Starch Grains of Leguminous Seeds

By

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Introduction

Starch is perhaps the most widely distributed substance in the vegetable kingdom and occurs in almost every species of green plants. It is stored in the form of grains in aerial stems (e. g. *Cycas revoluta* THUNB., *Metroxylon sagu* ROTTB., *Maranta* sp., *Poa bulbosa* L.), subterranean stems (e. g. *Manihot esculenta* CRANTZ, *Solanum tuberosum* L.), corms (e. g. *Colocasia* sp.), rhizomes (e. g. *Phragmites communis* TRIN.), fruits (e. g. *Artocarpus heterophyllus* LAMK.), and seeds (e. g. *Phaseolus mungo* L., *Vicia faba* L.). The starch grains are usually characteristic of individual species. A comparative study of the form of starch grains and the type of striations is of great value in determining the identity of taxa, especially when starch is used as an adulterant with flour, etc. According to WHISTLER & PASCHALL 1965, starches can be incorporated into a scheme of classification that makes it possible to determine their origin from the particular species of plants.

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Previous Work

Towards the end of last century, the morphology of starch grains of endosperm was studied in connection with the systematic classification of grasses. HARZ 1880 was among the earliest to recognize the taxonomic value of starch grains. He classified the starch grains of grass endosperm into three groups: (I) compound grains, (II) simple and rounded or elliptical grains, (III) simple, angular grains. Following HARZ 1880, HACKEL 1896 remarked further that the characters of the starch grains are constant within most of the genera and sometimes even in entire tribes and, therefore, are valuable in systematics. He also made use of such characters in framing his classification of the family. REICHERT 1913 produced a monumental work in which, besides summarizing the earlier work and systems of classification, showed that generic, specific and even varietal criteria of starch grains may be derived from a careful study of the particular group. In recent years,

the different forms of starch grains have been mentioned in the works of BOWER 1939, FOSTER 1942, EAMES & MCDANIELS 1947, METCALFÉ & CHALK 1950, ESAU 1953, MCLEAN & IVIMEY-COOK 1956, BOR 1960, CARLQUIST 1962, BURSTRÖM & ODHNOFF 1963, and FLEMION & TOPPING 1963.

In the family *Gramineae,* HAYEK 1925 found that the simple starch grains were characteristic of the tribes *Bambuseae, Maydeae, Andropogoneae, Paniceae* and *Zoysieae,* while compound ones occurred in *Oryzeae, Phalarideae, Agrostideae, Aveneae,* and *Chlorideae.* According to TATEOKA 1962, four types of starch grains can be noted in the family *Gramineae:* **A.** *Triticum* Type: The grains are simple and elliptic, round or reniform. Their size is much variable, even within the same species. They are characteristic of the tribe *Triticeae,* but occur occasionally in some members of the tribe *Festuceae,* e. g. *Bromus* and *Brachypodium.* **B.** *Panicum* Type: The grains are simple and angular. They are found in the tribes *Paniceae, Andropogoneae, Eragrosteae, Chlorideae* and *Arundinelleae.* The grains vary from 4—10 μ in diameter, sometimes reaching about 40 μ, e. g. *Sorghum* and *Cenchrus.* **C.** *Miscanthus* Type: Both simple and compound grains occur in the same species. The compound grains consist of 2—4 granules. TATEOKA 1955 reported this type for the first time in some genera of *Andropogoneae.* This type is of sporadic occurrence in various members of the family *Gramineae,* but has not been recorded in the subfamilies *Festucoideae, Oryzoideae* and *Bambusoideae.* The simple grains of this type are 15—40 μ in diameter. **D.** *Festuca-Eragrostis* Type: This type is characterized by compound grains only. It has been noted in the subfamilies *Eragrostoideae, Festucoideae, Arundinoideae, Oryzoideae* and *Bambusoideae.* In their revision of the *Gramineae* of temperate North America, STEBBINS & CRAMPTON 1961 are in agreement to TATEOKA's conclusions. They consider the tribe *Festuceae* as a natural group except for the genera *Bromus* and *Brachypodium,* both of which have simple starch grains.

Material and Methods

In view of the encouraging results obtained by TATEOKA, a systematic survey of the starch grains in the family *Leguminosae* was undertaken. In all, 76 species belonging to 39 genera have been studied. The seeds were collected from the Botanical Garden at the University of Delhi, Delhi. In some cases, seeds from herbarium specimens were also examined. However, only mature seeds were used in this study. The seeds were soaked in water and thinly sectioned with a hand-razor. The sections were stained with Iodine solution.

Observations

The present study shows that starch grains are of sporadic occurrence in the family *Leguminosae.* Out of 76 species examined, 30 species belonging to 14 genera revealed their presence in seeds. The starch grains were usually absent in the ligneous taxa. However, a few woody plants like *Dalbergia sissoo* ROXB., *Pongamia pinnata* PIERRE, *Acacia auriculiformis* A. CUNN. and *Pithecellobium dulce* BENTH. were found to contain starch grains. The following observations deal with their occurrence and morphology in relation to systematics.

Papilionaceae

Tribe *Phaseoleae*

In the genus *Phaseolus,* starch grains are oblong, circular or reniform, e. g. *P. lunatus* L. In *P. mungo* L. and *P. aconitifolius* JACQ., their shape is highly irregular. In *P. calcaratus* ROXB. and *P. limensis* MACFAD., the simple grains are of common occurrence. Occasionally, the compound grains are also met with. In other species, e. g. *P. aconitifolius* JACQ., *P. aureus* ROXB. and *P. multiflorus* WILLD., only simple grains were observed. The grains of *P. mungo* L. show only linear hilum and the striations are not visible. In *Phaseolus* sp., triangular grains are also found (see Table 1).

Table 1

Starch Grains of *Phaseolus* seeds

Name	Shape of simple grains					Hilum & Striations	Type
	Circular	Oblong	Irregular	Triangular	Reniform		
P. aconitifolius JACQ.	9%	12%	52%	—	27%	distinct	simple
P. aureus ROXB.	28%	30%	4%	—	38%	distinct	simple
P. calcaratus ROXB.	24%	35%	17%	—	24%	distinct	simple & compound
P. limensis MACFAD.	34%	33%	11%	—	22%	distinct	simple & compound
P. lunatus L.	36%	40%	1%	—	23%	distinct	simple
P. multiflorus WILLD.	46%	36%	4%	—	14%	distinct	simple
P. mungo L.	5%	8%	71%	—	16%	striations indistinct	simple
Phaseolus sp.	22%	40%	—	19%	19%	distinct	simple

The genus *Dolichos* is characterized by simple grains only. It is possible to distinguish the two species of *Dolichos,* namely *D. biflorus* L.

and *D. lablab* L. on the basis of their starch grains. In *D. biflorus* L., the grains are usually reniform or oblong with an even surface, and distinct hilum and striations. The hilum is sometimes triradiate. In *D. lablab* L., the grains are usually irregular in shape and possess indistinct hilum and striations. Their surface is somewhat uneven (see Table 2).

Table 2

Starch Grains of *Dolichos* seeds

Name	Shape of simple grains: Irregular	Reniform	Oblong	Circular	Hilum & Striations	Type
Dolichos biflorus L.	14%	45%	28%	13%	distinct	simple
D. lablab L.	63%	22%	8%	7%	indistinct	simple

Tribe *Cajaneae*

Both simple and compound grains have been found even in the same genus, e. g. *Rhynchosia* and *Cajanus*. In *Moghania*, however, only simple grains have been noted. The hilum of the grains is linear as well as triradiate. The two species of *Rhynchosia*, namely *R. capitata* DC. and *R. minima* DC. can be distinguished on the basis of their starch grains. In *R. minima* DC. only simple grains are seen, whereas both the kinds are found in *R. capitata* DC. In *Moghania bracteata* Li, the starch grains are simple and usually irregular in shape. The hilum is distinct but not the striations (see Table 3).

Table 3

Starch Grains in the Tribe *Cajaneae*

Name	Shape of simple grains: Reniform	Irregular	Oblong	Circular	Rhomboidal	Hilum & Striations	Type
Cajanus cajan L.	40%	—	43%	16%	1%	distinct	simple & compound
Moghania bracteata Li	30%	52%	8%	10%	—	striations indistinct	simple
Rhynchosia capitata DC.	20%	10%	38%	18%	14%	distinct	simple & compound
R. minima DC.	12%	48%	22%	18%	—	indistinct	simple

Tribe *Vicieae*

In the tribe *Vicieae* to which belong the genera *Cicer, Lathyrus, Lens, Pisum* and *Vicia,* the grains are of undefined shapes and sometimes oblong.

Cicer: In *C. arietinum* L., both simple and compound grains are found. The grains vary from 16 × 13 μ to 40 × 23 μ in size and are mainly oblong. The hilum and striations are usually distinct. The compound grains are composed of 2—5 granules.

Lathyrus: Only simple grains have been noted in *L. aphaca* L. and *L. sativus* L. However, both simple and compound grains are found in *L. odoratus* L. Usually, the grains possess oblong, reniform or irregular shapes. The hilum and striations are distinct only in *L. aphaca* L. and *L. sativus* L. Sometimes, a triradiate hilum is also visible. In *L. odoratus* L., large-sized grains with an irregular or rhomboidal outline are also found. The hilum and striations are indistinct in this species.

Lens: The starch grains of *Lens culinaris* MEDIK. are simple, and mostly circular and oblong with prominent hilum and striations. The hilum is sometimes tri- or tetraradiate. The starch grains vary from 30 × 16 μ to 53 × 33 μ in size.

Pisum: Both compound and simple grains are found in *Pisum sativum* L. The compound grains have either several hila or they are composed of 2—4 distinct granules. A few striations are seen around each hilum and some common to all the hila of the granules. The striations are more distinct near the hilum. The grains vary from 10 × 10 μ to 40 × 23 μ in size. In most of the rounded grains, the hila are triradiate and in oblong ones linear in outline. The percentage of compound grains is rather low.

Vicia: In *V. pisiformis* L., the grains are usually of undefined shapes, simple, and without prominent hilum and striations. *V. faba* L. also shows a very high percentage of undefined grains. In this species, simple grains are present without prominent hilum and striations. Their size varies from 23 × 16 μ to 53 × 36 μ. *V. hirsuta* S. F. GRAY, however, differs to a great extent from the other two species. The grains are usually renform; hilum and striations being distinct. Their size varies from 13 × 13 μ to 36 × 23 μ. The grains of *V. sativa* L. do not differ greatly from those of *V. pisiformis* L. and *V. faba* L. These are all simple and irregular in outline (see Table 4).

Tribe *Dalbergieae*

The starch grains were observed only in *Dalbergia sissoo* ROXB. Here the grains are rather small, oblong and remain aggregated in the cells.

Table 4

Starch Grains in the Tribe *Vicieae*

Name	Shape of simple grains: Circular	Oblong	Reniform	Irregular	Rhomboidal	Hilum & Striations	Type
Cicer arietinum L.	11%	49%	14%	26%	—	distinct	simple & compound
Lathyrus aphaca L.	5%	—	40%	44%	11%	distinct	simple
L. odoratus L.	5%	40%	10%	20%	25%	indistinct	simple & compound
L. sativus L.	19%	33%	22%	26%	—	distinct	simple
Lens culinaris MEDIK.	40%	33%	20%	4%	3%	distinct	simple
Pisum sativum L.	30%	36%	26%	8%	—	distinct	simple & compound
Vicia pisiformis L.	3%	2%	12%	83%	—	indistinct	simple
V. faba L.	5%	15%	9%	71%	—	indistinct	simple
V. hirsuta S. F. GRAY	15%	28%	44%	11%	2%	distinct	simple
V. sativa L.	5%	5%	15%	45%	30%	indistinct	simple

Tribe *Lonchocarpeae*

According to HUTCHINSON 1964, the tribe *Lonchocarpeae* includes *Pongamia*. The starch grains from the cotyledons of *P. pinnata* PIERRE were examined. These are rather minute, ranging from 2—14 μ in diameter.

Caesalpiniaceae

Seeds of 10 species belonging to the genera *Cassia, Bauhinia, Delonix* and *Poinciana* have been examined. These were all found to be devoid of starch grains.

Mimosaceae

The starch grains could not be noted in the seeds of the following taxa: *Acacia leucophloea* WILLD., *A. modesta* WALL., *Albizia lebbeck* BENTH., *A. procera* BENTH., *Desmanthus virgatus* WILLD., *Leucaena leucocephala* WIT, *Mimosa hamata* WILLD., *M. pudica* L., *M. rubicaulis* LAMK., *Prosopis glandulosa* TORR., *P. juliflora* DC., and *P. cineraria* DRUCE (syn. *P. spicigera* L.).

In *Acacia auriculiformis* A. CUNN., the starch grains are usually oblong and minute, ranging from 7—14 μ in diameter. Being too minute, the hilum and striations are not visible.

In *Pithecellobium dulce* BENTH., the grains are somewhat larger, ranging from 4 × 2,5 μ to 1,9 × 1,1 μ in diameter. The grains are of various forms, namely triangular, rhomboidal, spherical and oval. The hilum and striations are visible in most of the grains.

Discussion

During recent years, much attention has been focussed towards the comparative study of basic metabolites, secondary compounds and macromolecules in relation to taxonomic problems. BATE-SMITH 1953 remarked that leuco-anthocyanins occur more commonly in the tissues of woody plants. BATE-SMITH & LERNER 1954 further observed that in the family *Papilionaceae,* the ligneous members like *Cladrastis, Robinia, Wisteria, Derris, Machaerium* and *Pterocarpus* are positive for leuco-anthocyanins, whereas *Sophora* and *Laburnum* are negative. Among the herbaceous members, the tribe *Hedysareae* is persistently positive for leuco-anthocyanins. PECKET's 1959 work on the leaf extracts of *Lathyrus* also showed that leuco-anthocyanins are of rare occurrence in herbaceous taxa. From the botanical point of view, tannins are phenolic substances which give a characteristic appearance in microscopical preparations such as those which have been fixed and stained with ferric chloride or ferric acetate. BATE-SMITH & METCALFE 1957 showed that the presence of tannins can be used as a taxonomic character. An interesting parallelism has been noted between the occurrence of tannins and phylogenetic status of the families in which they occur. These authors believe that the capacity to synthesize tannin is a primitive character which is lost along with an increasing phylogenetic specialization. The reduction or complete loss of tannin production is often most marked in herbaceous plants and not so in the woody ones. Recently DEWET & SCOTT 1965 have shown that the essential oils extracted from the inflorescences of *Bothriochloa* spp. can be used as taxonomic criteria. The chromatograms could be reproduced exactly from samples of the same collection obtained at different times from the field or under glass. The chemical data were often found to be more reliable than gross morphology in determining taxonomic affinities. All these works give an indication of the utility of such biochemical data in the field of systematics.

The recent work of TATEOKA 1962 suggests that in the family *Gramineae,* certain features of the starch grains are common to most of the members of a particular tribe or a subfamily. In a few cases, the starch grains of the genera within a tribe or the species within a genus also differ in some respects. However, the present study of the starch grains in the family *Leguminosae* shows that such a generalisation cannot be made. In contrast to other biochemical products like leuco-anthocyanins and tannins, the starch grains are of common occurrence in the advanced taxa of the *Papilionaceae.* These are, to a lesser extent,

found in the *Mimosaceae* and none to be observed in the *Caesalpiniaceae*. Out of twenty ligneous species examined by us, only four showed the presence of starch grains. It is believed that starch grains are of rare occurrence in the woody plants of the family *Leguminosae* (see Table 5).

Table 5

Occurrence of Biochemical Products in *Leguminosae*

Biochemical Products	Herbaceous taxa	Ligneous taxa
Leuco-anthocyanins	Rare	Abundant
Tannins	Rare	Abundant
Starch grains	Abundant	Rare

Summary

The paper deals with the occurrence and morphology of starch grains in the seeds of the family *Leguminosae*. Out of 76 ligneous and herbaceous taxa examined in this study, 30 species belonging to 14 genera showed their presence in seeds. In contrast to other biochemical products like leuco-anthocyanins and tannins, the starch grains are of common occurrence in the herbaceous taxa of tropical legumes. The taxonomic value of starch grains in the genera *Phaseolus, Dolichos, Rhynchosia, Cajanus, Moghania, Cicer, Lathyrus, Lens, Pisum* and *Vicia,* is discussed. The starch grains were not observed in the following taxa: *Cassia tora* L., *C. obtusifolia* L., *C. occidentalis* L., *C. surattensis* BURM. f. var. *surattensis, C. auriculata* L., *C. artemisioides* GAUD. ex DC., *Poinciana pulcherrima* L., *Delonix regia* RAF., *Bauhinia purpurea* L., *B. racemosa* LAMK., *Acacia leucophloea* WILLD., *A. modesta* WALL., *Albizia lebbeck* BENTH., *A. procera* BENTH., *Desmanthus virgatus* WILLD., *Leucaena leucocephala* WIT, *Mimosa hamata* WILLD., *M. pudica* L., *M. rubicaulis* LAMK., *Prosopis glandulosa* TORR., *P. juliflora* DC., and *P. cineraria* DRUCE (syn. *P. spicigera* L.). However, a few woody plants like *Dalbergia sissoo* ROXB., *Pongamia pinnata* PIERRE, *Acacia auriculiformis* A. CUNN., and *Pithecellobium dulce* BENTH. indicate the presence of starch grains in seeds. It is believed that starch grains are of rare occurrence in the woody plants of the family Leguminosae.

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