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The Role of Callose in the Seed Coat of a Fast Growing Legume – *Sesbania speciosa*

By

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With 1 Figure

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Summary

SETH N. & VIJAYARAGHAVAN M. R. 1990. The role of callose in the seed coat of a fast growing legume – *Sesbania speciosa.* – Phyton (Horn, Austria) 30 (2): 299–303, 1 figure. – Englisch with German summary.

The impermeability of the seed coat of *Sesbania speciosa (Fabaceae)* is shown to be related to callose deposition. Callose is found in the macro-, and osteo-, sclereid layers of impermeable seeds. It is suggested that callose is one of the candidates that impart temporary impermeability to *Sesbania speciosa* seed coat.

Zusammenfassung

SETH N. & VIJAYARAGHAVAN M. R. 1990. Die Rolle der Callose in der Samenschale einer schnellwüchsigen Leguminose – *Sesbania speciosa.* – Phyton (Horn, Austria) 30 (2): 299–303, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Es wird gezeigt, daß die Wasserundurchlässigkeit der Samenschale von *Sesbania speciosa (Fabaceae)* mit der Ablagerung von Callose zusammenhängt. Callose wird in den Makro- und Osteosklereidschichten der undurchlässigen Samen gefunden. Es wird angenommen, daß Callose eine der Ursachen für die zeitweise Undurchlässigkeit der Samenschale von *Sebania speciosa* ist.

1. Introduction

The family *Leguminosae (Fabaceae)* is characterized by hard-seededness i. e. inability of the seed coat to absorb water and germinate. Seed coat impermeability is apparently controlled by the nature of macrosclereid

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layer which lies beneath the cuticle covering (GRAAFF & VAN STADEN 1983, DELILAH 1984, JAIN & VIJAYARAGHAVAN 1985). Water impermeability in such hard seeds is also attributed to the zone of the light-line (COE & MARTIN 1920, FREY-WYSSLING 1959) and hard pectic caps (RALEIGH 1930, WERKER & al. 1973). MARBACH & MAYER 1974 hypothesized that oxidation of phenolic compounds to coloured lipophilic substances by catechol oxidase during seed dehydration causes water impermeability in *Pisum* seeds. The present investigation aims in establishing in situ the metabolite responsible for seed coat impermeability in yet another important fast growing legume – *Sesbania speciosa.*

2. Material and Methods

The hard seeds of *Sesbania speciosa* were collected and fixed in 10% aqueous acrolein. After dehydration in methoxyethanol series they were infiltered and embedded in glycol-methacrylate resin mixture. Polymerization was accomplished at 40° C for 24 hours and later at 60° C for 48 hours. The embedded material was sectioned using a Spencer (A. O.) rotary microtome fitted with a locally made glass-knife adaptor. Two micron thick sections were cut. The sections were pre-treated with bromine vapours for 30 seconds to remove the plastic and then washed with acetone and distilled water respectively for 2 minutes. These treated sections were stained with 0.005% water soluble aniline blue (in 0.15 M K_2HPO_4 at pH 9.0). The slides were observed using ultraviolet H365, BP365, FT395, L939 filter sets for epi-fluorescence condenser IV FI in a Carl Zeiss D-7082 OberKochen microscope to localize the callose.

3. Observations

The seed coat of *Sesbania speciosa* is well differentiated into an outer tightly packed macroslereid layer, below which is present the typical dumbbell/bone-shaped osteosclereid layer. The macrosclereid layer is overlaid by a thin waxy cuticle. In a fully mature hard seed the testa shows an intense deposition of callose (Fig. 1A). The macro-, and osteo- sclereids show polarized distribution of callose (Fig. 1B, C). The callose deposition is maximum at both ends of the cell; minimum at the cell core and fluoresces yellowish white (Fig. 1C, D). At the hilum region, two palisade layers are present, one of the hilum i. e. hilar palisade and other of the seed coat i. e. counter palisade. The hilar palisade lacks fluorescence but the counter palisade fluoresces yellowish white indicating callose. Callose deposition is only observed in the testa of a fully mature seed. Besides the tight packing of the macrosclereid layer, it is the callose that is present in both the macro- and the osteosclereid layers that causes impermeability.

4. Discussion

Callose is chemically a 1,3-β-glucan and functionally considered as a temporary sealing or plugging compound. Callose is generally present in

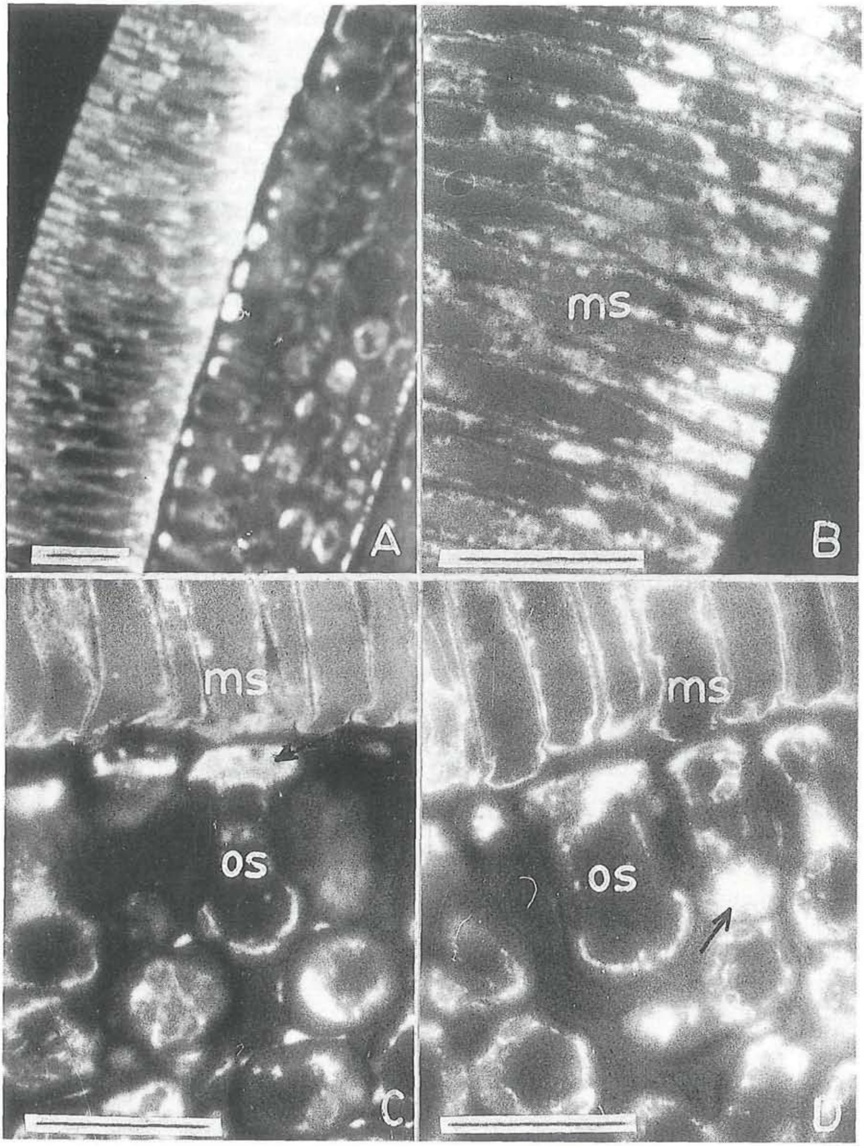

Fig. 1. Seed coat of *Sesbania speciosa,* aniline blue-induced fluorescence. A: Portion of seed coat at mature dicotyledonous embryo stage to show the callose deposition both in macrosclereid and osteosclereid layers (× 400). B: A few macrosclereids from figure A, enlarge to show the polarized deposition of callose (× 1000). C, D: Osteosclereid cells showing polarized deposition of callose. Excessive deposition occurs at both the ends (arrow) of the cells and meagre amount in the centre. ms = macrosclereid layer, os=osteosclereid layer, index bars = 25 μm.

small quantities in many structurally different plant tissues, and is a substance which has special physical and physiological properties.
It is rapidly synthesized and degraded with equal ease. Callose appears for a short time when needed and disappears. Its appearance for short duration has been well documented during microsporogenesis (WATERKEYN 1962, VIJAYARAGHAVAN & SHUKLA 1978), megasporogenesis (RODKIEWICZ 1970), mechanical damage (DEKAZOS & WORLEY 1967), in response to stress (THOMAS & HALL 1979), wounding (SCHNEIDER 1980), and infection by pathogens (PENNAZIO & al. 1981). Many roles has been ascribed to callose wall during microsporogenesis and megasporogenesis. It has been suggested that the callose wall protects the differentiating sporogenous cells from the hormonal and nutritional influences of the surrounding vegetative cells (GODWIN 1968) and also provides genetic autonomy to the meiocytes (HESLOP-HARRISON 1968). It supplies carbon compounds, like glucose for the development of cellulosic primexine (LARSON & LEWIS 1962), and acts as a template for the future exine pattern (WATERKEYN & BEINNFAIT 1970). In *Sesbania speciosa* callose wall may act as a filter decreasing the imbibition of the cell wall and thus the permeability of the testa. The presence of callose in the testa of *Sesbania speciosa* hinders movement of water into the seed and thus causes impermeability as also observed in *Trifolium subterraneum* (BHALLA & SLATTERY 1984) and *Sesbania punicea* (BEVILACQUA & al. 1987). In the seed coat of other legumes such as *Melilotus alba* (HAMLY 1932), *Gleditsia triacanthus* (CAVAZZA 1950) and *Rhynchosia minima* (RANGASWAMY & NAND KUMAR 1985), however, callose was not seen. It appears that callose is one of the candidates that imparts temporary impermeability to hard seededness in *Sesbania speciosa.*

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