The Morphological Study of Amyloplast Distribution in *Crocus sativus* L. Fibrous Roots

Behrooz Sh. Behboodi[1] and Leili Samadi [1,2]
[1]Cytology Lab., Department of Biology, Faculty of Science,
University of Tehran, Tehran,
Iran
[2]Section of Cell Biology, Department of Biology, Faculty of Science,
Razi University, Kermanshah
Iran

Keywords: amyloplasts, PAS staining

Abstract

The fibrous roots of *Crocus sativus* L. emerge from a single ring of its corms and these roots are not branched. We studied the distribution of amyloplasts in these roots by using periodic acid Shiff staining (PAS). As the result of PAS staining the amyloplasts are colored purple. Four morphogenic regions can be distinguished in proliferation region of a fibrous root: Cap, Meristem, Cortex initiation, and central cylinder initiation. Results of PAS staining shows that the amyloplasts are stored in cap cells, mostly. The amyloplasts were partly observed in meristem, cortex and central cylinder as well. The size of amyloplasts in cap cells is larger than amyloplasts of cortex and central cylinder initiation. There is no amyloplast in caliptra and meristem cells which evident that the formation of these grains may occur during differentiating stages.

INTRODUCTION

Crocus sativus L. is a monocotyledon, and a member of Iridaceae family that has permanent underground stem bases, called bulbs or corms. These corms produce two modes of structural and functional different roots: fibrous roots and contractile roots (Cannon, 1949). The fibrous roots emerge from a single ring at the base of the corms. These roots are straight and thin (about 1 mm thickness). At the longitudinal sections two main regions can be recognized: proliferation region and elongation region (Clows, 1959). There are four morphogenic zones in the proliferation region, include: cap, meristem, cortex initiation zone and central cylinder initiation (Clows, 1961).

Amyloplasts are non-pigmented plastids, which synthesize and store starch. About a century ago, the starch statolith hypothesis (SSH) was established (Sack, 1991), which states that the starch grain-filled plastids (amyloplasts) interact with other cell components, and provide the cell with information about its orientation (Hart, 1991). Specific cells in plant organs contain amyloplasts. Sometimes these amyloplasts combine with each other and form formless starch grains, named statoliths (Hart, 1991). Our purpose was to study the distribution of amyloplasts in root proliferation cells of fibrous roots and possibly formation of statoliths in these cells.

MATERIALS AND METHODS

Two years old *Crocus sativus* corms were raised in distilled water for 48 h, at room temperature (RT), in dark. Terminal 5 mm of the grown fibrous roots were separated after 2 days, and fixed immediately in a Formalin- Acetic acid- Ethanol solution (5%, 10%, 85%) overnight at 4 ºC (Jensen, 1962). The samples then were dehydrated through a graded ethanol series (20, 50, 75, 95, 100, 100, and 100 % in distilled water), infiltrated through a paraffin series (25, 50, 75, and 100 % in Toluol), and embedded in paraffin. After that they were sectioned longitudinally to 6µm thickness.

Sections were later deparafinized in toluol and hydrated through a graded ethanol series (100, 100, 100, 75, 50 and 0 % in distilled water). Afterwards they were oxidized by a 1% periodic acid solution (MERK B 383224, in distilled water) for 15 min, at RT

and after washing were sank in a reactive Shiff solution for 45 min, at RT, in dark (Kerr et al., 1994). The sections then were washed repeatedly by distilled water, dehydrated in ethanol, cleared in toluol and covered with cover slips. This reaction, entitled PAS (Periodic Acid Shiff), is a cytochemical method to stain polysaccharides in which amyloplasts are seen purple.

RESULTS

The distribution of amyloplasts were studied in different zones of root proliferation region, which are described below:

Meristem

The meristematic cells are isodiametric and have central nuclei. They are commonly unfilled by amyloplasts (Fig.1 A).

Cortex Initiation

Central cylinder encircled cortex initiation cells have no amyloplast. The outer layers of cortex initiation have a few small and distinct amyloplasts (Fig.1 B). In the layer immediately before epidermis the statoliths were observed (Fig.1 C). These statoliths are amorphous and large.

Central Cylinder Initiation

In central cylinder initiation zone most layers contain amyloplasts (Fig.1 D, E), except tracheary element cells (Fig.1 F). Amyloplasts in central cylinder initiation cells are positioned right away at the side of the cell wall and are separated from each other (Fig.1 E).

Cap

Our examination showed that the saffron root cap includes 12 cell layers: 4 layers of meristematic cells that are called caliptra, one layer of differentiating cells, 5 statolith layers, and 2 layers of secretory cells. These cells come from a continuous developmental event that successively occurs during root growth, from division of caliptra, differentiation of their derivatives into amyloplast contain cells (statocytes), transformation of statocytes into secretory cells, and finally into sloudhed cells (Fig.1 A). This event is the normal cap development.

The structure of cap meristem cells, caliptra, in the main feature is typical for cells of meristem type. But in spite of meristem cells, these cells are polygonal. Their nucleus is large, spherical and center-positioned. No amyloplast constantly is found in the caliptra (Fig.1 G). In the differentiating layer of cap, rare numbers of very small amyloplasts were observed in the cell periphery (Fig. 1 G). These grains combine and form statoliths in the statocyte layers (Fig. 1 H). Although, separated amyloplasts were also observed in these cells. In the secretory cap cells, the statoliths are larger and deformed. The cell wall of these cells is very thick and their nuclei are condensed and marginated (Fig. 1 I).

In general, meristematic cells of root proliferation region, which include central meristem and caliptra, have no amyloplast (Fig.2). Cap is the main amyloplast storage zone (Fig. 2). The volume and number of amyloplasts are increased from caliptra to secretory cells (Fig. 3).

DISCUSSION

Despite the results of several authors (Moctezumz and Feldman, 1999; Jourdan et al., 2000; Kordyum and Guikema, 2001; Kutschera, 2001), amyloplasts in saffron root cells are not sedimented and are scattered through out the cells. Although in downward layers of cap, the amyloplasts or statoliths are deposited in the corner of the cells in the lowest region of cytoplasm. Appearance of amyloplasts in differentiating cells and their absence in meristematic cells may evident the formation of these grains during differentiating stages.

Literature Cited

Cannon, W.A. 1949. A tentative classification of root systems. Ecology 30: 542-548.
Clows, F.A.L. 1959. Apical meristem of roots. Biol. Rev. 34: 501-519.
Clowes, F.A.L. 1961. Apical meristems. Oxford, Blackwell.
Hart, J.W. 1990. Gravitropism. In: Hart J.W. (ed.) Plant tropisms and other growth movements. Unwin Hyman, London.
Jourdan, C. Michaux-Ferrie, N. Perbal, R. and Perbal, G. 2000. Root system architecture and gravitropism in the oil palm. Ann. Bot. 85: 861-868
Kerr, J.F. Winterford, C.M. and Harmon, B.V. 1994. Cell Biol: A Laboratory Handbook, Acad. Press Inc., London.
Jensen, W.A. 1962. Botanical Histochmistry. Freeman and Co., London.
Kordyum, E. and Guikema, J. 2001. An active role of the amyloplasts and nuclei of root statocytes in graviperception. Adv. Space Res. 27: 951-956.
Kutschera, U. 2001. Gravitropism of axial organs in multicellular plants. Adv. Space Res 27: 851-860.
Moctezumz, E. and Feldman, L.J. 1999. The role of amyloplasts during gravity perception in gynophores of the peanut Plant (*Arachis hypogaea*). Ann. Bot. 84: 709-714
Sack, F.D. 1991. Plant gravity sensing. Int. Rev. Cytol. 127: 193-252.

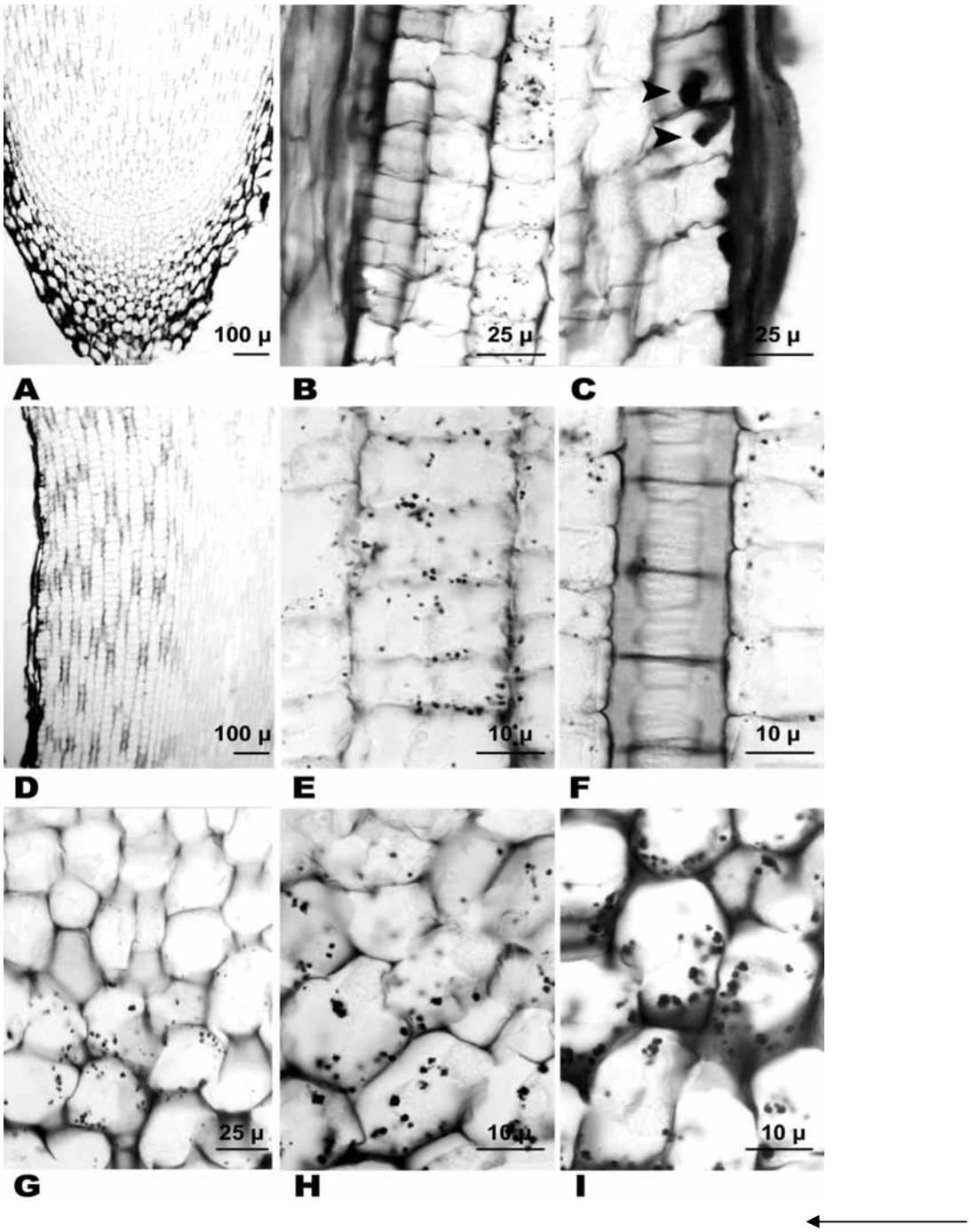

Fig. 1. Results of PAS staining, Amyloplasts and cell walls are seen purple. A. Root cap and meristem cells. B. The outer layers of cortex initiation zone. C. The cortex layer immediately before epidermis. The statoliths are observed in these cells (arrows). D. Cortx and central cylinder initiation zones. E. The cells of central cylinder initiation zone. F. A row of tracheary element cells. G. Root cap meristem (caliptra). H. Statoliths in the cap statocyte layers. I. Cap secretory cells. Longitudinal section.

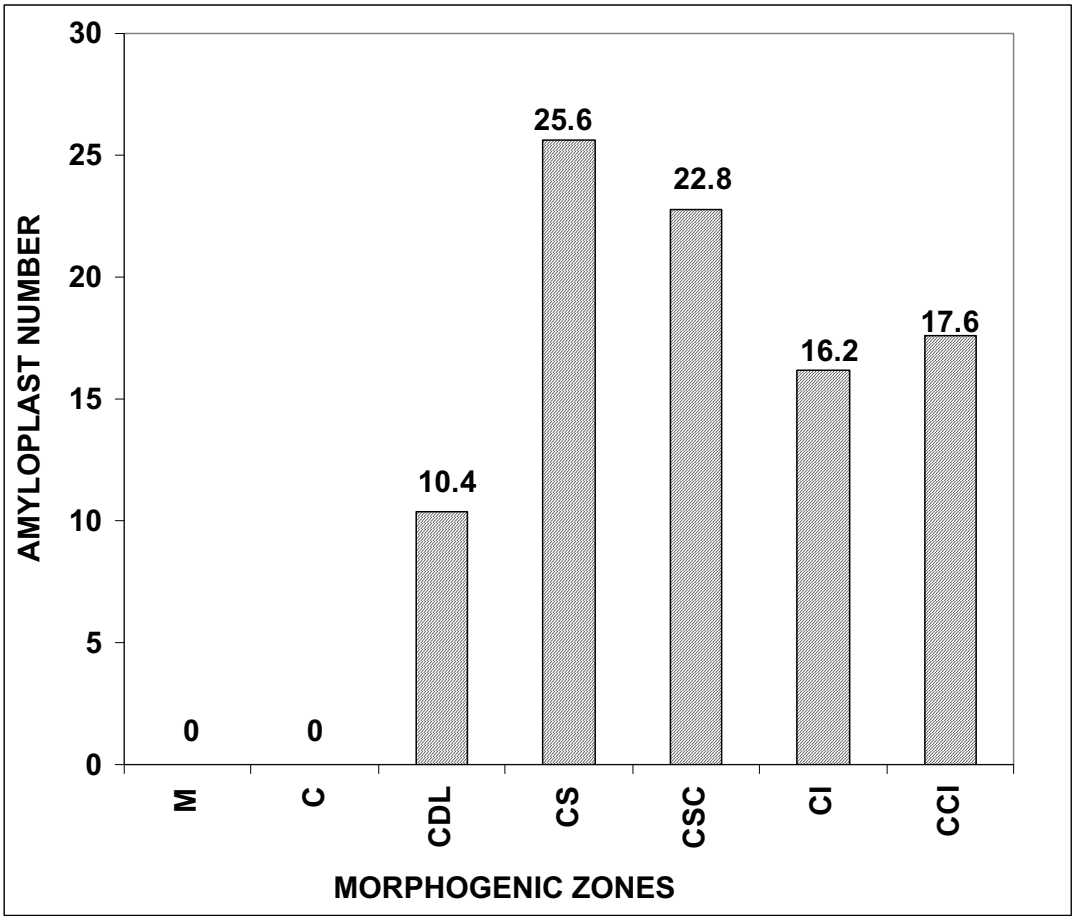

Fig. 2. The average number of amyloplasts per cell in root proliferation zones. M: meristem, C: caliptra, CDL: cap differentiating layer, CS: cap statocytes, CSC: cap secretory cells, CI: Cortex initiatrion, CCI: Central cylinder initiation.

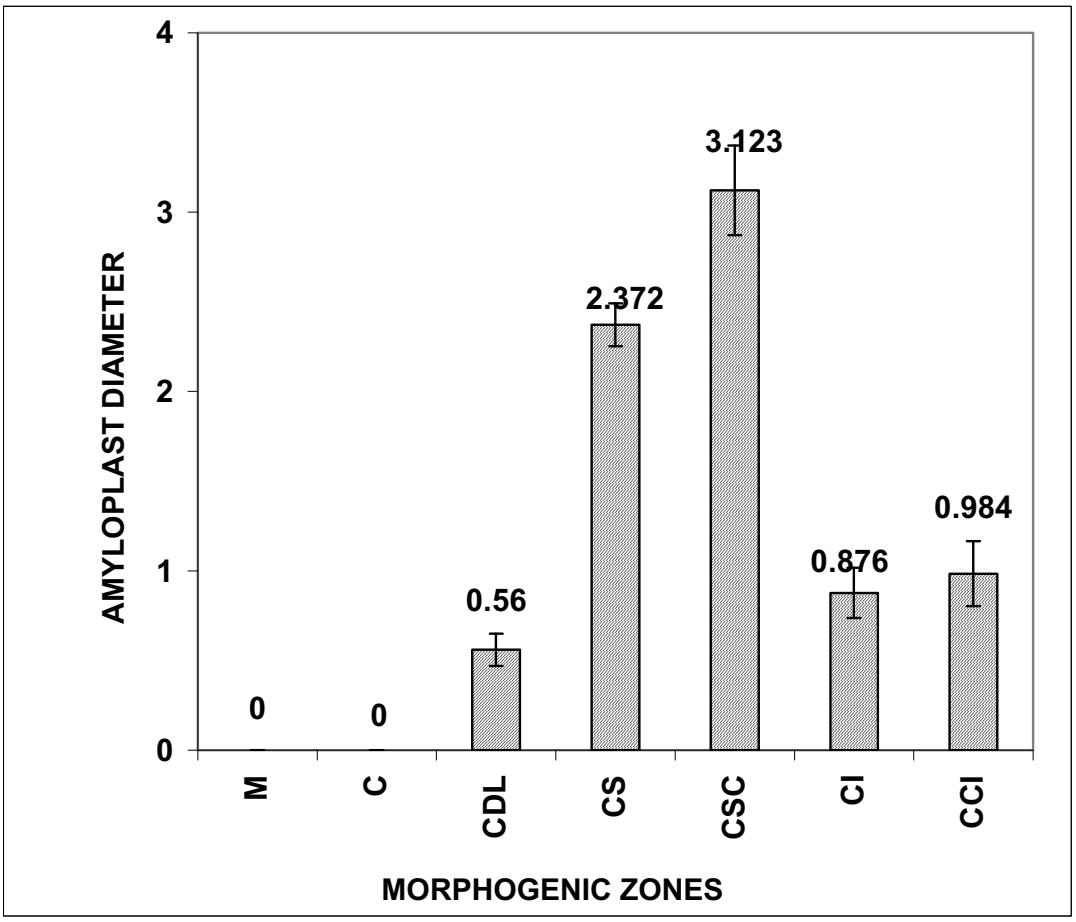

Fig. 3. The average diameter of amyloplasts per cell (μm) in root proliferation zones of saffron root. M: meristem, C: caliptra, CDL: cap differentiating layer, CS: cap statocytes, CSC: cap secretory cells, CI: Cortex initiatrion, CCI: Central cylinder initiation.