A COMPARATIVE STUDY ON THE ROOT AND STEM ANATOMY OF ASPARAGUS *(Asparagus officinalis* L.) EXVITROPLANTLETS AND VITROPLANTLETS

Florica HURGOIU[1] & Monica ŞIPOŞ[2]

[1]PHD Student,University from Oradea
2 University from Oradea

Abstract. We made this study for make the comparison between anatomy of *Asparagus officinalis* L. vegetative organs, roots and stems, which were harvested from the exvitroplantlets and from the vitroplantlets, normal and vitrified. Roots and stems harvested from the exvitroplantlets and normal vitroplantlets have shown the same primary monocotyledon structure, these two types of plantlets being in two different stages of growth. The structure differences and cytological modifications were observed in the vitrified stems of vitroplantlets, where bigger intercellular spaces denote tissular fragility and the ratio of cortex / central cylinder will modify. The vitrified stems remained in a less advanced ontogenetic growing stage comparatively with those normal vitroplantlets stems.

INTRODUCTION

In the specialty literature, were made concerning about the anatomic structures of asparagus stems *(Asparagus officinalis* L.), made by Grinţescu, 1985. Our studies were centered on an anatomic research of asparagus plantlets stems and roots derived from normal and vitrified vitroplantlets cultures, comparatively with the anatomy of the acclimatized exvitroplantlets in greenhouse conditions.

MATERIAL AND METHOD

The vegetal material, roots and stems of asparagus, were harvested from fresh, normal or vitrified vitroplantlets, after 90 days of vitrocultures, and from plantlets grown "in vitro" for 45 days, then were acclimatized and grown for 45 days in greenhouse conditions.

The cross sections were made on harvested fragment, in the middle side of these vegetative organs, with the help of a blade.

To remove the cellular content which diminishing the transparency degree of the sections these were covered with some few milliliters Javel water (a 10% NaOCl solution), in 5-10 minutes, this procedure being known as the clarification process. After the removing of the NaOCl solution, a repeated washing of these sections was made with distilled and sterile water, to remove the chloral disinfectant solution from the cells, the hipochloric solution spots discolor or breaching the sections after short time of coloration. For an easier differentiation of the tissular structures was precede a doubled coloration of the sections.

The doubled coloration was made by an application on the clarified sections of a dyestuff or staining, 3% red Congo solution, for one minute, a solution which colors in red the primary cells walls. The remove of the staining was made by aspiring of this with a pipette, than the segments were washed with distilled water and with the help of an spatullated needle were transferred in a watch glass, dropping on it 1% green iodine solution, which color the lignin of the cell walls with secondary modifications in green. This second staining was left to action in 30 seconds, after that it was decanted, and the sections were washed repeated times.

The sections and some parts of this were examined to an optical microscope, with 4x and 10x objectives, and for details with 20x and 40x, were photographed and this make the object of the 1-3 figures.

RESULTS AND DISCUSSIONS

The sections of the greenhouse acclimatized exvitroplantlets of asparagus *(Asparagus officinalis* L.) were used for exam their structure.

In cross sections the root has a round form (fig. 1) and appears to be formed of a series of concentric cylinder.

First layer, the rhizoderma, was represented by small cells, some of them became absorbing hairs by elongation.

The cortex was observed with 10x objective; it was pluristratified and divided in 3 subsectors: exoderma, cortical parenchyma and endoderma.

Exoderma consists in 3-4 ranks of suberificated cells. With the 40x objective was observed the cell walls, it being a suber with primary origin.

The cortical parenchyma had 15-20 ranks of oval cells, with intercellular spaces between them and these cells were deposited the reticence substances, the asparagus roots being metamorphosed, more exactly tuberified.

The last layer of cortex, the endoderma, was formed from cells with lateral walls with suberous thickenings called *Casper's gangs*, and in the cross section *Casper's points* interrupted from passage cells. The central cylinder (fig. 1) includes the pericycle, the ligneous fascicles, the liberian fascicles, medullar rays and the medullar parenchyma.

The pericyclei, is the peripheral layer and it is consist from small cells with cellulose walls. In some sections was observed that from this layer will form radicells, that why as known as a rizogene layer. In the root section was observed, the ligneous and the liberian fascicles, which alternate between them, they being separated from the primary medullar rays, composed from oval cells, with cellulose walls. It has observed 13-15 ligneous fascicles and liberian fascicles, in the same number, in the asparagus exvitroplantlet root sections.

The ligneous fascicles were easy to distinguish through the lignified callous walls, colored in green with green iodine. The extern vessel beside the pericycle, had a small diameter, forming the protoxylem, these being the first vessels which differentiate. The subsequent differentiated vessel to the root center, had a bigger diameter and integrates the metaxylem. Around the metaxylem vessels are the ligneous parenchymatic cells found.

The liberian fascicles were smaller, with cellulose walls, colored in red with Congo red. The smaller vessels form the protophloem and are beside the pericycle, and the vessels with a bigger diameter are disposed inside, shaping the metaphloem.

The center of root has been proved to be a parenchyma formed from round and oval cells, with cellulose walls.

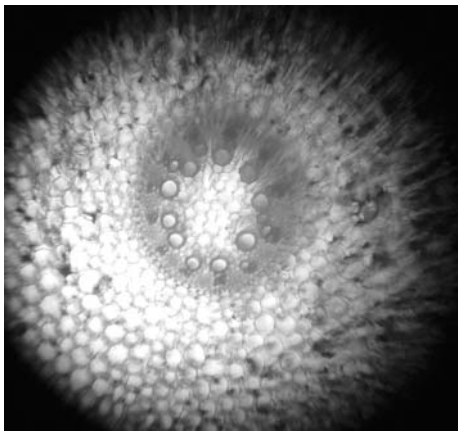

Fig.1 Transversal section through tuberified root at the asparagus (*Asparagus officinalis* L.*)* exvitroplantlets – the vision on the cylinder center

The stem has a circular perimeter in cross section (fig.2).

The epiderma is composed from a cell layer which has no intercellular spaces, interrupted site by site from stomata and covered by a cuticle. The cortex was dignified being composed from one chlorenchyma sector, integrated from 3-4 cell layers full with chloroplasts and one schlerenchyma sector with 2-3 cell layers with strong cellular walls callous with lignin. It has not been observed the endoderma and the pericycle, the limit between the cortex and the central cylinder being an imaginary line which passes by those more external fascicles periphery.

In the central cylinder, through a fundamental parenchyma, it were observed that were disposed, in three ranks, the libero-ligneous collateral closed conducting fascicles. The 8 biggest fascicles were disposed to the stem ax and the external two ranks covered smaller fascicles. This type of the central cylinder is known as atactostel. The wood had a V form, with protoxylem vessels, with small lumenum inside. To the opening of the V is it founded the phloem, with the metaphloem inside and with the protophloem outside.

The central cylinder was good represented comparatively with the cortex, the cortex/ central cylinder ratio being 1:4 (fig.2).

The normal asparagus vitroplantlets were small in the length and diameter, these being in different stage of growth, less advanced comparatively with the exvitroplantlets. To settle the growth process, it will be measured one or more parameters, for example the length, weight, area, and volume of these plants or vegetal organs. (Taji et. al, 2002). It had been observed the same anatomic structure with the normal exvitroplantlets, the primary structure, with the specific characters of monocotyledons, but normal vitroplantlets were another ontogenetic stage.

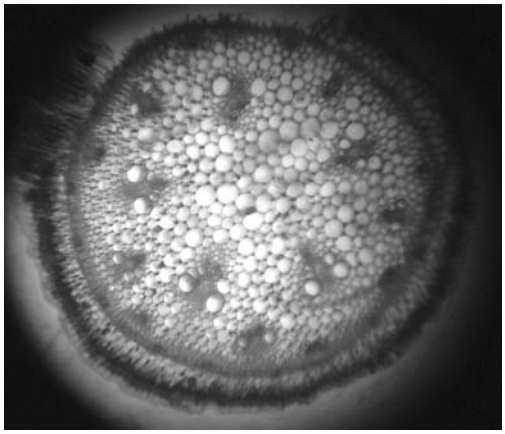

Fig.2 Transversal section through the asparagus *(Asparagus officinalis* L.*)* exvitroplantlets stem

The root, with a circular contour, was easy tuberified, the cortical parenchyma being powerless represented to those plants roots acclimatized in the greenhouse, and the number of the fascicles from the central cylinder was smaller, 10-11 lignified fascicles, and also same liberians too.

As regards the stem, the sector outline has a rounded form, the epiderma being less cutinized. The atactostel was presented inside a number of 5 libero-ligneous fascicles, with big dimensions, and outside, a big number of smaller fascicles. The libero-ligneous fascicles, disseminated in the fundamental parenchyma, were placed in two ranks.

The asparagus vitroplantlets which were vitrified, they no had roots, finally were made it transverse sections only in those stems. As results from fig.3, the stem has an irregular contour.

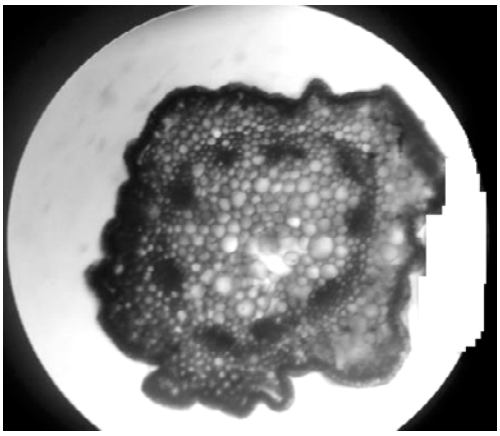

Fig.3 Transversal section through the stem of the vitrified asparagus *(Asparagus officinalis* L.*)* vitroplantlets.

The chlorenchyma from the cortex was good represented to the exvitroplantlets acclimatized in the greenhouse, as well as the normal vitroplantlets, being composed from 6-8 cell layers, between exists big intercellular spaces from the outside they being fraught in chloroplasts, comparatively with the inside layers. The schlerenchyma was less differentiated, in a sclerification process. From the specialty literature is known the fact that the vitrification and the hyperhidration of the tissues suppose the growth of the water quantity, the replacing air from the intercellular spaces with water. The histological and cytological modifications from the vitrified vegetal tissues were studied by many authors. Hereby, Cachiță and Crăciun, 1990, in the

researches made it with electronic microscope with carnation vitrified leaves comparatively with the normal in vitro leaves, the intercellular spaces from mezophil are bigger, the cells were extremely poor in the cytoplasma, the chloroplasts had the whole structure deteriorated, and the plasmalema is separating from some places of the cell wall, inserting to the center of cells.

The collateral libero-ligneous conducting fascicles from the central cylinder were placed in one single rank, in near of schlerenchyma. The ratio between the cortex and central cylinder it was 1:1 (fig. 3).

CONCLUSIONS

The same anatomic structure, the specific primary anatomic structure of the monocotyledons, was observed at *90 days* of growth, to the tuberified roots and also to the stems of the asparagus exvitroplantlets which were acclimatized in the greenhouse conditions, as well as the vegetative organs of the normal vitroplantlets, these two types of plants being in two different growing stages.

The structure differences and cytological modification were observed in the vitrified stems of vitroplantlets, where bigger intercellular spaces denote tissular fragility and the ratio of cortex / central cylinder will modify. The vitrified stems remained in a less advanced ontogenetic growing stage comparatively with those normal vitroplantlets stems.

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