribes of the sub-family. Galactomannan occurs very generally and in large quantities in the well-developed endosperm of species belonging to the tribes *Cassieae* and *Eucaesalpinieae*, while the seeds of the *Swartzieae* contain starch as a reserve polysaccharide.

The conclusions with regard to the occurrence of amyloid in the *Leguminosae-Caesalpinioideae* cannot be final since only part of the species were studied. Of the about 730 species belonging to the tribes *Amherstieae-Cynometreae-Sclerolobieae* about 110 species have been investigated; the investigated species belong to about 57 out of the 92 genera. Of the roughly 700 species belonging to the other tribes 76 species were investigated, belonging to 45 out of the 62 genera.

B. Other plant families

In other plant families amyloid was found in cotyledons or endosperm of the species listed in Table v. About 2500 species belonging to 208 different families of the *Spermatophyta*, were tested. In 16 dicotyledonous families amyloid-containing species were found, but none in the *Monocotyledones*, of which 25 families were studied.

TABLE V

Amyloid-containing species in taxa other than *Leguminosae-Calsalpinioideae*

Acanthaceae
Anisacanthus virgularis Nees
  wrightii Nees
Beloperone californica Benth.
Drejerella guttata (T.S. Brandegee) Brem.
Hypoëstes phyllostachya Bak.
  sanguinolenta Hook.
Peristrophe bicalyculata Nees
Ruspolia seticalyx Milne-Redhead
Schaueria calycotricha Nees
Schwabea ciliaris Nees
Annonaceae
Annona cherimolia Mill.
  montana Macfad.
  muricata L.
  reticulata L.
  squamosa L.
Artobotrys brachypetalus Benth.
  uncinatus Merrill.
Asimina triloba Dun.
Cananga odorata Hook. f. et Th.
Hexalobus glabrescens Hutch. et Dalz.
Mezzettia parviflora Becc.
Monodora crispata Engl.
  myristica Dun.
  tenuifolia Benth.
Polyalthia littoralis Boerl.
  suberosa B. et H.
Popowia caffra Hook. f.
Rollinia emarginata Schlecht.
Stelechocarpus schefferi Boerl.
Unona discolor Vahl
Uvaria macrophylla Roxb. var. microcarpa Finet et Gagn.
  rufa Blume
Xylopia aethiopica A. Rich.
  mendoncae Exell.
  villosa Chipp.
Balsaminaceae
Impatiens balsamina L.
Limnanthaceae
Limnanthes douglasii R. Br.
Linaceae
Linum bienne Mill.
  grandiflorum Desf.
  perenne L.
  usitatissimum L.
Melianthaceae
Melianthus major L.
  minor L.
Myrsinaceae
Ardisia acuminata Willd.
  crenata Sims
  humilis Vahl
  polycephala Wall.
  wallichii D.C.

TABLE V (*continued*)

Maesa alnifolia Harv.
argentea Wall.
indica (Roxb.) Wall.
lanceolata Forsk.
perlarius (Lour.) Merr.
Myrsine africana L.
Rapanea neriifolia Mez.
urvillei Mez.
Papilionaceae
Goodia lotifolia Salisb.
Mucuna urens Medic
Pedaliaceae
Ceratotheca triloba E. Mey.
Harpagophytum peglerae Stapf
Josephinia imperatricis Vent.
Sesamum indicum L.
orientale Sieber
radiatum Schum.
Primulaceae
Anagallis arvensis L.
foemina Mill.
Androsace maxima L.
Ardisiandra wettsteinii J. Wagner
Asterolinum stellatum (L.) Link et Hoffm.
Centunculus minimus L.
Coris monspeliensis L.
Cortusa matthioli L.
Cyclamen cilicium Boiss. et Heldz.
europaeum L.
graecum Link.
neapolitanum Tenore
persicum Mill.
hybr.
Dodecatheon meadia L.
Douglasia laevigata A. Gray
vitaliana Rox.
Glaux maritima L.
Hottonia palustris L.
Lysimachia lichiagensis Forrest.
mauritiana Lam.
punctata L.
thyrsiflora L.
Primula officinalis Jacq.
sinensis Lindl.
uralensis Fisch.
Samolus valerandi L.
Soldanella alpina L.
carpatica Vierh.
montana Willd.
Steironema ciliatum (L.) Raf.
Trientalis europaea L.
Ranunculaceae
Paeonia albiflora Pall.
anomala L.
beresowskii Komarov.
corallina Retz.
coriacea Boiss.
decora Anders.
delavayi Franch.
lusitanica Mill.
lutea Del.
macrophylla (Alb.) Lomak
mlokosewitschi Lomak
mollis Anders.
moutan Sims.
obovata Maxim.
officinalis L.
paradoxa Anders.
peregrina Mill.
potanini Komarov.
tenuifolia L.
triternata Pall.
trollioides Stapf.
veitchii Lynch.
wittmanniana Hartw.
woodwardii Cox.
Sapindaceae
Cardiospermum halicacabum L.
hirsutum Willd.
Sapotaceae
Achras sapota L.
Dipholis montana Griseb.
salicifolia A.D.C.
Illipe spec.
Mimusops balata Miq.
elengi L.
hexandra Roxb.
Omphalocarpum ahia A. Chev.
anocentrum Pierre
Sideroxylon australe Benth.
foetidissimum Jacq.
inerme L.
quadriloculare Pierre
Theophrastaceae
Jacquinia pungens A. Gray
Thunbergiaceae
Thunbergia alata Boj. ex Sims subspec. alata Brem.
subspec. reticulata Brem.
fragrans Roxb.
Tropaeolaceae
Tropaeolum majus L.
minus L.
peltophorum Benth.
peregrinum L.
speciosum Poepp. et Endl.

a. *Acanthaceae* (sensu Brem.)

Among sixty-two species belonging to 49 different genera 10 amyloid-containing species were found; the latter species appeared to occur in the tribe *Justicieae* (sensu Brem.). The amyloid occurs in the cell-walls of the cotyledons.

b. *Annonaceae*

The cell-wall of the endosperm of all investigated *Annonaceae* (25 species) stained brownish-violet with the reagent. The reaction was judged to be positive in all cases.

c. *Balsaminaceae*

In the cotyledons of *Impatiens balsamina* amyloid was found; in six other *Impatiens* species and in *Hydrocera triflora* the reaction was negative.

d. *Leguminosae-Papilionatae*

Goodia lotifolia has amyloid in the cotyledons in contrast to *Goodia pubescens* in which it is absent.

Mucuna urens was the only one out of four *Mucuna* species which proved to contain amyloid. Of 104 genera of *Papilionatae* 126 species have been tested.

e. *Limnanthaceae*

Of this small family the only species investigated appeared to have amyloid-containing cotyledons.

f. *Linaceae*

The endosperm cell-walls of 3 out of 11 *Linum* species gave a positive reaction. The amyloid-containing species belong to the section *Eulinum*. Amyloid was not found in *Hugonia swynnertoni* nor in *Radiola linoides*.

g. *Melianthaceae*

Of this small family two *Melianthus* species were tested; the endosperm cell-walls of both species contain amyloid.

h. *Myrsinaceae*

All species investigated have amyloid-containing endosperm. The *Maesa* species, however, show only a slightly positive reaction.

i. *Pedaliaceae*

The cotyledon cell-walls of the six investigated species give a very slightly positive reaction.

j. *Primulaceae*

The endosperm cell-walls of all investigated species react positively.

k. *Ranunculaceae*

All the *Paeonia* species investigated have endosperm cell-walls with amyloid. The other *Ranunculaceae* that were tested (30 species belonging to 27 genera), have no amyloid.

l. *Sapindaceae*

Of 28 species (belonging to 18 genera) only *Cardiospermum hali-*

cacabum and, very slightly, *C. hirsutum* were found to have amyloid-containing cotyledon cell-walls.

m. *Sapotaceae*

Twenty-three species (belonging to 11 genera) were tested; 12 species (belonging to 6 genera) contain amyloid.

n. *Theophrastaceae*

One member of this small family was tested; the endosperm cell-walls contain amyloid.

o. *Thunbergiaceae* (sensu Brem.)

Three *Thunbergia* species were tested which appeared to have cotyledons with thickened cell-walls, containing amyloid.

p. *Tropaeolaceae*

All the investigated species have amyloid-containing cotyledon cell-walls.

In some instances a correlation exists between the taxonomic position and the occurrence of amyloid.

Of the families belonging to the order *Primulales* (*Primulaceae*, *Myrsinaceae* and *Theophrastaceae* the endosperm of all investigated species has cell-walls containing amyloid.

The families *Linaceae*, *Tropaeolaceae*, *Limnanthaceae*, *Balsaminaceae*, *Melianthaceae* and *Sapindaceae* are related to each other. According to DALLA TORRE and HARMS (1900–1907) they belong to the orders *Geraniales* and *Sapindales*.

The families *Acanthaceae*, *Thunbergiaceae* and *Pedaliaceae* are interrelated and belong to the order *Tubiflorae*.

In the *Ranunculaceae* only the genus *Paeonia* was found to have species with amyloid-containing seeds. On account of its morphological characters this genus occupies an isolated place in the family, and it has been proposed to exclude the genus from the *Ranunculaceae* (e.g. by DAVESNE, 1957).

The genus *Cardiospermum* was the only one of the *Sapindaceae* where amyloid-bearing species were encountered, but of the tribe *Paullinieae* to which *Cardiospermum* belongs, only one more species, viz. *Serjania clematidifolia*, was tested. Perhaps an investigation of more species of this tribe will reveal other amyloid-containing genera.

The amyloid-positive species of the *Sapotaceae* are spread over the subfamilies *Mimusopoideae* and *Chrysophylloideae*; in the *Madhucoideae* amyloid positive species were not found. In the *Chrysophylloideae* also a number of negative species were encountered.

As already stated, the seeds of all the investigated species of the *Annonaceae* give a brownish violet colour with the iodine reagent. A study of the structure of this cell-wall substance which has been classified among the amyloids, is under way.

In summarizing the results obtained with plant taxa other than the *Leguminosae-Caesalpinioideae* we may say that a number of plant taxa seem to consist exclusively of amyloid-containing species (*Annonaceae, Primulaceae, Myrsinaceae* and perhaps *Theophrastaceae, Limnanthaceae, Tropaeolaceae, Melianthaceae, Pedaliaceae, Thunbergiaceae*), while in other families amyloid occurs more or less occasionally (*Linaceae, Balsaminaceae, Sapindaceae, Sapotaceae, Ranunculaceae, Acanthaceae, Leguminosae-Papilionatae*).

Plant species with seeds containing amyloid will certainly appear to be much more numerous than we know at present (the number is now about 230). Besides the possibility that more plant families will prove to comprise amyloid-containing species, the fact that of the species of the amyloid-containing families only a fraction has been tested, leaves but little doubt that still numerous amyloid-positive species will be discovered.

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SUMMARY

By the application of potassium triiodide to dry sections of seeds amyloid was observed in the cell-walls either of the cotyledons or of the endosperm of the following plants:

1. All genera of the tribes *Cynometreae-Amherstieae-Sclerolobieae of the Leguminosae-Caesalpinioideae* with the exception of two groups of genera (Table II and III); in the other tribes of this subfamily no amyloid was found (Table IV).
2. All investigated species of the *Primulales*, the *Anonaceae, Limnanthaceae, Melianthaceae, Pedaliaceae Thunbergiaceae* and *Tropaeolaceae* (Table V).
3. A number of species of *Balsaminaceae, Acanthaceae, Leguminosae-Papilionatae, Linaceae, Ranunculaceae, Sapindaceae* and *Sapotaceae* (Table V).

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