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DETERMINATION OF THE CAPACITY OF ABSORPTION OF THE NEUTRAL RED VITAL DYE IN THE EMBRYONIC AND SECONDARY ROOTS OF MAIZE (ZEA MAYS) SEEDLINGS

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ABSTRACT. To have valuable experimental results and to have the possibility to ensure the repeatability of this research, as experimental model we used seedlings, respectively their roots, especially the embryonic ones. The device with which the amount of neutral red was determined was a Spekol spectrophotometer. The determination of the concentration of neutral red solution was done using a standard curve which was "constructed" using the photometric data obtained by reading solutions of different concentrations of neutral red. From the physiological point of view, the method of staining of seedling tissue, or their organs, with a neutral red solution (prepared in tap water) can be regarded as being used as a method of evaluating the capacity of absorption of a compound and the reaction of certain living organs and tissues, according to the external conditions, more or less harmful, in comparison with similar cells or organs located in an optimal environment, taken as a control group.

Keywords: neutral red, embryonic roots, absorbtion

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 (see Pop and Soran 1960, 1961).

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. 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.

In solution, vital dyes dissociate, exhibiting certain properties that allow their division into basic, acidic or neutral. If the chromophore group is linked to the anion the dye is acid, and if the chromophore is linked to the cationic group, the dye is basic. To this last category belongs neutral red, substance often encountered in use in the research laboratories. Neutral red is an azine derivative, which turns its color under the influence of acid or alkaline reagents in red-purple at acid pH to brick-red, or yellow, at alkaline pH. It is a hydrochloride 3-amino-6-dimethylamino-2-methyl phenazine (Calan, 1942; Pop et al. 1961,1963 a and b; Soran 1959 a and b).

MATERIALS AND METHODS

To have valuable experimental results and to have the possibility to ensure the repeatability of this research, as experimental model we used seedlings, respectively their roots, especially the embryonic ones. Maize has and embryo with a single cotyledon, which is interposed between the reserve tissue of endospermic type, which contains mainly starch, and some unsaturated lipid deposits.

Both the size of seeds and that of the embryo, respectively seedlings is quite different, as is the that of embryonic root and areas held by these roots as embryonic root and the secondary ones, arising from this.

In the following we will refer to the working methods used in the experiments in this thesis.

In order to obtain seedlings used for the experiment, the seeding material was placed to germinate on filter paper moistened with tap water; the paper was placed in plastic containers, at laboratory temperature. Experiments were performed to determine the absorption capacity (see Soran 1960) of seedlings used as plant material, analyzing the variations of dry weight recorded at their level during germination, therefore seedlings, respectively their organs were dried.

The device with which the amount of neutral red was determined was a Spekol spectrophotometer.

The determination of the concentration of neutral red solution extracted from the roots was done using a standard curve which was "constructed" using the photometric data obtained by reading solutions of different concentrations of neutral red. Neutral red solutions, for obtaining – in equal parts- the standard curve, were also made in the mixture of ethyl alcohol 70^0 and acetic acid with which the vital dye extraction was performed from the tissues of the plant material. Root extract was made by boiling them a few minutes on the water bath.

Based on the readings we calculated:

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• the total absorption, which is the amount of neutral red vital dye (mg) permeated and accumulated in the cells of the analyzed organ per time unit (abbreviated mg/2h/organ);

• the specific absorption, which refers to the amount of neutral red vital dye permeated and accumulated in the organ analyzed per unit of time and related to the dry weight of the plant material from which the dye has been extracted (abbreviated mg/g/2h/organ).

Calculations were done using the following formula:

$$A_t = \frac{C \bullet V}{n} [mg / organ]$$

At = total absorption

As = specific absorption

V = total volume of the solution collected from a sample

C = concentration read on the standard curve

N = number of analyzed organs

g = dry weight of the plant material

RESULTS AND DICUSSIONS

In the case of maize seedlings, as can be seen in Figure 1 A and B, three days from germination, the embryonic root had a size of 3 cm, with 4-5 secondary roots and at hypocotyl level, close to the kernel, two adventitious roots form, some even penetrating inside the pericarp of the maize grain.

Seven days after germination, on the embryo root are distinguished the secondary roots presence which have a characteristic structure of the radicular apex, namely at their level (as well as at the tip of the adventitious roots) the calyptral tissue is distinguished, that protects the root meristem, apically located, but subterminal, which continues with the zone of elongation of the root, followed by the zone of differentiation.

Figure 2 shows graphically the total absorption of neutral red in maize roots under immersion of the plants at 3, 5, 7, 9 and 11 days after germination in vital dye solution prepared at a concentration of 100 mg / l, with tap water.

By analyzing the histograms shown in Figure 2 it can be inferred that, with the advancement of germination there is a gradual increase in the total absorption of neutral red on the overall root system, but no other part of the maize seedling is not stained, reason why we could not show values of absorption of the dye in the other organs of the seedlings.

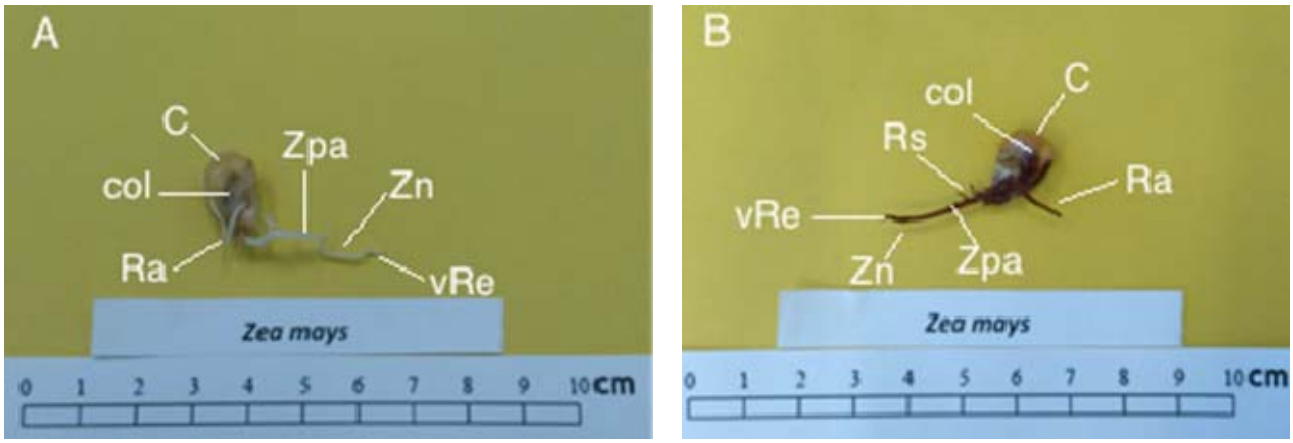

Fig. 1. Aspect of maize (*Zea mays*) seedlings found on the 3rd day of germination: A– non-colored seedling and B– colored seedling (abbreviations: C– caryopsis; vRe – tip of the embryonic root; Rs–secondary roots; Ra – adventitious roots; Zn – zone of elongation; Zpa – zone of absorbent hairs; col - coleoptile).

But we need to specify that, in the case of maize, vital staining method can be performed only on maize seedlings that do not have vacuolar cell dye in the coleoptile cells or in the radicle as can be found such situations in some varieties of maize, and in the seedlings of beetroot or red radish.

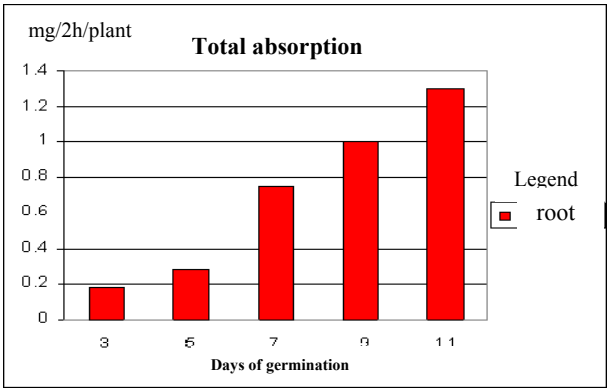

Fig. 2. Data regarding the capacity of total absorption (expressed in mg/2h/plant) of the root system of maize (*Zea mays*) seedlings during the first 11 days of

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germination.

From the physiological point of view, the method of staining of seedling tissue, or their organs, with a neutral red solution (prepared in tap water) can be regarded as being used as a method of evaluating the capacity of absorption of a compound and the reaction of certain living organs and tissues, according to the external conditions, more or less harmful, in comparison with similar cells or organs located in an optimal environment, taken as a control group.

CONCLUSIONS

Examination of the absorption capacity of the seedlings with the vital staining procedure, using a neutral red aqueous solution at a concentration of 100 mg / l, prepared in tap water, allowed the identification of the amount of the dye absorbed and accumulated in the absorbent organs, as macroscopically could be identified their location, the non-absorbent tissues did not color in the presence of this reagent.

From the physiological point of view, the method of staining of seedling tissue, or their organs, with a neutral red solution (prepared in tap water) can be regarded as being used as a method of evaluating the capacity of absorption of a compound and the reaction of certain living organs and tissues, according to the external conditions, more or less harmful, in comparison with similar cells or organs located in an optimal environment, taken as a control group.

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