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Structure and Breakdown of Protein Bodies in Cotyledonary Cells During Seed Germination in *Lens culinaris* MEDIC.

By

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With 1 Plate (6 Figures)

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Summary

VIJAYARAGHAVAN M. R. & JAIN A. 1985. Strucutre and breakdown of protein bodies in cotyledonary cells during seed germination in *Lens culinaris* MEDIC. – Phyton (Austria) 25 (2): 273–276, with 6 figures. – English with German summary.

Protein bodies in *Lens culinaris* are of homogeneous type and lack globoid and crystalloid inclusions. Protein bodies lyse from the centre. In germinated seeds protein body breakdown occurs in a wave – (i) in the embryonic axis starting from the radicular end onwards, and (ii) in cotyledonary storage parenchyma tissue from the outer epidermis and towards the central storage cells.

Zusammenfassung

VIJAYARAGHAVAN M. R. & JAIN A. 1985. Struktur und Abbau der Proteinkörner in Keimblattzellen von *Lens culinaris* MEDIC. während der Keimung. – Phyton (Austria) 25 (2): 273–276, mit 6 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Proteinkörner in *Lens culinaris* sind homogen, Globoid- und Kristalleinschlüsse kommen nicht vor. Die Proteinkörper lösen sich von einem Zentrum ausgehend. In keimenden Samen erfolgt der Abbau wellenförmig: 1. in der Embryonalachse ausgehend vom Radicula-Ende aufwärts und 2. im cotyledonaren Speicherparenchym von der äußeren Epidermis gegen die zentralen Speicherzellen. (Editor transl.)

1. Introduction

Seed storage reserves like proteins, carbohydrates and lipids are lysed during seed germination for the growth and development of the new

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sporophyte. Proteins are stored in a seed within the specialised cell organelles called protein bodies. Degradation of protein bodies is under the control of some factor as evidenced by their specific pattern of degradation during seed germination. Breakdown of protein bodies results in a central large vacuole within the cell. The degradation of protein bodies has been observed in a number of plants (BRIARTY & al. 1970, HARRIS & al. 1975, DHAR & VIJAYARAGHAVAN 1979, GIFFORD & al. 1983, BHANDARI & CHITRALEKHA 1984, VIJAYARAGHAVAN & GARG 1984).

2. Materials and Methods

The seeds of *Lens culinaris* MEDIC. were sown on moistened filter paper in petri dishes and were kept in controlled temperature and light conditions (25±2° C, 75–80 lux). The germinated seeds at an interval of 6 hr, 1 day, up to 10 days respectively were fixed in 10% acrolein. The seeds were dehydrated in the butanol series and infiltered in pure glycol methacrylate. The material was embedded in monomer mixture (glycol methacrylate: Azobis: polyethylene glycol 400) (FEDER & O'BRIEN 1968). The sections were cut at 2 μm using glass knives. Total proteins were localised with coomassie brilliant blue (FISHER 1968).

3. Observations

In the dormant seeds of *Lens culinaris*, the cotyledonary cells with the onset of imbibition increase in size. Six hours after imbibition the cotyledonary cells become fully turgid. A large amount of small protein bodies are interspersed with the starch grains (Plate 1A). The protein bodies appear as distinct round bodies, a day after imbibition and stains homogeneously with coomassie brilliant blue. The fusion and swelling of protein bodies also occur, in the cotyledonary peripheral storage parenchyma cells, a day after imbibition (Plate 1B). Two days later protein bodies fuse in the central region of the cotyledonary cells. Vacuolization within the protein bodies starts 2 days after imbibition (Plate 1C). The protein body integrity is lost after 5 day of imbibition. At this time many vacuoles are formed within the fused protein bodies (Plate 1D). The protein masses that are formed by coalescence of protein bodies, 7 days after imbibition, disintegrate leaving a network of small protein particles (Plate 1E), and a day later protein particles show lysis from the core of the vacuoles (Plate 1F). Nine days later these particles disappear from the centre of the vacuoles. A few protein particles are, however, present in the peripheral portion of vacuoles that later degrades completely leaving a big vacuole.

4. Discussion

Protein bodies are generally distributed throughout the protein-storage tissue though certain cells might be richer than others. Protein bodies are

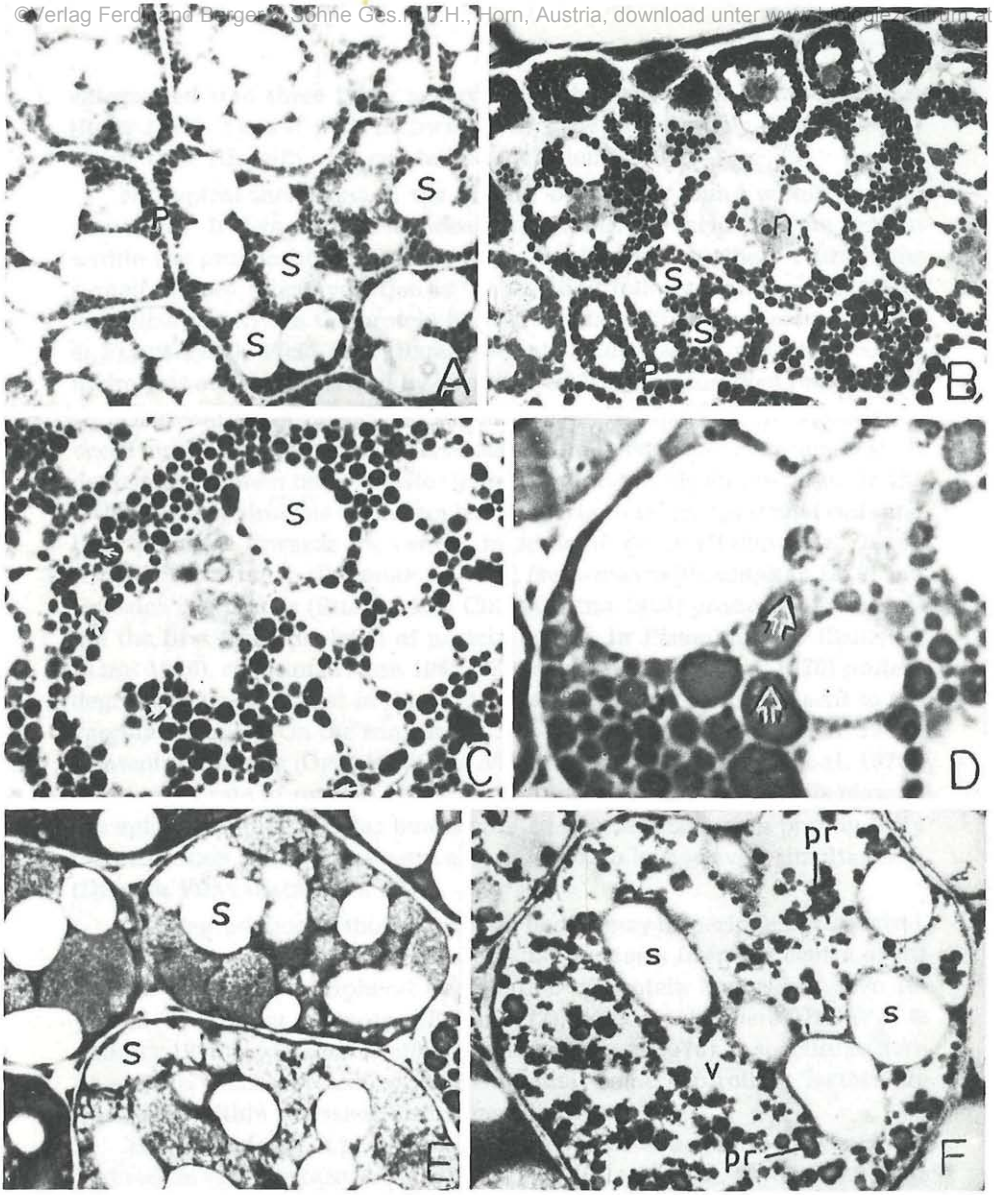

Plate 1

Plate 1 A. *Lens culinaris,* L. S. of a portion of cotyledonary tissue (6 hr after soaking) showing distinct protein bodies (P) engorged with starch grains (S) (× 690).

B, C. Same, one and two days after soaking. In C the fusion (arrows) within the protein bodies is noteworthy. In the centre a nucleus (n) is marked in B (× 690).

D. Part of cotyledonary tissue, (5 days after soaking) to reveal the presence of large number of tiny vacuoles (arrows) within the fused and enlarged protein bodies (× 1100).

E–F. Portion of cotyledonary tissue (7 and 8 days after soaking). Degraded proteins in the form of a network is present inside the vacuoles in E. Remnants of protein particles (Pr) show lysis from the centre of the cell vacuoles (v) in F (E × 690; F × 900).

categorised into three types according to the types of inclusions present (ROST 1972). Type I: with no inclusions; Type II: with only globoid inclusions; Type III: with only crystalloid inclusions.

No typical inclusions in the protein bodies are found within the *Leguminosae*. In *Lens culinaris* (present work) also no inclusions are present within the protein bodies. In this taxon the first change that occurs in the protein bodies after hydration is the swelling followed by the fusion and vacuolization within the protein bodies. Similarly in *Pisum arvense* (SMITH & FLINN 1967), *Vicia faba* (BRIARTY & al. 1970), events of protein bodies hydrolysis are characterized by swelling, coalescence and then breakdown.

Degradation of protein bodies in *L. culinaris* (present work) does not occur simultaneously in all the tissues of the seed. The embryonal axis is depleted of protein bodies earlier than that of the cotyledonary tissue. In the latter tissue hydrolysis of protein bodies starts from the epidermal cells and later proceeds towards the centre. In seeds of *Yucca* (HARRY & al. 1966), *Alyssum maritimum* (PRABHAKAR 1979), *Iberis amara* (PRABHAKAR 1979) and *Brassica campestris* (BHANDARI & CHITRALEKHA 1984) procambium strands are the first to be depleted of protein bodies. In *Pisum arvense* (SMITH & FLINN 1966), soybean (TOMBS 1967), *Vicia faba* (BRIARTY & al. 1970) protein degradation occurs first in the epidermis and in those cells adjacent to the vascular bundles. On the contrary in *Arachis hypogea* (BAGLEY & al. 1963), *Phaseolus vulgaris* (ÖPIK 1966) and *Vigna unguiculata* (HARRIS & al. 1975), the slowest rate of protein bodies breakdown occurs in those cells close to the epidermis and vascular bundles. In *Linum usitatissimum* protein body disintegration in the endosperm and the embryo is, however, simultaneous (DHAR & VIJAYARAGHAVAN 1979).

The degradation within the protein bodies may be peripheral or central. In *Lens culinaris* (present work) degradation starts from the centre of the protein body. The peripheral degradation of protein bodies has been reported in *Setaria lutescens* (ROST 1972), *Yucca schidigera* (HORNER & ARNOTT 1965) and *Lupinus albus* (MLODZIANOWSKI 1978). A specific pattern of protein bodies breakdown indicates that some controlling factors are operating within the seeds during germination.

The breakdown of protein bodies is reported to be under the control of embryonic axis (BRYANT & HACZYCKI 1976, HALMER & al. 1978). Cytokinins from the embryonic-axis is the likely candidate as the regulator of food reserve breakdown in germinating seeds (HUTTON & VAN STADEN 1982).

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References

BAGLEY B. W., CHERRY J. H., ROLLINS M. L. & ALTSCHUL A. M. 1963. A study of protein bodies during germination of pea nut *(Arachis hypogea)* seed. – Am. J. Bot. 50: 523–532.

BHANDARI N. N. & CHITRALEKHA P. 1984. Degradation of protein bodies in germinating seeds of *Brassica campestris* L. var. sarson PRAIN. – Ann. Bot. 53: 793–801.

BRIARTY L. G., COULT D. A. & BOULTER D. 1970. Protein bodies of germinating seeds of *Vicia faba.* Changes in fine structure and biochemistry. – J. exp. Bot. 21: 513–524.

BRYANT J. A. & HACZYCKI S. J. 1976. Studies on the interactions between the embryonic axis and the cotyledons during germination in *Pisum sativum* L., the influence of incubation conditions. – New Phytol. 77: 757–760.

DHAR U. & VIJAYARAGHAVAN M. R. 1979. Ontogeny, structure and breakdown of protein bodies in *Linum usitatissimum* Linn. Ann. Bot. 43: 107–111.

FEDER N. & O'BRIEN T. P. 1968. Plant microtechnique: Some principles and new methods. – Am. J. Bot. 55: 123–142.

FISHER D. B. 1968. Protein staining of ribboned epon sections for light microscopy. – Histochemie 16: 92–96.

GIFFORD D. J., GREENWOOD J. S. & DEREK B. J. 1983. Vacuolation and storage protein breakdown in the castor bean endosperm following imbibition. – J. exp. Bot. 34: 1433–1443.

HALMER P., BEWLEY J. D. & THORPE T. A. 1978. Degradation of the endosperm cell walls of *Lactuca sativa* L. cv. Grand Rapids. – Planta 139: 1–8.

HARRIS N., CHRISPEELS M. J. & BOULTER D. 1975. Biochemical and histochemical studies on protease activity and reserve protein metabolism in the cotyledons of germinating cowpeas *(Vigna unguiculata).* – J. exp. Bot. 26: 544–554.

HARRY T., HORNER J. R. & ARNOTT H. J. 1966. Histochemical and ultrastructural study of pre- and post-germinated *Yucca* seeds. – Bot. Gaz. 127: 48–64.

HORNER H. T. Jr. & ARNOTT H. J. 1965. A histochemical and ultrastructural study of *Yucca* seed proteins. – Am. J. Bot. 52: 1027–1038.

HUTTON M. J. & VANSTADEN J. 1982. Cytokinins in germinating seeds of *Phaseolus vulgaris* L. Transport and metabolism of $8^{14}C$ t-Zeatin applied to the radicle. – Ann. Bot. 49: 693–699.

MLODZIANOWSKI F. 1978. The fine structure of protein bodies in lupine cotyledons during the course of seed germination. – Z. Pflanzenphysiol. 86: 1–13.

ÖPIK A. 1968. Development of cotyledon cell structure in ripening *Phaseolus vulgaris* seeds. – J. exp. Bot. 19: 64–76.

PRABHAKAR (née CHEEMA) K. 1979. Histochemical and ultrastructural studies in some *Cruciferae:* Zygote to seedling. – Ph. D. Thesis, University of Delhi.

ROST T. L. 1972. The ultrastructure and physiology of protein bodies and lipids from hydrated dormant and nondormant embryos of *Setaria lutescens (Gramineae).* – Am. J. Bot. 59: 607–616.

SMITH D. L. & FLINN A. M. 1967. Histology and histochemistry of the cotyledons of *Pisum arvens* L. during germination. – Planta 74: 72–85.

TOMBS M. P. 1967. Protein bodies of the soybean. – Plant Physiol. 42: 797–813.

VIJAYARAGHAVAN M. R. & GARG M. L. 1984. Protein bodies of seeds. – Ind. Rev. Life Sci. 4: 255–278.