

SHORT RESEARCH PAPER

The protective shell: sclereids and their mechanical function in corollas of some species of *Camellia* (Theaceae)

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ABSTRACT

Studies of rain-wash effects on pollen have shown that flower structures can protect susceptible pollen from rain. It remains unclear, however, how a thin corolla can withstand external force and perform its protective function. The sclereids in petals of several species of *Camellia* (Theaceae) were anatomically investigated to determine their mechanical properties. To examine the effects of changing physical environment on the occurrence of sclereids in petals, sclereid density in petals of six species, including wild samples from different rainfall zones and samples from a greenhouse under mild conditions without wind and rain, were examined and statistically analysed. The results showed that the occurrence of sclereids in petals varied with physical environment. The number of sclereids in the same species increased with the increasing rainfall. There were abundant sclereids in petals of the wild species, but few or no sclereids in species cultivated in the greenhouse. Moreover, the anatomical features of sclereids, especially the unique distribution pattern that has not hitherto been described, were correlated with external environmental pressures. Our observations reveal a novel mechanical system in the corolla and provide further evidence for the hypothesis that flower structures may protect rain-susceptible pollen.

INTRODUCTION

In addition to biotic factors, flowers are also under strong selection from forces in their physical environment; rain is one such external, abiotic force (Kerner 1902; Corbet 1990; Huang *et al.* 2002; Sun *et al.* 2008; Mao & Huang 2009). To protect against the deleterious effects of rain, which can lead to the physical removal of pollen, dilution of nectar and a decrease in pollen viability due to excess water, flowers have evolved various adaptive strategies. The bracts or corollas of downward-facing flowers act as shielding umbrellas (Sun *et al.* 2008), while some upright flowers close their corolla to shelter the stamens from rain (Hart 1990; Simons 1992; Dafni 1996). Experimental studies have shown that these adaptive strategies are efficient ways to protect reproductive structures and thus enhance reproductive success (Bynum & Smith 2001; Huang *et al.* 2002; Hase *et al.* 2006; He *et al.* 2006; Mao & Huang 2009).

The studies mentioned above focused mainly on fully opened flowers, with very few studies on the pre-anthesis stage. On the one hand, the response of pollen to rain has been investigated extensively, but the corolla as a rain shelter has received less attention. Traditionally, the anatomical structure of the petal is considered to be extremely simple. It is made up of several layers of cells: ground parenchyma, a more or less branched vascular system and an epidermis. In addition, crystal-containing cells, laticifers, tannin cells and

other idioblasts may occur in the ground tissue in some special cases, but the petals have generally been considered to lack mechanical tissue (Esau 1965; Fahn 1990). Thus, there is apparently no information concerning how the soft structure of petals performs its protective function.

The occurrence of sclereids is a widespread phenomenon among higher plants (Foster 1944; Tomlinson 1959; Rao 1965; DE Roon 1967; Rao & Das 1979; Kiew & Ibrahim 1982; Franceschinelli & Yamamoto 1993; Trift & Anderberg 2006; Zhang *et al.* 2009). They are of special interest because of their large size, bizarre forms and sharp demarcation from neighbouring tissue elements. Sclereids are generally considered to be of mechanical significance in view of their lack of living plasma, their elasticity and thick secondary walls (Esau 1965; Fahn 1990). Most of what we know about sclereid function comes from foliar sclereids, although sclereids are widely present throughout the plant body. However, sclereids in other plant parts have received little attention (Bender 1967, 1975).

In this paper we report anatomical investigations of the corolla of several species of *Camellia*, typical winter-flowering plants distributed mainly in subtropical areas in China, where winter rainfall frequently occurs. We provide the first full description of sclereid features in the corolla of *Camellia* and discuss their protective functions, which might shed light on the hypothesis that flower structures have evolved partly to protect susceptible pollen from rain.

MATERIALS AND METHODS

Study species and sites

Camellia species are evergreen shrubs or small trees that flower mainly in winter, from November to February of the next year. The flowers are usually white or pink, axillary or subterminal, solitary or up to three in a cluster; typically with two to three bracteoles, dispersed on the pedicel; five to six nearly verticillate and often persistent sepals; five to six or more petals in single or double whorls that close in humid environments. The best-known species of this genus is the tea plant, *Camellia sinensis* L.

Material of six species of *Camellia* from five wild localities and several samples of one species growing in the greenhouse were selected (Table 1). Fresh, fully opened and closed corollas were fixed in formalin–acetic acid–alcohol (FAA) solution for at least 48 h and stored in 70% alcohol.

To examine whether sclereid occurrence is primarily influenced by genetic traits or by environmental factors, or, more precisely, to what extent sclereid features are influenced by either factor, the following observations and comparisons were made: (i) Sclereids from different species in the same site with the same climate conditions were examined and compared; (ii) Sclereids of the same species from different climate conditions, especially in terms of rainfall, were examined and compared. Because most *Camellia* species have restricted distributions, only one species, *C. sinensis*, the widely cultivated tea plant, was selected from the following sites: Menghai in Yunnan, Yongchuan in Chongqing and Conghua in Guangdong, with average winter rainfall of 48, 85 and 91 mm, respectively; and (iii) To examine the occurrence of sclereids in conditions without wind and rain, we transplanted several samples of *C. japonica* to a greenhouse, cultivated them for 2 years and recorded their morphology and number of sclereids in petals each year.

Tissue clearing and sectioning

For clearing, corollas stored in 70% alcohol were rinsed in water, cleared in 5% NaOH and left overnight in an oven at 45 °C. The NaOH was changed twice a day until clearing was

satisfactory. After washing in distilled water several times, the corollas were transferred to 15% NaClO for 2 h to remove any cloudiness. Cleared and bleached corollas (or portions of them) were then rinsed in water, dehydrated through an ethanol series, stained with 1% safranin and 0.5% fast green, then mounted on slides with neutral balsam for observation. Materials were sectioned using standard methods for paraffin embedding and serial sectioning at 12- μ m thick.

Sclereid number counts

Sclereid density counts were taken from five random fields (field size = 1 mm²) per slide using a microscope (Axio imagerA1, Carl Zeiss Co., Shanghai, China).

Statistical analysis

All data were examined for normal distribution with a one-sample Kolmogorov–Smirnov test. The data in species pairs were examined with an independent-samples t-test to identify any significant differences between each pair. One-way ANOVA was performed to identify any significant differences among sclereid number (mm⁻²) of the same species from different sites. All analyses were performed in SPSS (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA) and data were plotted using SIGMAPLOT (version 10.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Form of corolla sclereids

The sclereids were observed both in fully opened and closed corollas (Fig. 1d,e). They exhibited great diversity of form due to variations in body shape and branch number. In general, the sclereids could be divided into two main groups: monomorphic sclereids characterised by a long base with short processes and polymorphic sclereids with a complex base and long arms. A typical monomorphic sclereid has a spindle-like body without obvious branches (fusiform sclereid, Fig. 1i); polymorphic sclereids have a columnar body with branches at either (rhizo-sclereid) or both ends (ramiform sclereid) (Fig. 1g,j). Irrespective of form, both types

Table 1. Sclereid features in different material from various localities.

species	sclereid form	distribution pattern	density (mm ⁻²)	locality
<i>Camellia assamica</i>	polymorphic	restricted to central part	60	Menghai, introduced from Changning, Yunnan, China
<i>Camellia grijsii</i>	monomorphic	restricted to central part	89	Jinhua, Zhejiang, China
<i>Camellia gymnogyna</i>	polymorphic	restricted to central part	80	Pingbian, Yunnan, China
<i>Camellia japonica</i>	polymorphic	restricted to central part	52	Yongchuan, Chongqing, China
<i>Camellia japonica</i>	monomorphic	restricted to central part	15 ^a	Greenhouse of Beijing botanical garden, Beijing, China
<i>Camellia japonica</i>	monomorphic	scattered in central part	4 ^b	Greenhouse of Beijing botanical garden, Beijing, China
<i>Camellia kiss</i>	monomorphic	restricted to central part	77	Jinhua, Zhejiang, China
<i>Camellia sinensis</i>	polymorphic	restricted to central part	54 ^c	Menghai, introduced from Luxi, Yunnan, China
<i>Camellia sinensis</i>	polymorphic	restricted to central part	61 ^c	Yongchuan, Chongqing, China
<i>Camellia sinensis</i>	polymorphic	restricted to central part	66 ^c	Conghua Guangdong, China

^a1 year after transplanted.

^b2 years after transplanted.

^cin the inner (closed) petal.

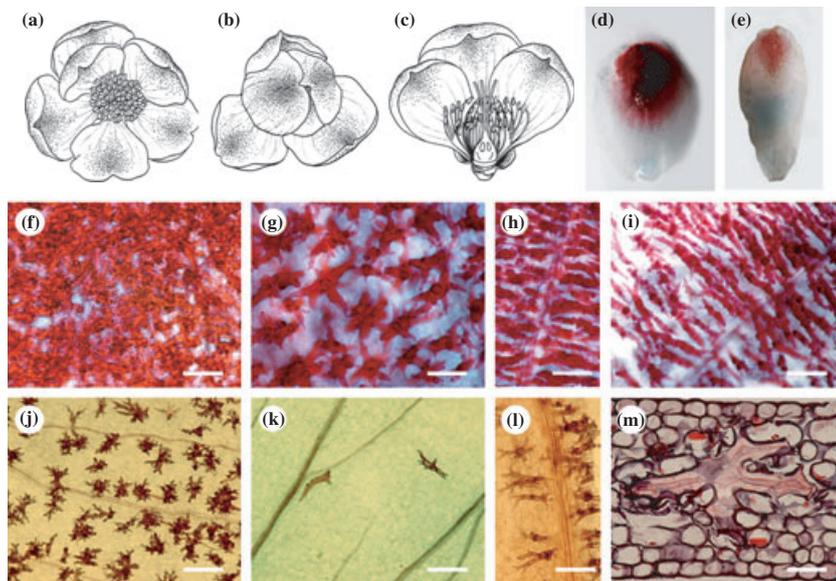


Fig. 1. Sclereids and their distribution in the corolla of species of *Camellia*. a–c: illustrations showing distribution of sclereids in fully opened and closed corolla. d–l: sclereids in cleared petals of species of *Camellia*, d,e: entire cleared petal, d: outer petal, e: inner petal; f–l: central region of petal. d, e, h: *C. grijsii*; i: *C. kissi*; f: *C. gymnogyna*; g: *C. japonica* from wild; j, m: *C. sinensis*; k: *C. japonica* from greenhouse; l: *C. assamica*. m: sclereid in transverse section of petal. Scale bars = 200 μm (f–l) and 50 μm (m).

have thick striated walls and a lumen of irregular width (Fig. 1m).

Disposition and distribution of corolla sclereids

The surface distribution pattern of sclereids is also worthy of special attention. Despite their great diversity in morphology, sclereids were distributed consistently in certain definable regions of the petal blade (Fig. 1a–e). The cleared petal showed abundant sclereids in its apical or central region, both in the inner (if any) and outer petals (Fig. 1d,e), where the elongated branches of the sclereids became entangled and collectively formed a loose, net-like layer (Fig. 1f). When the corolla is closed, this layer forms a mechanical shell that covers the ovary and stamens (Fig. 1b,c). On the surface, sclereids were found scattered singly in the petal mesophyll (Fig. 1g–l) with their main axis approximately parallel to the surface of the lamina and more or less perpendicular to the vein (Fig. 1h,i,l). In transverse section, the petal mesophyll is made up of several layers of parenchyma cells with the sclereids disposed in the middle part of the mesophyll. The sclereid branches usually extended into neighbouring intercellular spaces and also penetrated between adjoining mesophyll cells (Fig. 1m).

Variation in corolla sclereids in different species and different environments

Sclereids constantly occurred in petals of all six wild species studied, which cover three sections of the genus *Camellia*.

Table 2. Analyses of variance for sclereid number from three species pairs.

site	species pair	Levene's test for equality of variances		t-test for equality of means	
		F	P	df	P
Menghai	<i>Camellia sinensis</i> versus <i>Camellia assamica</i>	0.019	0.894	12	0.007
Jinhua	<i>Camellia grijsii</i> versus <i>Camellia kissi</i>	0.045	0.836	12	0.000
Yongchuan	<i>Camellia sinensis</i> versus <i>Camellia japonica</i>	0.395	0.541	12	0.000

Species of the same section had the same sclereid type, e.g. *C. grijsii* Hance and *C. kissi* Wallich of section *Paracamellia* had mainly monomorphic sclereids, while *C. assamica* (Masters) Hung T. Chang and *C. sinensis* (L.) Kuntze of section *Thea* had mainly polymorphic sclereids. The sclereids of three species pairs from three sites showed some differences in numbers. The sclereid number showed no obvious difference within a species ($P > 0.05$), however, there was a significant difference between species pairs at the same site ($P < 0.05$) (Table 2). The above results suggest that genetic traits of one species have some influences on sclereid morphology and density.

The average sclereid number in *C. sinensis* from Menghai, Yongchuan and Conghua was 54, 61 and 66, respectively, being significantly positively correlated with the average winter precipitation (Fig. 2). The average number of petal sclereids decreased significantly after *C. japonica* was transplanted to a greenhouse and grown for 2 years; falling from 52 prior to greenhouse treatment to 15 in the first year and to four in the second year (Table 1). In addition, the morphology changed from polymorphic prior to greenhouse growth to monomorphic after 2 years in a greenhouse (Table 1, Fig. 1k).

DISCUSSION

Sclereids are widely distributed in leaves and other organs of about 430 plant genera belonging to 110 families and covering over 47 orders of angiosperm (Rao & Bhattacharya 1978). The most vital question regarding sclereids is: why do plants possess sclereids? In other words, what selective advan-

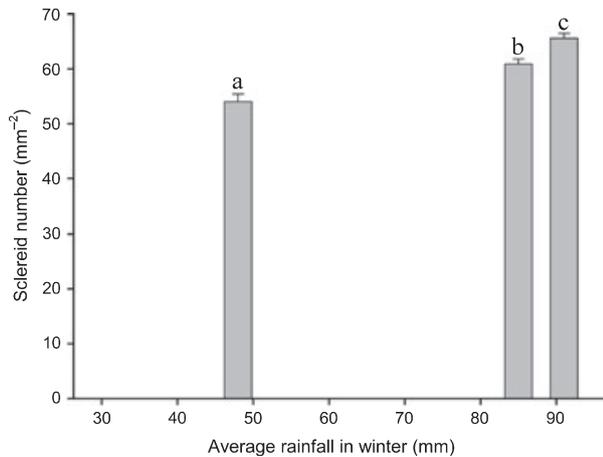


Fig. 2. Comparison of sclereid number among *Camellia sinensis* from three sites with different average rainfall in winter. a: Menghai, b: Yonchuan, c: Conghua.

tage does the presence of sclereids confer (Rao & Bhattacharya 1978)? By virtue of their thick lignified secondary wall, they are considered to have mainly mechanical properties, protecting soft tissue from mechanical or herbivore damage (Grant 1950; Rao 1957; Esau 1965; Metcalfe 1979; Fahn 1990; Nyffeler *et al.* 1997). Moreover, they may function as vein extensions, conducting water to the epidermis and directly to both palisade layers (Heide-Jorgensen 1990). Recently, it was suggested that they play an optical role, guiding light to mesophyll areas with insufficient light supply (Karabourniotis *et al.* 1994; Karabourniotis 1998). Most of what we know about sclereid function comes from foliar sclereids. The function of sclereids in the ephemeral corolla of flowers is little understood.

Our investigation of six species of *Camellia* indicates that sclereids are a constant anatomical character. The species investigated are typical winter-flowering plants that are subject to frequent winter rain and moisture. Field observations have determined that the flowering period of a flower of *Camellia* is about 4 or 5 days, and about 10 days before the flower opens, the anthers have already developed pollen. This period between pollen production and anthesis is vital for successful reproduction and thus any possible damage from outside may be fatal. Fortunately, the corolla is mainly closed during this period, and even at anthesis the corolla closes again in humid weather. Corolla movement in such a manner has been suggested to be a means for protecting pollen from rain and moisture during inclement weather (Bynum & Smith 2001; Hase *et al.* 2006; He *et al.* 2006; Mao & Huang 2009). When these hypotheses are considered in conjunction with the mechanical property of sclereids in *Camellia* petals, it is reasonable to assume a correlation between the presence of sclereids and the protective function of the petals.

Petals are made up of several layers of cells, and to counteract outside pressure, the petals of *Camellia* have developed sclereids and other related properties. First, the unique surface distribution pattern of sclereids is worth some attention. Sclereids are distributed abundantly and exclusively in the central region of the petal (Fig. 1a–e). When the petals are closed, the regions overlap and form a rigid sclereid shell

(Fig. 1b,c), giving additional strength and rigidity to resist external forces. In addition, the main axis of the sclereids is oriented approximately parallel to the surface of the lamina and more or less perpendicular to the vein (Fig. 1h,i,l). This arrangement is obviously able to withstand much more external force. Moreover, the sclereid arms and processes extend into neighbouring intercellular spaces and also penetrate between adjacent mesophyll cells, uniting the cells and tissues (Fig. 1m).

Previous studies revealed sclereid differentiation in response to changing physiological environment. Foard (1958, 1959) observed that high sugar concentrations in the medium inhibited sclereid differentiation in the leaves of *Camellia*. He later showed that sclereid formation was determined by position along the *Camellia* leaf margin. Through histochemical testing, Mia & Pathak (1965) showed intensified enzyme activity of cytochrome oxidase was correlated with sclereid initials. Some other authors (Al-Talib & Torrey 1961; Savidge 1983), however, claim that plant hormones, especially IAA, were essential for sclereid occurrence. These views have been confirmed here from our results for petal sclereids in *Camellia* species.

The number of petal sclereids in three populations of *C. sinensis* from different climatic zones increased with increasing rainfall (Fig. 2). This indicates that changing physiological environment influences sclereid occurrence. Moreover, we compared variations in the sclereids in *C. japonica* from wild and greenhouse plants and present further functional evidence for their initiation. In the wild, the corolla must develop structures to protect the reproductive parts from possible external pressure, especially rain. In contrast, the greenhouse provides a mild and stable environment without wind and rain, where it appears unnecessary for the corolla to expend energy to develop useless mechanical support systems. One may argue that the glass of the greenhouse might block particular wavelengths that are required to stimulate production of sclereids, since it has been found that flavonoid production decreases in greenhouse plants due to the filtered light (Raviv & Antignus 2004; Lau *et al.* 2006). However, we examined sepals of the same flower from the greenhouse plant, and found a large number of sclereids, as in wild plants.

In view of our investigations and statistics of variation in petal sclereids both in the field and in artificial conditions, and in combination with anatomical mechanically properties, it is reasonably to suspect that sclereid occurrence in *Camellia* is strongly function-related. We thus present evidence consistent with the hypothesis that flower structures can protect susceptible pollen from rain. Sclereids in the mesophyll of the corolla constitute a novel mechanical system, making the corolla rigid enough to withstand all possible external pressures. Thus, sclereids in the corolla of species of *Camellia* are a strikingly novel example of how seemingly delicate petals respond to the pressures of their environment by functioning as organs for protection in addition to their usual role as agents of attraction for pollinators.

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