Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 23, issue 3, 2013, pp. 357-359

DETERMINATIONS OF THE NEUTRAL RED VITAL DYE ACCUMULATION IN THE ROOTS OF CASTOR OIL PLANT (*RICINUS COMMUNIS*) COMPARED TO THE EXTRA-RADICULAR ABSORPTION RECORDED IN THE AERIAL PARTS OF THE PLANT

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ABSTRACT. These studies and the obtained results have beside a practical value as "investigative tools" in phytophysiology, also a theoretical one, since they allowed the identification of differentiation of seedling organs, tissues and cells capacity during sprouting , of the transformations undergone in the course of physiologic processes that take place at the stage of germination, to accumulate in varying amounts the neutral red dye, depending on the type of cells found in the structure of tissues and organs, and their degree of differentiation.

Keywords: castor oil plant, total absorption, specific absorption, vital dyes, neutral red

INTRODUCTION

According to Drawert (1948), the first experiments with vital dyes were still performed by Unger, data published in 1848. Subsequently, Pfeffer (1886) used vital dyes for exploring the structure of plant cells to investigate their functioning.

Vital dyes were used in the research of cellular permeability and absorption, to obtain data on the degree of vitality of the tissues or cells and to acquire information about the chemical composition of vacuolar fluid etc. They accumulate in living cells without damaging them. There are, however, some limits of tolerance depending on the nature of the dye used and its concentration, and the duration of action on the tissue or cells. As the dye concentration is higher, and as the duration of contact with the vegetable tissue increases, as some aspects of cell intoxication become more apparent, and their intensity varies in relation to the chemical nature of the dye.

Vital dyes are organic substances, which have in their molecule a chromophore group. This group selectively absorbs light radiation, the dye showing a specific absorption spectrum.

Neutral red accumulated in the vacuole can be in diffuse form or in the form of corpuscles (aggregates, crystals, and so on). Prolonged staining always causes the occurrence of vacuole contraction phenomenon and cytoplasm inhibition (Drawert, 1948).

MATERIALS AND METHODS

After seeds germination, seedlings raise their cotyledons above the ground, these turn green and the autotrophic nutrition processes start, respectively photosynthesis.

For the experiment conducted with seedlings of castor oil plant material we used castor oil plant seeds germinated and seedlings of different ages between the 3rd and 12th day after their germination.

Before the plant material had to be placed in the experimental process, in the neutral red solution, seed coat and the endosperm of the cotyledons of the seedlings were removed with a spatulated needle. After the 10th day from placing the castor oil plant beans to germinate this operation was not necessary because seed coat fell off and endosperm was already almost entirely consumed by the seedlings, becoming mucilaginous. The duration of keeping the seedlings in the solution of the dye was fixed at 2 hours.

For each age, in part, the experiment was conducted with 50 seedlings, subdivided into five lots of ten seedlings each. The final expression of the results was to calculate the absolute values (arithmetic average) of the total absorption and specific absorption; in the second case, the data obtained by calculating the total absorption was related to the dry weight of organs the dye was extracted from, with reference to an individual. Data were expressed at relative values, obtained by mathematical calculations of reporting the results in percentage per whole seedling, values considered as 100%.

Measurements were made on the 3rd, 5th, 7th, 9th and 11th day of germination. The graphs in Figure 3 show the variation of the absorption capacity of the seedlings and their organs at different ages from the placing of seeds to germinate, expressed in absolute values, of mg/2h of absorption, representing the total absorption of the embryo radicle, together with that of the secondary roots, and the rest of the seedling, or by calculating the percentage of absorption by the whole seedling, values considered to be 100%; calculating the total absorption of the root and, separately, the absorption done by the hypocotyl and cotyledons and a part of the epicotyl, data represented graphically in Figures 3-6.

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Article received: May 2013; Published: September 2013

RESULTS AND DICUSSIONS

From microscopy images (Fig. 2 B-E) is clear that neutral red was fixed in all the cells that make up the tissues of the tip of the root, both the embryonic radicle and the secondary ones, generated at the level of the embryo ones. As shown in the macroscopic images (Fig. 1 and Fig. 2) illustrating the appearance of the castor oil plant seedlings at 3 and 7 days of germination, the secondary roots are well developed, which, at 7 days of germination, favored the formation of highly branched root system, which explains the high capacity of the roots in this species (Fig. 3-6), both regarding the total absorption and the specific absorption.

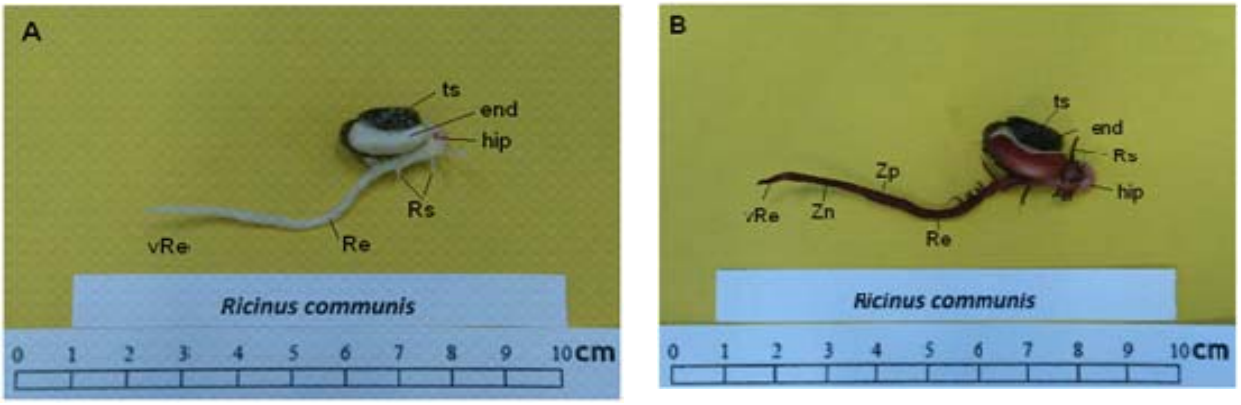

Fig. 1. Aspect of the castor oil plant (Ricinus communis) seedling on the 3rd day of germination; A – non-colored seedling and B – colored seedling (abbreviations: end – endosperm; hip – hypocotyl; Re-embrionary root; Rs – secondary roots; vRe – tip of embrionary root; Zn – zone of elongation; Zp – zone of absorbent hairs; ts – seed coat).

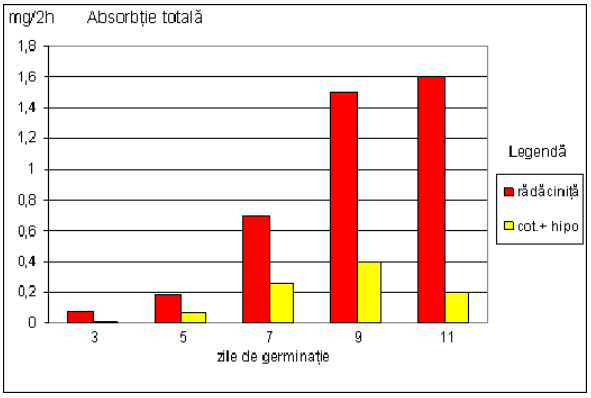

Fig. 3 Comparative results regarding the total absorption (in mg/2h) of the neutral red vital dye in roots, respectively seedling of castor oil plant (*Ricinus communis*) recorded during the first 11 days of germination.

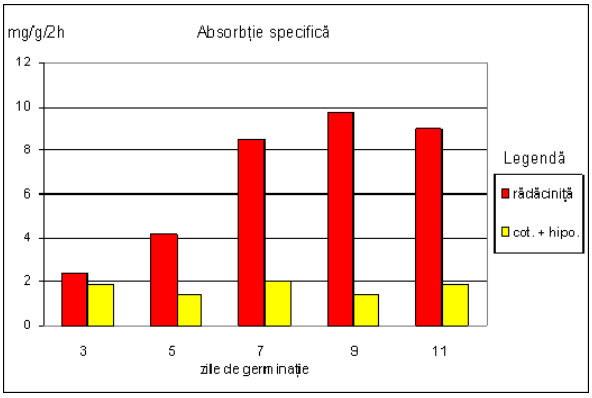

Fig. 4. Comparative results regarding the specific absorption (in mg/g/2h) of the neutral red vital dye in roots, respectively seedling of castor oil plant (*Ricinus communis*), during the first 11 days of germination (abbreviations: cot – cotyledons; hipo – hypocotyl).

The analysis of the graph shown in Figure 3 which shows the evolution of the total absorption of neutral red on root tissue, helps us conclude the fact that, during the first 5 days (the 3rd, 4th and 5th day after germination), the largest amount of the dye was recorded at the mass of the root of the seedling of castor oil plant and to a lesser extent in the cotyledons and hypocotyl (Fig. 4).

In the case of castor oil plant, the embryonic root and its ramifications, i.e. seedling root system of different ages (3, 5, 7, 9 or 11 days after germination), dominates the parameter values registered in other organs, which is proof to the importance of this morphophysiological "compartment" in the nutrition and metabolism of these plants. It was not until the 10th day of germination when cotyledons started to turn green and to become organs that assimilate photosynthetically.

ACKNOWLEDGEMENTS

This work was supported by Structural Funds POSDRU/CPP107/DMI 1.5/S/77082 "Doctoral scholarship complex training in ecoeconomy and bioeconomy for safety, food security and feed from anthropic ecosystems".

CONCLUSIONS

These studies and the obtained results have beside a practical value as "investigative tools" in phytophysiology, also a theoretical one, since they allowed the identification of differentiation of seedling organs, tissues and cells capacity during sprouting, of the transformations undergone in the course of physiologic processes that take place at the stage of germination, to accumulate in varying amounts the

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