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Studies on the Sponge Gourd (*Luffa cylindrica*) Pollen during Storage at Different Humidity Levels

By

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With 3 Figures

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Summary

SETIA N., GREWAL M. & MALIK C. P. 1990. Studies on the sponge gourd (*Luffa cylindrica*) pollen during storage at different humidity levels. – Phyton (Horn, Austria) 30(1): 75–81, 3 figures. – English with German summary.

In vitro germination and tube growth of sponge gourd (*Luffa cylindrica*) pollen decreased continuously following storage at various humidity levels. Pollen stored at 55% RH maintained better germinability. Compared to fresh, the stored pollen exhibited greater leakage of electrolytes and metabolites across the membrane. Activities of invertase, acid phosphatase, malate dehydrogenase and glucose-6-phosphate dehydrogenase also changed in stored pollen. Changes in the lipid composition were also observed in stored pollen.

Zusammenfassung

SETIA N., GREWAL M. & MALIK C. P. 1990. Untersuchungen an Luffa-Pollen (*Luffa cylindrica*) während der Lagerung bei verschiedener Feuchtigkeit. – Phyton (Horn, Austria) 30(1): 75–81, 3 Figuren. – Englisch mit deutscher Zusammenfassung.

Nach Lagerung der Pollen von *Luffa cylindrica* bei verschiedener Feuchtigkeit (0–55% rel. F.) nehmen in vitro-Keimung und Pollenschlauchwachstum kontinuierlich ab. Bei 55% rel. F. gelagerter Pollen hat die bessere Keimfähigkeit. Im Vergleich zum frischen Pollen verlieren Pollen nach Lagerung in Wasser mehr Elektrolyte und Stoffwechselprodukte. Die Aktivitäten von Invertase, saurer Phosphatase, Malat-Dehydrogenase und Glucose-6-P-dehydrogenase ändern sich während der Lagerung. Auch in der Lipidzusammensetzung gelagerter Pollen wurden Veränderungen beobachtet.

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1. Introduction

The ability of pollen to germinate declines during storage under various conditions (STANLEY & LINSKENS 1974, SHIVANNA & JOHRI 1985). Though a number of studies dealing with physiological changes during storage of pollen have been carried out, the actual mechanism or events leading to loss of pollen viability are little understood. Mature pollen grains are relatively quiscent containing full compliment of organelles and reserve substances. Germination of pollen involves activation of enzymes of various metabolism, synthesis of proteins and nucleic acids (MASCARENHAS 1975, SHIVANNA & JOHRI 1985). Loss of pollen viability has been correlated with deficiency of respiratory substances, irreversible loss of membrane permeability, inactivation of various enzymes and hormonal imbalance (SHIVANNA & JOHRI 1985). This paper reports some metabolic changes that take place in sponge gourd pollen during storage at various humidity levels.

2. Material and Methods

Pollen of sponge gourd (*Luffa cylindrica* ROEM cv. Pusa chikni), collected from freshly opened flowers, was stored at various levels of relative humidity (% RH) maintained in the desiccators kept in the refrigerator by using saturated solutions of calcium chloride and calcium nitrate for 35 and 55% RHs, respectively, and dry silica for 0% RH. Germinability and tube growth of stored pollen samples was tested on every alternate day upto 10 days after storage. Before culturing, stored pollen grains were equilibrated by exposing to high humidity (95–100%) for 30 min. Pollen was germinated in two replicates in the liquid culture medium comprising 15% sucrose and 0.01% boric acid. From each replicate over 100 pollen grains were scored for germination and tube lengths of 50 tubes were measured with the ocular micrometer using randomly selected microscopic fields after incubation for 180 min at 25±2° C.

For estimating the electrolyte leakage into pollen steep water, 25 mg pollen was taken in 10 ml deionized water and kept for 20 min with occasional shaking. Conductivity of pollen steep water was recorded on conductivity meter and data is expressed in terms of percentage leakage of electrolytes over fresh pollen following the method given by STEWART & BEWLEY 1980. Pollen steep water was also analysed for total soluble sugars (CLEGG 1956) and amino acids (LEE & TAKAHASHI 1966). Modified method of FOLCH & al. 1957, was used for extraction of lipids from fresh and stored ungerminated pollen. Quantitative determinations of phospholipids (AMES 1966), glycolipids (ROUGHAN & BATT 1968), sterols (STADMAN 1957) and total free fatty acids (LOWRY & TINSLEY 1976) were also carried out. Procedures for extraction and assaying the activities of invertase, acid phosphatase, malate dehydrogenase, glucose-6-phosphate dehydrogenase and peroxidase were followed as described in MALIK & SINGH 1980.

3. Results

Two parameters, per cent germination and tube length, were used to measure pollen vigor following storage at different humidity levels. The germination percentage of pollen declined gradually with increasing period

of storage at various RHs (0, 35 and 55%) and decreased by about 75–80% after 10 days of storage (Fig. 1). There was also decrease in pollen tube length following storage, pollen stored for two days at 35 and 55% RHs exhibited increase in tube length by 5 and 28%, respectively, followed by a gradual decline thereafter (Fig. 2). The pollen stored at 55% RH maintained better germinability and tube growth compared to other RHs.

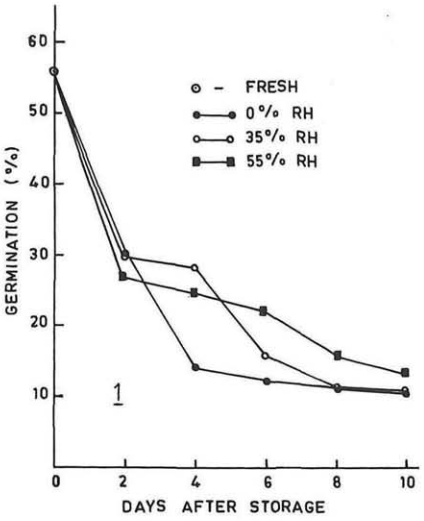

Fig. 1

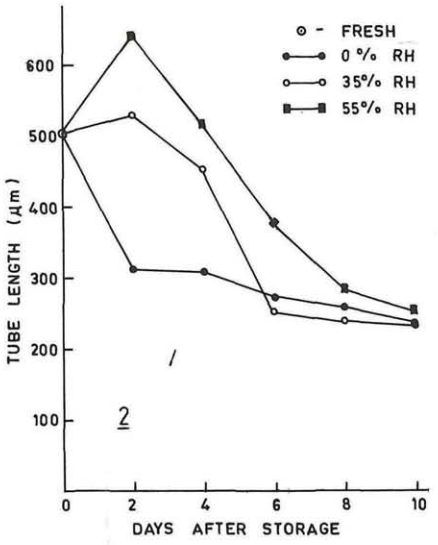

Fig. 2

Fig. 1. Effect of storage at different humidity levels (% RH) on germination (%) in sponge gourd pollen.

Fig. 2. Effect of storage at different humidity levels (% RH) on tube length (µm) in sponge gourd pollen.

The data on electrical conductivity of pollen steep water, expressed as per cent increase over control (fresh pollen) indicated that leaching of electrolytes increased with increasing storage period; maximum leaching occurring in pollen stored at 0% RH and minimum at 55% RH (Fig. 3). The leaching of metabolites like sugars and amino acids into steep water was more from stored (for 4 days) than fresh pollen (Table 1).

The investigated lipid components (total lipids, phospholipids, glycolipids, sterols, triglycerides, and total free fatty acids) in fresh and stored ungerminated pollen showed only small and not significant differences in both directions (in most cases <10%), and a common trend was not recognizable.

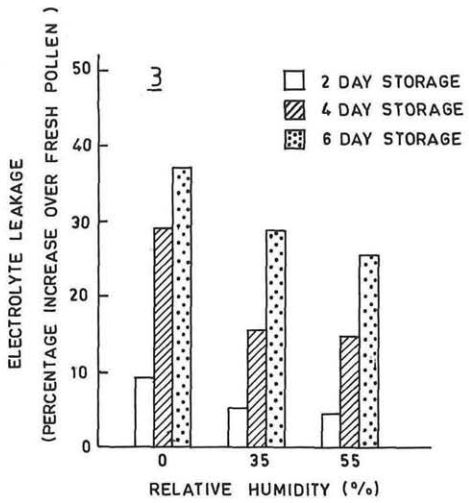

Fig. 3. Effect of storage at different humidity levels (% RH) on electrolyte leakage in sponge gourd pollen.

Table 1

Effect of storage at different humidity levels (% RH) on the leakage of sugars and amino acids (from 25 mg pollen in 10 ml deionized water) in sponge gourd pollen.

Storage treatment (% RH)	Sugars (µg glucose)	Amino acids (µg glycine)
Fresh pollen	1250	465
0	1800	560
35	2100	520
55	1880	490

Table 2 shows the activities of various enzymes in ungerminated and germinated (for 60 min) fresh and stored (at 0% RH for 4 days) pollen samples. Activities of invertase and acid phosphatase increased with storage in ungerminated pollen compared to fresh pollen. During germination the activity of these enzymes increased only in fresh pollen and remained low in stored germinating pollen sample. The activity profiles of malate dehydrogenase, glucose-6-phosphate dehydrogenase and peroxidase were low in fresh ungerminated pollen but increased substantially during germination. Comparatively the activity of these enzymes was low in stored ungerminated and germinated pollen (Table 2).

Table 2

Effect of storage (at 0% RH) on activities of various enzymes in ungerminated and germinated (60 min) pollen of sponge gourd

		Fresh	Stored
Invertase	ungerminated	205	320
µg glucose/mg protein	germinated	235	235
Acid phosphatase	ungerminated	200	265
µmol paranitrophenol released/mg protein. h	germinated	599	255
Malate dehydrogenase*	ungerminated	200	100
	germinated	820	350
Glucose-6-phosphate-dehydrogenase*	ungerminated	100	90
	germinated	152	16
Peroxidase*	ungerminated	19	45
	germinated	112	35

* = Enzyme activity expressed as enzyme units per mg protein
(1 enzyme unit = 0,001 change in O. D.)

4. Discussion

Germination and tube growth of sponge gourd pollen decreased following storage at different humidity levels. Pollen stored at 55% RH maintained better germinability and tube growth. The decreased germination capacity of pollen was accompanied by increased leakage of electrolytes and metabolites (soluble sugars and amino acids) in the pollen steep water indicating thereby loss of membrane integrity of pollen during storage. The leakage was minimum in pollen stored at 55% RH. The increased leakiness of the membrane as a result of storage appears to be associated with changes in lipid composition. JAIN & SHIVANNA 1988, have correlated the loss of pollen viability during storage (in organic solvents) to the changed phospholipid composition in *Crotolaria retusa*. Similarly, the loss of seed viability has been related with loss in phospholipids especially phosphatidyl choline, and enhanced leakiness of the membrane (PRIESTLEY & LEOPOLD 1979). Further, poor germinability of stored pollen samples can also be due to decline in activity of membrane bound enzymes as a result of membrane deterioration (DALGARN & NEWMAN 1986).

Low activities of various enzymes in stored sponge gourd pollen indicate their poor ability to metabolize substrates required for supporting germination and tube growth. Pollen is known to contain large number of enzymes to metabolize external and internal substrates essential for germination and tube growth (SHIVANNA & JOHRI 1985, STANLEY & LINSKENS

1974). Reduced activity of glucose-6-phosphate dehydrogenase, a key enzyme of pentose phosphate pathway, in stored pollen indicate poor source of reducing power and various pentose phosphates needed for biochemical reactions. Low activity of malate dehydrogenase is indicative of reduced respiration; peroxidase is involved in the defence of aerobic cells against H_2O_2, a product of partial reduction of oxygen (BREWBAKER 1971). Though it is very essential to recognize the regulatory mechanism in and around pollen which influence the activity of various enzymes, the decreased activities of various enzymes following storage are difficult to explain as several factors are involved in regulating the activity pattern of enzymes (SHOPFER 1977).

5. Acknowledgement

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6. References

AMES B. N. 1966. Assay of inorganic phosphate, total phosphates. In: NEUFELD E. & GINSBERG V. (Eds.) Methods in Enzymology, Vol. 8, pp 115–118. – Academic Press Inc., New York.

BREWBAKER J. L. 1971. Pollen enzymes and isoenzymes. In: HESLOP-HARRISON J. (Ed.) Pollen – Development and Physiology, pp 156–170. – Butterworth, London.

CLEGG K. M. 1956. The application of anthrone reagent to the estimation of starch in cereals. – J. Sci. Food Agric. 7: 40–44.

DALGARN D. S. & NEWMAN D. W. 1986. Ecophysiology of plant membrane lipids. – Agro Botanical Publishers, India.

FOLCH J., LEES M. & SOLANE-STANLEY G. H. 1957. A simple method for isolation and purification of total lipids from animal tissues. – J. Biol. Chem. 226: 497–509.

JAIN A. & SHIVANNA K. R. 1988. Storage of pollen grains in organic solvents: Effect of organic solvents on leaching of phospholipids and its relationship to pollen viability. – Ann. Bot. 61: 325–330.

LEE Y. P. & TAKAHASHI T. 1966. An improved colorimetric determination of amino acids with use of ninhydrin. – Anal. Biochem. 14: 71–77.

LOWRY R. R. & TINSLEY I. J. 1976. Rapid colorimetric determination of free fatty acids. – J. Amer. Oil Chem. Soc. 53: 470–473.

MALIK C. P. & SINGH M. B. 1980. Plant enzymology and histoenzymology. – Kalyani Publishers, New Delhi.

MASCARENHAS J. P. 1975. The biochemistry of angiosperm pollen development. – Bot. Rev. 41: 259–314.

PRIESTLEY D. A. & LEOPOLD A. C. 1979. Absence of lipid oxidation during accelerated aging of soybean seeds. – Plant Physiol. 63: 726–729.

ROUGHAN P. G. & BATT R. D. 1968. Quantitative analysis of sulpholipids and galactolipids in plant tissues. – Anal. Biochem. 22: 74.

SCHOPFER P. 1977. Phytochrome control of enzymes. – Ann. Rev. Plant Physiol. 28: 223–252.

SHIVANNA K. R. & JOHRI B. M. 1985. The angiosperm pollen structure and function. – Wiley Eastern Limited, New Delhi.

STADTMAN T. C. 1957. Preparation and assay of cholesterol and ergosterol. In: COLOWICK S. P. & KAPLAN N. O. (Eds.) Methods in Enzymology, Vol. 3, pp 392. – Academic Press, New York.

STANLEY R. G. & LINSKENS H. F. 1974. Pollen biology, biochemistry and management. – Springer-Verlag, Berlin.

STEWART R. R. C. & BEWLEY J. D. 1980. Lipid peroxidation associated with accelerated aging of soybean axes. – Plant Physiol. 65: 245–248.

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Recensiones

BINZ-REIST Hans-Rudolf 1989. Mechanische Belastbarkeit natürlicher Schilfbestände durch Wellen, Wind und Treibzeug. Veröffentlichungen des Geobotanischen Institutes der ETH Stiftung Rübel, Zürich, H. 101. – 8°, 536 Seiten mit zahlreichen Abbildungen und Tabellen, broschiert. – sFr 60,–. – ISSN 0254-9439.

Das Schilf, *Phragmites australis* (CAV.) TRIN. ex STAUDEL (= *P. communis* TRIN.) bildet an Seenufern und an feuchten Standorten ausgedehnte, fast undurchdringliche, meist artenarme Bestände. Seit den sechziger Jahren ist ein Rückgang der Schilfbestände, ein „Schilfsterben", in ganz Mitteleuropa zu beobachten. Dabei spielt die mechanische Festigkeit des Schilfes eine zentrale Rolle, die auch in der vorliegenden Veröffentlichung in den Vordergrund der Untersuchungen gestellt wurde. Im 1. Teil der Arbeit werden die Anatomie, die Lebensweise, die Standortsansprüche und die bisher bekannten Ursachen des Schilfsterbens behandelt. Das Schilf ist, außer einer direkten Zerstörung, durch Siedlungsdruck, Bautätigkeit, Erosionstätigkeit des Wassers, durch chemische Abwässer sowie durch den Erholungsbetrieb u. a.