Seed Development in *Phaseolus vulgaris* L., *Populus nigra* L., and *Ranunculus sceleratus* L. with Special Reference to the Microtubular Cytoskeleton

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Summary

Seed plays an important role in the sexual reproduction of higher plants. A seed consists of a seed coat enveloping the endosperm and the embryo which is the new generation of the plant. To understand seed formation, differentiation, and maturation, three species, i.e. celery-leafed buttercup (*Ranunculus sceleratus* L.), bean (*Phaseolus vulgaris* L.), and poplar (*Populus nigra* L.), were selected for comparative cytological and immunocytochemical investigations. Special attention has been paid to embryo and endosperm development and to the configurations of the microtubular cytoskeleton during these processes.

The embryogeny of celery-leafed buttercup belongs to the Onagrad Type. However in this study, the pattern of embryo development appeared different from the Onagrad Type. From three-celled linear proembryos, the basal cell gave rise to a multicellular suspensor that exhibited limited development, whereas the middle and upper cells formed the embryo proper. Therefore, the embryo proper is not only formed by the apical cell but also by the sub-apical cell. The endosperm occupies the greater part of the mature seed, and is filled with lipid and starch. Endosperm cells surrounding the embryo suspensor persist whereas those surrounding the embryo proper degenerate, indicating a site-specific interaction between the endosperm and the embryo. The distributions of wall ingrowths of the transfer type and multivesicular structures are analyzed in relation to metabolite transport. Wall ingrowths are abundant in the antipodal cells and point to the transfer of metabolites from the apoplast to the symplast of the megagametophyte (Chapter 2). During the process of seed coat differentiation the microtubular cytoskeletons of the seed coat cells exhibit various configurations, functioning in cell and in the formation of wall ingrowths of a non-transfer-type. During cell aging microtubular organizations became disturbed in the seed coat cells (Chapter 4).

In bean, the embryo exhibits three cytomorphological zones: the embryo proper, the suspensor neck zone and the suspensor basal zone. The ultrastructure, including microtubular cytoskeleton, was analyzed. The results indicate that the structural differentiation of the embryo zones is related to the

functional differentiation of the three zones. Also the microtubular cytoskeleton is involved in the structural differentiation of the three zones. In particular, it functions in the development of the shape of the embryo proper and the suspensor. Taken the distribution of wall ingrowths in the embryo into account, it is plausible from the structural point of view, too, that the embryo suspensor functions in the transport of nutrients from the integuments and nucellus cells towards the embryo proper (Chapter 3).

The endosperms of celery-leafed buttercup (Chapter 5), bean (Chapter 6) and poplar (Chapters 7 and 8), all belong to the Free Nuclear Type which is characterized by an initial coenocytic phase in which free nuclei are distributed throughout the cytoplasm of the fertilized central. The coenocytic phase is followed by cellularization, studied in detail in this work.. In the process of cellularization, three sub-types of developmental patterns are distinguished, i.e., the Peripheral Type as seen in e.g., celery-leafed buttercup and poplar, the Lingering Type as seen in e.g., bean, and the Linear Type as seen in e.g., sunflower. In the Peripheral Type, the free nuclei of the endosperm surround the central vacuole and give rise to alveoli all around. Cellularization proceeds centripetally. In the Lingering Type, the free nuclei surround the central vacuole but the endosperm only forms alveoli in the micropylar area, whereas the chalazal area remains free nuclear. Cellularization proceeds from the micropylar side towards the chalazal side. In the Linear Type, the free nuclei are only present in the micropylar area where they gave rise to alveoli. Cellularization proceeds from here towards the chalazal side. Endosperm development is investigated with special attention to the process of cellularization. The Peripheral and Linear Types belong to the synchronous cellularization type, and the Lingering Type is an asynchronous type (Chapter 9).

Although tissue development proceeds differently, the process of the nuclear endosperm cellularization starts with the formation of alveoli, and proceeds by both alveolus formation and cell formation. The formation of alveoli, termed *alveolation*, includes three steps: (1) mitosis-cytokinesis derived and non-mitosis-cytokinesis derived cell plate formation in internuclear spaces, (2) fusion of cell plates at the lateral sides of the alveoli, and fusion of the extending cell plates with the wall of the fertilized central cell, and (3) elongation of alveoli by the extension of the freely growing wall edges of the anticlinal walls at the side towards the central vacuole of the central cell. Cellularization of the endosperm ends when the elongating alveoli meet in the central part of the original central vacuole of the central cell.

During the endosperm alveolation, the freely growing edges of the anticlinal walls are associated with extensive MTs with mirror symmetric organization with the respect of the wall plane. In the areas where cell plates fuse, Y-shaped junctions of walls are usually formed. Here, populations of microtubules run well parallel to the bisectors of the wall angles, forming an axial symmetric, Y-shaped organization, too, staggering with the Y-shaped walls (Chapter 6). The microtubules in mirror symmetric and axial symmetric configurations form one phragmoplast covering the freely growing wall edges of all the alveoli. Such phragmoplast is termed *alveolar phragmoplast* (*a-phragmoplast*) in this thesis. It functions as a mechanically supporting framework for cell plate growth throughout the process of alveolation. The a-phragmoplast and the mitosis-cytokinesis related phragmoplast, termed *m-phragmoplast* in this thesis, are characterized as functionally different structures. It is suggested that the term phragmoplast should be maintained as a general term for both types of phragmoplasts (Chapter 8).

菜豆，欧洲黑杨和芹叶毛茛的种子发育及其微管骨架

（摘要）

种子在高等植物有性生殖中起着重要作用．它由种皮及其包被的胚乳和胚构成，其中胚是新一代的植物个体．本文以毛茛，菜豆和欧洲黑杨为材料，对种子的产生，分化和成熟过程进行了细胞学和免疫细胞化学的比较研究．

芹叶毛茛的胚胎发育属柳叶菜形．但本文报道了一种不同于柳叶菜型的胚胎发育类型．自三细胞胚起，基细胞发育成多细胞构成的胚柄，而顶细胞和中间细胞共同发育为胚本体．因此胚本体不仅由顶细胞构成，而且亦由中间细胞参与构成．这与传统的顶细胞单独发育成胚本体的模式不同．在成熟的芹叶毛茛种子中，胚乳占据了绝大部分空间，并贮有大量脂类和淀粉．胚乳细胞在胚柄处保存完好，但在胚本体周围消耗，衰败．这显示了胚和胚乳相互作用中的位点特异性．本文还对传递细胞型细胞壁内生和多泡体结构与代谢物的运输之间的关系进行了研究．在反足细胞中，大量的细胞壁内生显示了代谢物在质外体和共质体之间的运输关系（第二章）． 种皮在胚乳发育期间分化，成熟．其间，微管骨架呈现出多种构型，并作用于非传递细胞型细胞壁的内生和细胞的形态建成．细胞在衰老过程中，微管骨架的组织形式遂失去常态并最终消失（第四章）．

菜豆的胚呈现出三个细胞形态不同的区域：胚本体，胚柄颈区和胚柄基区．对其超微结构包括微管构型的研究表明，胚胎结构的分化与这三个区的功能紧密相关．微管骨架参与这三个区的结构分化，其中，本文特别对在传递细胞型细胞壁内生的过程中以及在胚本体和胚柄在形态转变中的微管构型进行了研究．菜豆胚柄的超微结构显示了其具营养物传递的功能（第三章）．

芹叶毛茛，菜豆和欧洲黑杨的胚乳发育皆属于游离核型．在其共核体的初始期，胚乳游离核逐渐遍布于胚乳的周缘．细胞化过程随之开始．本文对胚乳的细胞化进行了重点研究．游离核型胚乳的发育可

分为三个亚型：向心型（例如芹叶毛茛和欧洲黑杨），延迟型（例如菜豆）和线型（例如向日葵）. 向心型的胚乳细胞化过程是围绕中央液泡的胚乳产生蜂窝状细胞壁并向心生长. 延迟型则是蜂窝状细胞壁只在珠孔端产生而合点端的胚乳保持游离核状态，细胞化过程由珠孔端向合点端发展. 线型的胚乳细胞化过程是胚乳游离核只分布在珠孔端并就地产生蜂窝状细胞壁；细胞化由珠孔端向合点端发展. 向心型和线型属于同步细胞化型，而延迟型属非同步化型（第九章）. 上述三种胚乳细胞化类型的共同点是细胞化都以蜂窝状细胞壁形成而产生蜂窝状细胞做为开端. 这一过程称为 "蜂窝化". 它包括三个步骤：(1) 有丝细胞分裂及非有丝细胞分裂所产生的细胞板在核间区形成；(2) 这些细胞板向周围延伸生长并于相邻的细胞板融合，导致产生蜂窝状细胞；(3) 这些融合后的细胞板形成垂直于胚囊壁的垂周壁并在胚囊内侧向心或向合点区生长. 这些垂周壁的自由生长端仍然处于细胞板状态. 胚乳的细胞化过程以垂周细胞壁在胚囊内侧的自由生长端于胚囊中央或合点端融合而告结束.

在胚乳蜂窝化过程中，垂周壁的自由生长端伴有大量密集的微管. 这些微管形成以细胞壁自由生长端的面而成镜像对称的构型. 在相邻三个蜂窝状细胞的垂周壁结合部，"Y" 型的细胞壁形成. 此处微管群平行于 "Y"型壁的角平分线，并在总体上形成轴向对称的"Y" 字构型，但与 "Y"型细胞壁交错 1/2个角度（第六章）. 上述两种构型的微管群交互结合在所有垂周细胞壁生长端上，形成一个蜂窝状成膜体--本文命名为 "A型成膜体". A型成膜体作为一个垂周细胞壁生长端的机械支撑网络在整个游离核胚乳蜂窝化过程中起作用. 与 A型成膜体相对应，有丝细胞分裂中的成膜体在本文中被称为 "M型成膜体". 其产生，组织形式和功能都与 A型成膜体有别. 鉴于此，"成膜体"一词今后应作为 A型及 M型成膜体的统称而不应仅指 M型成膜体.

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