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Variation in the Exine Ornamentation Patterns in Taro (*Colocasia esculenta* (L.) Schott.) with respect to Geographical Distribution

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Abstract

Light microscopic (LM) and scanning electron microscopic (SEM) observations were carried out in taro (*Colocasia esculenta)* pollen grains for detecting intra-specific variability as reflected in pollen features at Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India. The pollen grains were found to occur in monads and were inaperturate. Pollen diameter was found to be below 25μm and the shape was globular or spheroidal. Despite the uniformity in aperture type, pollen shape and size, wide intraspecific variations were observed with regard to the exine sculpturing. The echinate excrescence characteristic of the family Araceae was observed in all the accessions studied. The exine surface showed tilate (tile shaped) appearance with the arrangements of basal plates holding the column and wide variations were observed in the size, shape, arrangement and pattern of these basal plates. Based on these features five basic types of exine ornamentation pattern were observed in taro. The variations observed in the exine ornamentation pattern were correlated with the geographical distribution of the plants under study.

Key words: Taro, exine sculpturing, echinate excrescence, basal plate, tilate

Introduction

Taro (*Colocasia esculenta* (L.) Schott.) is an important tuber crop of the tropical and subtropical regions. It is a traditional food of cultural importance and is an integral part of social rituals in India and the South Pacific countries. The family Araceae, to which taro belongs is a herbaceous monocot with 106 genera and about 2500 species (Mayo et al., 1997). It is a vegetatively propagated crop with innumerable morphologically distinguishable types in terms of plant height, tillering, basal girth of the shoot, leaf size and shape, petiole length and colour and acridity of the plant parts, in addition to the size and shape of corms and cormels and size of the sterile tip of the spadix (Nusaifa Beevi, 2009). Accordingly, there are over thousand recognized cultivars of taro in the world. Pollen characters which have high taxonomic importance have been grouped into five categories, of which those relating to the germinal aperture are considered to be of primary importance, exine surface ornamentation, secondary and others such as exine strata, pollen size and shape, tertiary. The significance of variations of these attributes in cultivar taxonomy has been well documented in many genera (Nair, 1974; Nair and Kapoor, 1974). Studies with the aid of scanning electron microscope (SEM) and transmission electron microscope (TEM) in recent years have revolutionized the scope and depth of pollen study as they enable observations of exine sculpturing and exine strata with profound precision. The exine details are so specific and characteristic that they could be profitably used in plant identification

(Heywood, 1967). Electron microscopy has made it possible to describe exine architecture with precision, and has been used to detect even subtle variations of pollen wall features including the exine excrescence patterns.

The information available on the pollen morphology of taro is meagre and fragmentary. Nusaifa Beevi and Sreekumari (2006) made a preliminary study of the pollen morphology of taro and reported that the taro pollen grains occured in monads and were globose, inaperturate, mono to dimorphic with spinose excrescence system. A recent study on the pollen morphology of 12 taro accessions of the South Indian states showed that despite the constancy in aperture type, pollen shape and size, wide intraspecific variation was observed with regard to the exine sculpturing. The nature of interplate groove (or its absence) and the interspinal distance make the exine surface different from one another to a certain extent (Nusaifa Beevi and Sreekumari, 2009). Jayalakshmi (2006) characterized the pollen of some species of Araceae (Genera: Theriophonum; Typhonium) and observed the significance of spine in the taxonomy of the taxa. The present study deals with the variations observed in 12 taro accessions with regard to the exine ornamentation pattern with an attempt to classify the accessions in relation to the geographical variations by means of light microscopic and scanning electron microscopic studies.

Materials and Methods

The materials of the present study comprised of 60 taro accessions collected from different sources around the South Indian states (Kerala, Tamil Nadu and Karnataka). The accessions were conserved, multiplied and the experiment was laid out in Randomized Block Design in the experimental fields of Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India. Out of these 60, 12 accessions flowered, which alone were subjected to palynological studies. These included seven cultivated diploids, four wild diploids and one cultivated triploid, collected from different geographical regions of Kerala and Tamil Nadu (Table 1). During flowering, the fresh pollen grains were collected from live plants and fixed in glacial acetic acid. Pollen preparations were made by the acetolysis method standardized by Erdtman (1952). The acetolysed pollen was stored in 1:1 glycerin and distilled water for two weeks and permanent slides for LM studies were prepared by mounting it in glycerin jelly and sealed with paraffin wax. For SEM studies, the acetolysed pollen were dried and mounted on specimen studs, vacuum coated with gold using a JEOL-JFC-1200 Fine Coater (Walker, 1974) and observed under the Scanning Electron Microscope.

Morphological features of pollen grains including those relating to the aperture, exine ornamentation, pollen size and shape were studied from LM and/or SEM observations. For ascertaining the shape class, the classification suggested by Erdtman (1966) was followed. The pollen grains were grouped into different size classes following Walker and Doyle (1975). For describing the aperture types and exine ornamentation pattern of the pollen grains, the terminologies suggested by Punt et al. (1994) and Nair (personal communication) have been used. For LM studies, photomicrographs of the pollen preparations were made using an Olympus BH-2 photomicroscope at the Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. Using JEOL-SEM-5600LV scanning electron microscope, SEM pictures were taken at the Regional Research Laboratory, Pappanamcode, Thiruvananthapuram. Exine ornamentation pattern was studied in detail with the help of SEM pictures.

Results and Discussion

Light microscopic studies

With regard to the primary features, the grains were inaperturate and therefore variations in this feature were rather nil. However, some degree of variability was noticeable in the secondary feature namely the exine architecture. The study revealed that the excrescence system was echinate and the excrescence shape in the studied accessions was always spinate. Pollen size is a teritiary feature and for this, the grains ranged from small to medium. The pollen grains were monomorphic to dimorphic with regard to their size, but the degree of size variation was very marginal. The shape was globular or spherical. Monad nature and spherical shape of pollen grains were well illustrated (Fig. 1). Inaperturate pollen grains with globular or spherical, small to medium sized and spinous excrescence system was previously reported (Grayum, 1992; Jayalakshmi, 2006; Nusaifa Beevi and Sreekumari, 2006).

Table 1. Details of the 12 accessions of taro (cultivated and wild allies)

Code No. (ploidy status)	Source of collection	Germplasm type	Collection site	State of collection
Co-6 (2n)	Market	Cultivar	Kanayannoor, Ernakulam	Kerala
Co-8 (2n)	Field	Cultivar	Salem	Tamil Nadu
Co-13 (2n)	Field	Cultivar	Thrissur	Kerala
Co-19 (2n)	Market	Cultivar	Erode	Tamil Nadu
Co-22 (2n)	Field	Cultivar	Sivakasi, Virudhanagar	Tamil Nadu
Co-25 (2n)	Field	Cultivar	Nedumkandam, Idukki	Kerala
Co-28 (2n)	Backyard	Wild	Ranni, Pathanamthitta	Kerala
Co-31 (2n)	Backyard	Wild	Arcot, Vellore	Tamil Nadu
Co-39 (2n)	Roadside	Wild	Kadakkal, Kollam	Kerala
Co-41 (2n)	Roadside	Wild	Vazhayila, Thiruvananthapuram	Kerala
Co-44 (2n)	Market	Cultivar	Mannarkkadu, Palakkadu	Kerala
Co-52 (3n)	Field	Cultivar	Thiruvananthapuram	Kerala

The nature of pollen wall provided a multitude of phylogenetically important characters including pollen wall morphology, its structural components (various layers or strata) such as exine (pollen wall) stratification and external structural elements leading to exine structural sculpturing.

Exine stratification

Exine stratification refers to the various layers (strata) present in the pollen wall as observed morphologically, chemically and/or developmentally (teritiary feature). The exine of acetolysed angiosperm pollen grains

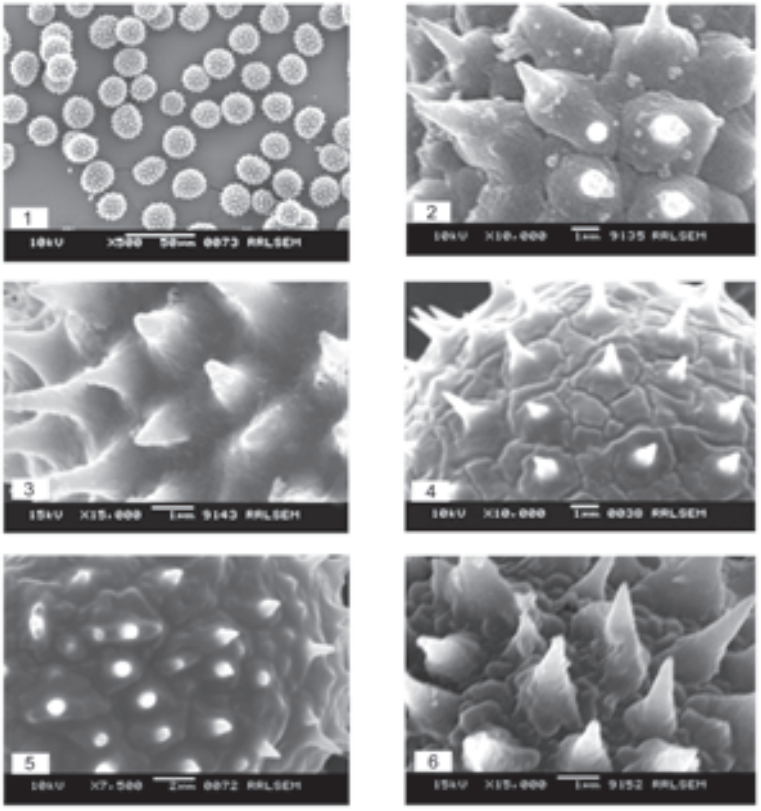

Fig. 1. General view of inaperturate pollen grains in monads
Fig. 2. Exine ornamentation pattern in Type 1 pollen
Fig. 3. Exine ornamentation pattern in Type 2 pollen
Fig. 4. Exine ornamentation pattern in Type 3 pollen
Fig. 5. Exine ornamentation pattern in Type 4 pollen
Fig. 6. Exine ornamentation pattern in Type 5 pollen

(acetolysis - resistant layer composed of oxidative polymers of carotenoids and/or carotenoid esters known as sporopollenin) as observed in the light microscope typically differentiated into two layers, an inner basal non sculptured layer called endexine or nexine, and an outer sculptured exine, the ektexine or sexine.

Scanning electron microscopic studies

Exine structural sculpturing

Study of the acetolysed pollen wall usually refers to the study of exine, since the inner wall, intine, which was more or less cellulosic was destroyed during acetolysis. So the present study explains only the external structural elements or exine sculpturing (secondary feature) as detailed below.

The basic excrescence system of taro

Exine sculpturing refers to surface topography of pollen grains. The echinate (height and basal diameter of spine more or less equal) excrescence characteristic of the family Araceae was observed in all accessions studied. The excrescence system observed in the present study was not simple, but a complex structure, which consisted of an external spine (column) and a basal hold (Fig.7). Basically each spine was the exposed portion and was slanting and wider towards the base. The basal hold was divided into two parts, an upper socket (basal collar) in which the basal part of the spine was incorporated and a basal plate from which the socket arose. A number of basal plates were arranged together to form the surface topology. In between two adjacent basal plates there was a groove, which may be deep or shallow. This was called the interplate groove. Difference in the size and shape of the basal plates and the depth or shallowness of the interplate groove made the floor surface entirely different from one another. The sides of the basal plate may be straight, zigzag or wavy. Totally, the exine surface made a tilate appearance which means that the arrangement looked like a tiled floor. But, there were accessions with no interplate groove, which made the general surface smooth.

Based on differences observed in the exine ornamentation pattern, five basic types of surface topology were observed in the present study as detailed below.

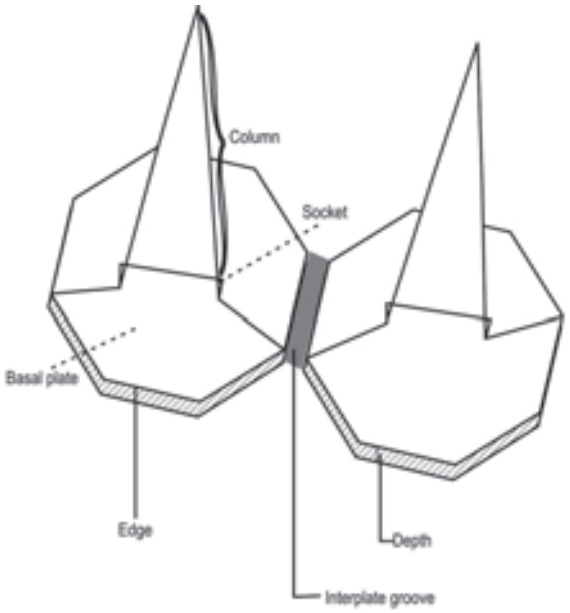

Fig.7. The basic excrescences system in taro (two adjacent basal plates are shown)

First type

Pollen grains small, slightly triangular and measured 17.0-20.0 μm ($\bar{x}$ = 18.0 μm) across. Spines 50-60 ($\bar{x}$ = 55) per hemisphere and monomorphic. Column spinate, straight and broader towards the base with a bulbous basal column (socket-base). Interspinal distance 1.6-2.7 μm ($\bar{x}$ = 2.1 μm). Floor tilate and each basal plate (tile) was monospinate, circular or slightly isodiametric and heteromorphic, with mildly sinuous edge. Each tile was bulbous or slightly cushioned with narrow, but deep interplate groove. Warted projections of varying size occurred on the surface (Fig.2). This exine surface pattern was observed in a single cultivated accession collected from Erode, Tamil Nadu (Co-19).

Second type

Pollen grains small and measured 15.0-20.0 μm ($\bar{x}$ = 17.5 μm) across. Spines 80-90 ($\bar{x}$ = 85) per hemisphere and monomorphic. Column spinate, straight and broader towards the base with a conical basal column. This basal column formed the floor of the pollen surface. Interspinal distance 1.0-2.0 μm ($\bar{x}$ = 1.5 μm). Floor non tilate with deep interspinal area. Minute warts or hairs were present throughout the surface (Fig.3).This was an entirely different exine surface pattern and was exhibited by a single cultivated accession collected from Palakkadu district (Mannarkadu) of Kerala state (Co-44).

Third type

Pollen grains small and measured 17.5-23.5 μm ($\bar{x}$ =21.0 μm) across (Fig.4). Spines 50-60 ($\bar{x}$ =55) per hemisphere and monomorphic. Column spinate, slanting and broader towards the base with a conical basal column. Interspinal distance 2.5-3.5 μm ($\bar{x}$ =3.0 μm). Floor tilate and each basal plate (tile) was monospinate, angular and heteromorphic, with straight or wavy edge. Each tile was more or less flat with deep and clear interplate groove (Fig. 4). This was the typical condition and was observed in a cultivated accession collected from Nedumkandam, Idukki of Kerala state (Co-25). Similar conditions were also observed in two more cultivated accessions, one from Kanayannoor, Ernakulam of Kerala (Co-6) and the other from, Sivakasi, Virudhu Nagar of Tamil Nadu (Co-22).

Fourth type

Pollen grains small and measured 17.0-22.0 μm ($\bar{x}$ =20.0 μm) across. Spines (together with spinules) 70-80 ($\bar{x}$ =75) per hemisphere, mono or slightly heteromorphic with regard to its shape and size. Column spinate, straight and broader towards the base with a conical basal column. Excrescence tip acute. Interspinal distance 1.5-3.2 μm ($\bar{x}$ =2.5 μm). Floor tilate and each basal plate (tile) monospinate and slightly elevated. Interplate groove was wide and shallow (Fig.5).

This pattern was observed in six accessions, two cultivated (Co-8 and Co-13) and four wild ones (Co-28, Co-31, Co-39 and Co-41) collected from different localities of Kerala and Tamil Nadu. The accession, Co-8 was from Salem, Tamil Nadu and Co-13 was from Thrissur, Kerala. Similarly out of the four wild accessions, Co- 28 was from Ranni, Pathanamthitta, Co-31 from Arcot, Velloore, Co-39 from Kadakkal, Kollam and Co-41 from Vazhayila, Thiruvananthapuram, Kerala.

Fifth type

Pollen grains small and measured 22.0-28.0 μm ($\bar{x}$ =24.0 μm) across. Spines 85-95 ($\bar{x}$ =90) per hemisphere and monomorphic. Column spinate, straight and broader towards the base with a pocket like basal column. Excrescence tip blunt. Floor non tilate and basal plate was not well developed. Column spinate, with a pocket like basal column but with an interspinal distance of 1.5 - 2.5 μm ($\bar{x}$ =2.0 μm). Interspinal area was completely occupied by small bud like warts or tubercles (Fig.6). This condition was shown by a single triploid accession (Co-52) collected from Thiruvananthapuram, Kerala.

In the five basic types of exine patterns, based on the variations in exine sculpturing, the first, second and fifth type of exine ornamentation pattern was unique for each single accession. The first type (Co-19) had somewhat different basal plate which was clearly bulbous. Here the pollen grains were slightly triangular in shape and with monomorphic spines (Fig.2). Interspinal groove was totally absent in the second type of pattern (Co-44). Here the number of spines was maximum per hemisphere and monomorphic. Interspinal distance was also least for this pattern. Minute hairs were present throughout the deep surface (Fig.3). While the fifth type was an entirely different one with a narrow basal plate area entirely filled with spinules or warts (Co-52, triploid) (Fig.6). Along with spines, spinules and warts appear in certain accessions, resulting in spine morphism, which was first reported in taro by Nusaifa Beevi and Sreekumari (2009).

In the third pattern found in three cultivated accessions, the floor was tilate and each basal plate (tile) was monospinate. The condition observed in Co-25 was considered as the basic type of excrescence system observed in taro (Fig.7). The other two accessions also resembled this but there was some sort of variation such as in Co-6, where a slightly cushioned basal plate was seen and Co-22, with not as much clear tiles. This difference might be due to their different geographical locations. The accession, Co-22 was the most different one and this cultivated accession was confined to Tamil Nadu, while, the other two looked more similar and were found in Kerala only. In the fourth pattern, which included majority of accessions (six), two cultivars (Co-8 and Co-13) and four wild (Co-28, Co-31, Co-39 and Co-41) from different localities of Kerala and Tamil Nadu, were grouped. The two cultivated accessions showed close similarities. In the same way the four wild accessions, viz., Co-28, Co-31, Co-39 and Co-41 also showed close similarities among themselves and this might be due to their common wild habitat.

In Araceae, the exine strata (tertiary feature) consisted of a perforated tectum, an insterstitium, formed generally by granular columellae and a robust foot layer together making the ektexine and a thin endexine (Grayum, 1992;

Hesse, 2002; 2006). Pollen wall or exine stratification study in detail (teritiary feature) needs transmission electron microscopic (TEM) observations. The intine is thin in members of Araceae (Hesse, 2002) and *Colocasia* was found to be less resistant to acetolysis (personal experience).

From the present study, certain correlations might be suggested with regards to the geographical distribution of the present flowering accessions and the exine ornamentation pattern. The simple tilate floor observed in Co-19 (first type) was entirely different from other simple tilate accessions, which might be correlated with the locality of this cultivar, which was collected from Erode of Tamil Nadu. Similarly, the simple tilate condition observed in Co-44 (second type) also had a unique pattern (non tilate exine surface) which was collected from Mannarkkadu, Palakkadu district of Kerala. The accession, Co-25 (third type), collected from Nedumkandam, Idukki district of Kerala state, a high range area, also showed an entirely different exine pattern, which was considered as the basic type of excrescence system observed in taro. The accession, Co-41 (fourth type) was a wild one collected from Vazhayila, Thiruvananthapuramm of Kerala state. It had an entirely different ornamentation with composite tilate floor. Composite tilate condition was generally observed in wild accessions (Nusaifa Beevi and Sreekumari, 2009). The exine ornamentation pattern observed in Co-52 (fifth type) was entirely different from the other cultivated or wild accessions. This unique exine pattern was seen only in a triploid cultivar. Even though some correlations were apparent between geographical distribution pattern and exine ornamentation pattern, present data is inadequate to make a concrete suggestion in this regard.

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