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# DISTRIBUTION OF ASCORBIC ACID IN THE DEVELOPING ANTHERS OF *CUCUMIS SATIVUS* L.

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## ABSTRACT

Ascorbic acid has been found to be present and essential to the normal functioning of all cellular units in the higher plants and animals including subcellular structure such as ribosomes and mitochondria. In plants, it appears to play an essential role in photosynthesis and is formed rapidly in all of the most active cells during the initial germination stage in seeds. Ascorbic acid (vitamin C) is an abundant component of plants. It has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth. In relation to cell growth, ascorbate is a cofactor for prolyl hydroxylase that posttranslationally hydroxylates proline residues in cell wall hydroxyproline-rich glycoproteins required for cell division and expansion. It occurs in all cell compartments including the cell wall. Additionally, high ascorbate oxidase activity in the cell wall is correlated with areas of rapid cell expansion. Emerging research results indicate that ascorbate, existing widely in plants as the abundant micromolecule substance, fulfils its essential roles in series of physiological processes such as plant defense against oxidization, co-factor of key enzymes, plant cell division, cell expansion, growth and development, and senescence (Smirnoff N (1996) and Horemans N, Foyer CH, Potters G, Asard H (2000b)). Ascorbate, at least in some plant species, is also the substrate for the biosynthesis of oxalate and tartrate (Loewus F.A., Loewus MW (1987) and Loewus F.A. (1999)). However, the multifunction of ascorbate makes it complicated to decipher its exact role under certain physiological process. Thus, there is need to increase our understanding of this enigmatic molecule since it could be involved in a wide range of important functions from antioxidant defense and photosynthesis to growth regulation. Present investigations pertain to the

histochemical assessments of general Ascorbic acid and their role in the differentiation of tissues during anther development in *Cucumis sativus* L.

**KEYWORDS:** Ascorbic acid *Cucumis sativus* L., anther tissue, histochemistry.

## INTRODUCTION

Ascorbic acid has been found to be present and essential to the normal functioning of all cellular units in the higher plants and animals including sub cellular structure such as ribosome's and mitochondria. In plants, it appears to play an essential role in photosynthesis and is formed rapidly in all of the most active cells during the initial germination stage in seeds. Ascorbic acid (vitamin C) is an abundant component of plants. It has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth. In relation to cell growth, ascorbate is a cofactor for prolyl hydroxylase that posttranslationally hydroxylates proline residues in cell wall hydroxyproline-rich glycoproteins required for cell division and expansion. It occurs in all cell compartments including the cell wall. Additionally, high ascorbate oxidase activity in the cell wall is correlated with areas of rapid cell expansion.

Emerging research results indicate that ascorbate, existing widely in plants as the abundant micromolecule substance, fulfils its essential roles in series of physiological processes such as plant defense against oxidization, co-factor of key enzymes, plant cell division, cell expansion, growth and development, and senescence (Smirnoff N (1996) and Horemans N, Foyer CH, Potters G, Asard H (2000b)). Ascorbate, at least in some plant species, is also the substrate for the biosynthesis of oxalate and tartrate (Loewus F.A., Loewus MW (1987) and Loewus F.A. (1999)). However, the multifunction of ascorbate makes it complicated to decipher its exact role under certain physiological process.

Arabidopsis thaliana mutants lacking ascorbate cannot grow well, but it is not known which function is critical: control of reactive oxygen or the proposed roles in modulating cell expansion and division (Smirnoff N. (2011). Thus, there is need to increase our understanding of this enigmatic molecule since it could be involved in a wide range of important functions from antioxidant defense and photosynthesis to growth regulation.

Present investigations pertain to the histochemical assessments of general Ascorbic acid and their role in the differentiation of tissues during anther development in *Cucumis sativus* L.

*Cucumis sativus* L, the garden cucumber, is a widely cultivated plant in the Gourd family (Cucurbitaceae), which includes squash, and in the same genus as the muskmelon. The cucumber likely originated in India, where it appears to have been cultivated for more than 3,000 years, and then spread to China. Plants are usually monoecious (male and female flowers on separate plants), but varieties show a range of sexual systems. *Cucumis sativus* L. has also served as a model system for sex determination.

## MATERIAL AND METHOD

The anthers from male flower buds of *Cucumis sativus* L. at successive developmental stages were collected during morning and fixed on the spot in acidified silver nitrate solution (Dave et.al. 1968) for seven days at $0^0$ C to $2^0$C.After seven days flower buds were washed thrice in 5% liquid ammonia in 50% alcohol for 15 minutes under red light in dark room, dehydrated in alcohol-xylene series, (Khasim 2002), embedded in paraffin. Five µm thick transverse sections of flower buds were taken with the help of semi automatic Leica Microtome for assessment of ascorbic acid.

## OBSERVATIONS

The floral meristem during the initiation of sepal and petal primordial is devoid of Ascorbic acid. When stamen buttresses make their appearance in the third whorl sporadic distribution of ascorbic acid is observed in the sepal primordial and in the subtending regions of stamen primordial. (Fig.1A) In subtending region of pedicel however rich accumulation of ascorbic acid is noticed (Fig. 1B) as the cells in stamen primordia proliferate, significant accumulation of ascorbic acid occurs in the derivatives of floral meristems (Fig.1C). Sporadic distribution of ascorbic acid is also observed in distal region of stamen primordium (Fig.1 C).

The development of stamen primordium is characterized by the rich accumulation of ascorbic acid in the filament (Fig.2A). However the ascorbic acid is absent in the anther including young sporogenous cells. (Fig.2A). As the sporogenous cells multiply (Fig.2B) and meiocytes differentiate (Fig.2C) ascorbic acid begins to accumulate in the tapetal tissue. During meiosis, only traces of ascorbic acid are seen in the tapetal tissues. (Fig.3A) At young and old microspore tetrad stages also, appreciable amount of ascorbic acid is not observed in any locular tissues of anther (Fig.3B and 3C). However at tetrad stage significant accumulation of ascorbic acid is observed in the connective (Fig. 4A).

Very rich accumulation of ascorbic acid is seen in the periphery of the vacuolated microspores (Fig. 4B). At this stage ascorbic acid is totally absent in the remaining parts of the anther. Rich peripheral distribution of ascorbic acid in the microspores persists at pollen mitosis stage (Fig. 4C). Significantly at this stage, discrete particles of ascorbic acid are also observed in the differentiating endothecium (Fig. 4C).The filament also is seen with the presence of ascorbic acid (Fig. 5A). However in mature stamen level of ascorbic acid significantly drops down in the filament (Fig.5B). Mature pollen grains contain rich content of ascorbic acid along their walls (Fig. 5C).The fully differentiated endothecium lacks ascorbic acid (Fig. 5C).

L.S. OF MALE FLOWER TESTED FOR ASCORBIC ACID

(s-sepal;p-petal;st-stamen;stp-stamen primordial;f-filament;spc-sporogenous cells;t-tapetum; tc-tapetal cells; co-connective; m-meiocyte;td-tetrads;ec-endothecial cells)

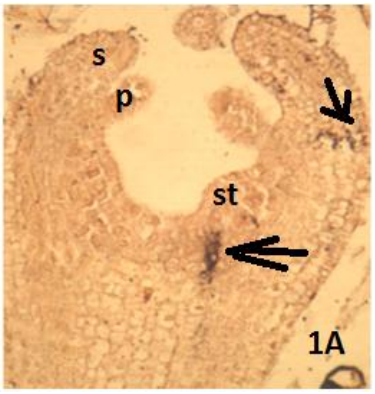


**Figure 1 A: Floral meristem showing sepal(s) petal (p) and stamen (st) primordial. Sporadic distribution of ascorbic acid is seen below stamen and sepal primordial**

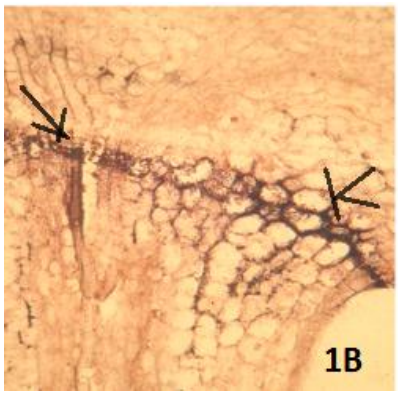


**Figure 1B portion of a pedicel showing accumulation of ascorbic acid (arrows)**

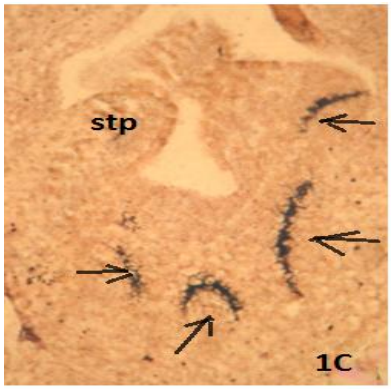


**Figure 1C: Growing stamen primordial (stp) showing Sporadic distribution of ascorbic acid (arrows) Ascorbic acid is also present in the subtending region of floral meristem (solid arrows)**

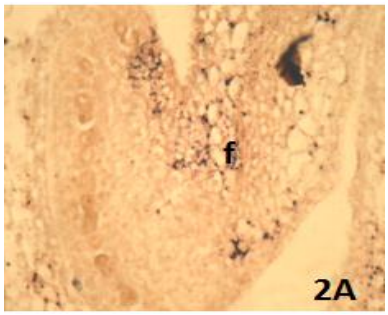


**Figure 2A: Ascorbic acid is present in the filament(f)**

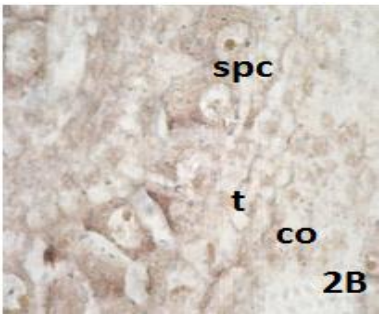


**Figure 2B: Sporogenous cells (spc) and tapetum (t) are poor in ascorbic acid. Ascorbic acid is absent in connective (co)**

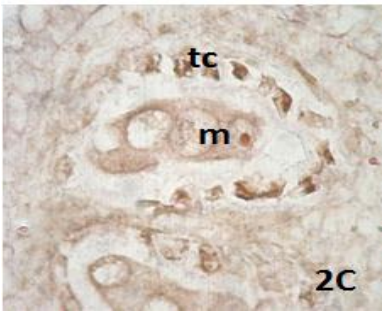


**Figure 2C :At meiocyte (m) stage, ascorbic acid accumulates in the tapetal cells (tc)**

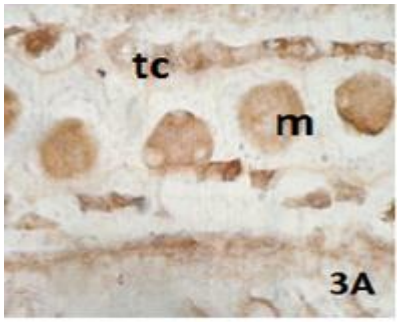


**Figure 3A :Tapetal cells (tc) telophase-II stage of meiocytes (m) contain only traces of ascorbic acid**

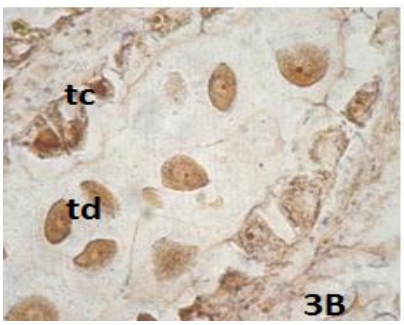


**Figure 3B: Except Tapetal cells (tc) anther wall layers and tetrads (td) lack ascorbic acid.**

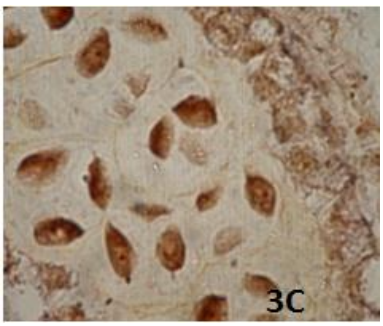


**Figure 3C: Similar situation prevails at the late tetrad(td) stage**

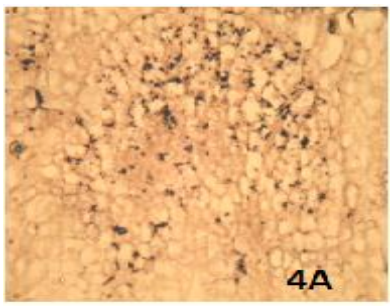


**Figure 4A: at the tetrad (td) stage ascorbic acid is present in connective (co)**

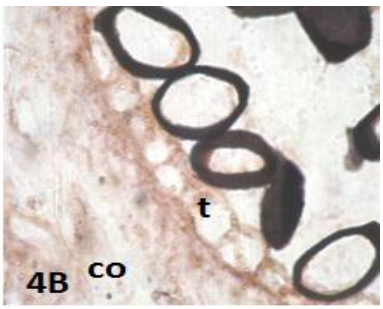


**Figure 4B: Vacuolate microspore showing rich accumulation of ascorbic acid at the periphery of cells.Tapetum (t)and connective (co) lack ascorbic acid**

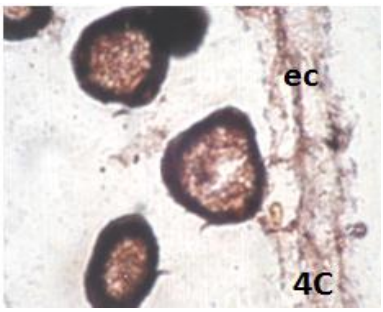


**Figure 4C: Pollen grains showing rich accumulation of ascorbic acid at the periphery. Endothecial cells (ec) also possess some ascorbic acid**

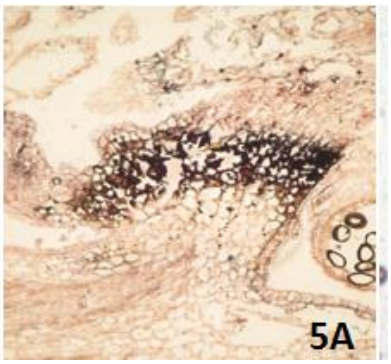


**Figure 5A :At microspore stage significant amount of ascorbic acid accumulates in the filament(f)**

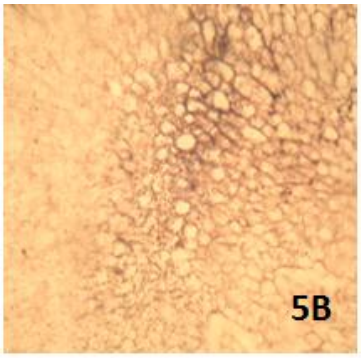


**Figure 5B: In the mature filament ascorbic acid drastically depletes**

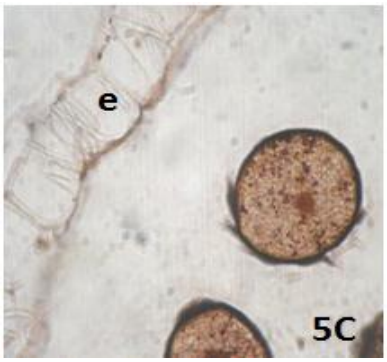


**Figure 5C: Mature pollen grain showing ascorbic acid confined along the exine. Endothecium (e) lacks ascorbic acid.**

## DISCUSSION

The stamen primordial develops after the development of sepal and petal primordial and has thin walled cells. But later the stamen primordium grows rapidly overtaking the petal primordial and this is made possible probably by the presence of ascorbic acid along with rich content of RNA and proteins. The differentiation of stamen primordium into filament and other correlates with the differential distribution of ascorbic acid along with starch, RNA and proteins. The cells of the filament are characterized by the presence of ascorbic acid and poor presence of RNA and proteins. On the contrary, cells of anther primordial are rich in RNA and proteins but lack storage carbohydrates and ascorbic acid. These histochemical differences between filament and anther reflect differences in their metabolic functions. The presence of rich content ascorbic acid in the filament presumably helps in its elongation, because the proper positioning of the anther is equally important as the production of viable pollen is. Filament also performs another important physiological function. The amylaceous content from the maternal plant have to pass through the filament (Clement et.al., 1994, 1996) and thus it functions as a conduct for the nutritional supply to anther tissues.

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