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DETERMINATION OF NEUTRAL RED AMOUNT IN THE ORGANS OF *PHASEOLUS VULGARIS* PLANTLETS DURING THE GERMINATION PROCESS

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ABSTRACT. The main focus of this study was to demonstrate that by using the *vital stain* technique it is easier to investigate the absorption capacity of both tissues and cells depending on their nature, thickness of the preparations, the available technique and the performance of laboratory instrumentation.

Key words: *Phaseolus vulgaris, vital stain, neutral red*

INTRODUCTION:

From a teoretical point of view, the mechanisms of *vital staining* phenomena remained unknown for a long time. At first, when the use of stains was generally empirical, the researcher's interest was limited to the observation and description of specific phenomena. The accumulation of experimental data and especially the recording of the first cyto-physiological successes in their use have pointed out the fact that the scientific value of the results obtained by using *vital staining* is determined by their causal interpretation. Thus, an intense and laborious campaign was undertaken to investigate the chemical and physicochemical properties of *vital stains*, advancing step by step towards the elucidation of the mechanisms of *vital staining*.

In 2005 Renmin Gonga *et al.* investigated the roles played by three major functional groups (amino, carboxy and hydroxy) present in the biomass of peanuts in the absorption of six dyes. These fuctional groups have been individually chemically modified to determine their contribution to the absorption of the ionic dyes.

Jian Zhang *et al*. in 2008 conducted a study in which the activated carbon made from a cheap and renewable carbon source - *Typha orientalis,* by activation with H_3P0_4 and then impregnated with different Mn salts, was tested for it's capability to adsorb the neutral red dye from wastewater. The thermodynamic parameters have indicated the the processes were spontaneous and endothermic and the activated carbon modified by Mn is a promising adsorbant for the removal of the neutral red dye from wastewater.

Furthermore, Han Runping *et al.* In 2008 performed a series of studies suggesting that peanut skins could be usefull as an adsorbant material for the adsorbtion of neutral red (NR) from aqueous solutions.

Stephen Trolove *et al.* in 2015 introduced two experiments that were performed to determine the staining technique, with a staining time of 15 minutes in order to observe the adsorption of macronutrients in 6 and 7 weeks old wheat plants (*Triticum aestivum* L.) cultivated in a solution growth media.

MATERIALS AND METHOD:

The plant material used was obtained from common bean plantlets grown from seeds that were germinated on filter paper moistened with tap water and placed in plastic containers at room temperature. The experiments were performed on dry plantlets to determine their adsorption capacity and the variation in weight.

The neutral red concentration was measured using a Spekol spectrophotometer at 540 nm detection wavelenght. For this, a calibration curve was constructed using the spectrophotometric data obtained from neutral red solutions of different concentrations. The solutions were prepared using a mixture of ethanol and acetic acid similar to that used to extract the vital stain from the tissues of the plant material.

RESULTS AND DISCUSSION:

It is well known that the seed coat of common bean can be either white or patterned with different colors and shades starting from white with brown or black patches to a uniform brown or black coloring.

The seeds of common bean (or beans) are of different sizes depending on the cultivar/variety of this species. The size of the beans influences the size and the weight of the embryo and the structure and the gloss of the seed surface influences the speed of seed imbibiton and the transition of the embryo from latent to active life.

The embryo of the common bean seed has two cotyledons that vary in size depending on cultivar. These organs contain reserve substances such as starch and proteins deposited in their parenchyma that by their hydrolysis supply the embryo cells with energy and also with other organic substances necessary for the embryo growth and cell differentiation.

As it can be observed in Figure 1, at day 3 of germination the bean has a grown rootlet, that already pierces the micropyle (present close to the bean hilum) at 30 hours from germination having a rapid growth.

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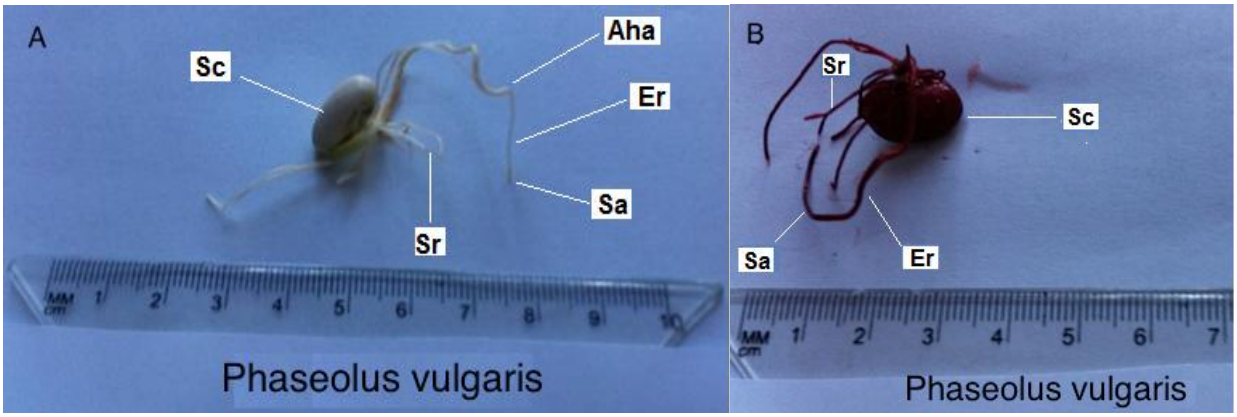

Fig.1 Common bean plantlets (*Phaseolus Vulgaris*) on day 3 of germination; A the plantlet before immersing in neutral red vital stain solution; B the coloring of the rootlets after a 2 h immersion in neutral red dye tap water solution (100mg/l) (abbreviations: Er - emrbryonic root; Sr - secondary root, Sa - smooth area, Aha - absorbing hairs area, Sc - seed coat)

On day 7 of germination, the common bean plantlet is already grown (Fig.2), the root system is well defined, the hypocotyl is long and robust and between the two cotyledons, the budlet can be seen.

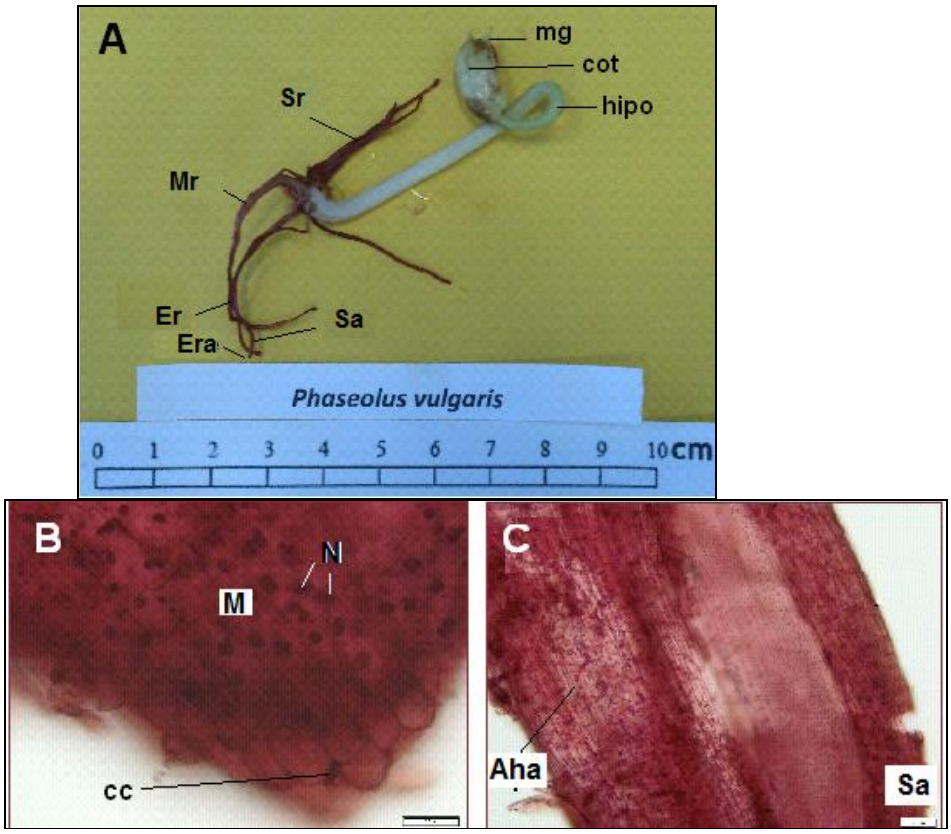

Fig. 2 Common bean (*Phaseolus vulgaris*) plantlets on day 7 of germination where: A – the plantlet before immersing in the dye solution and B – coloring of the rootlets after 2 h immersion in a tap water neutral red solution (100mg/l) (abreviations: cc – calyptra cells, hipo – hypocotyl, Er embryonic root; Sr – secondary rootlets; Era – embryonic root apex; Sa – smooth area; Aha – absorbing hairs area; M – meristem; N – nuclei; Mr – main root; b - budlet

Figure 2 shows the staining with neutral red both of the embryo rootlet and of the entire root system.

In Figure 2B and C are ilustrated examples showing the accumulation of neutral red in the rootlet apex in the calyptra and meristematic cells in which the presence of a large nucleus can be seen (Fig. 2B); Figure 2C shows a part from the smooth and of a root apex and piliferous layer that has adsorbed neutral red and even from the central area of this part of the rootlet.

As it can be seen from Figure 3B, the plantlets that have been immersed in a neutral red solution (100mg/l) have the rootlets strongly colored in dark red whereas the hypocotyl and most of the cotyledons are colorless.

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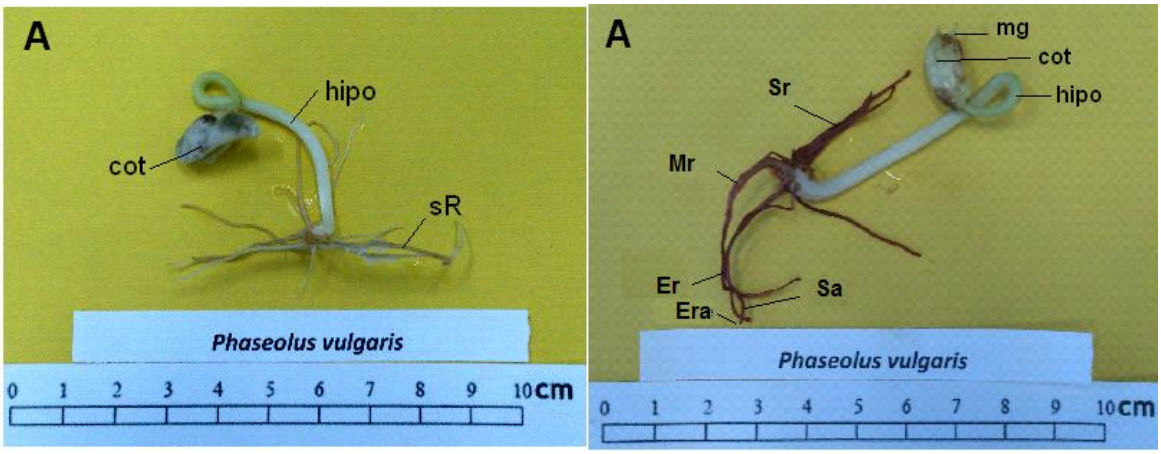

Fig. 3 Common bean (*Phaseolus vulgaris*) plantlets on day 7 of germination; A – the plantlet before immersing in the neutral red vital stain; B – the coloring of the rootlets after 2h immersion in a 100mg/l tap water neutral red solution (abbreviations: hipo – hypocotyl; Er – embryonic root, Sa – smooth area; Aha – adsorbing hairs area; cot – cotyledons)

Regarding the adsorption of the neutral red vital stain in common bean plantlets (Fig. 4, 5 and 6) it can be stated that the accumulation of the dye in the organ tissues of the plantets in the first 7 days of germination is low until day 6 (Fig. 5), after that being more intense due to the growth and branching of the rootlets. Further analysis of the neutral red adsorption data shown in Figures 5 and 6 (adsorption in roots, cotyledons, hypocotyl and budlet) clearly shows that the roots dominate substantially the dye adsorption compared with the rest of the plantlet.

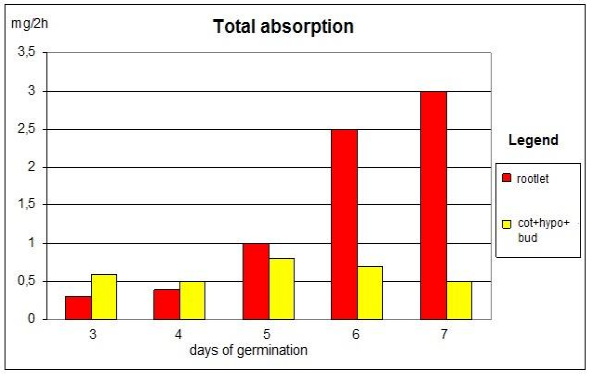

Fig. 4. Comparative results regarding *total absorption* (mg/2h) of the neutral red vital stain in the common bean (*Phaseolus vulgaris*) rootlets and plantlet, recorded in the first seven days of germination (abbreviations: cot – cotyledons; hypo – hypocotyl; bud - budlet) Total absorption;

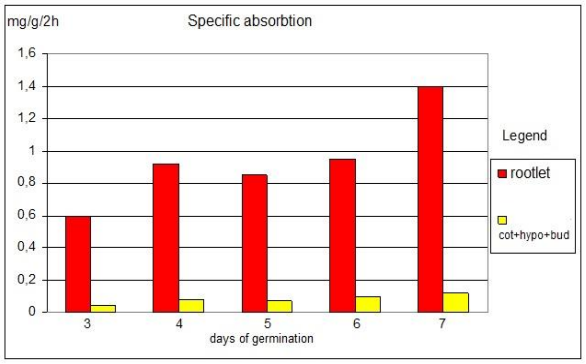

Fig. 5. Comparative results regarding the *specific absorption* (mg/g/2h) of the neutral red vital stain in both the rootlets and plantlet of common bean (*Phaseolus vulgaris*) in the first 7 days of germination (abbreviations: cot – cotyledons; hypo – hypocotyl; bud - budlet

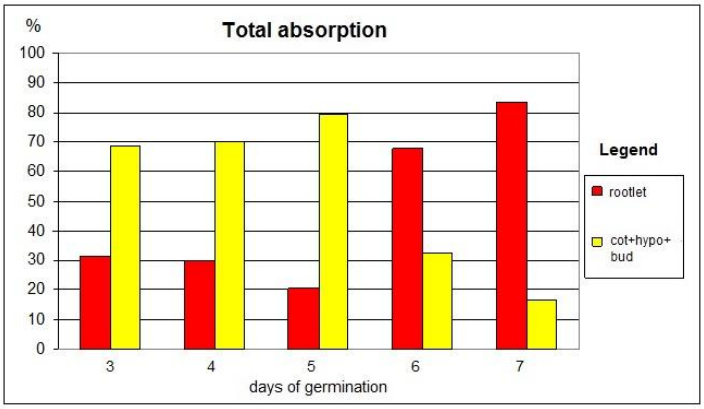

Fig. 6. Comparative results regarding *total absorption* (mg/2h) of the neutral red vital stain in both the rootlets and plant of common bean *(Phaseolus vulgaris)* in the first seve days of germination expressed as percentage values relative to the absorption and accumulation of the vital stain per whole plant, values considered to be 100%. (abbreviations: cot – cotyledons, hypo – hypocotyl; bud - budlet)

It is worth noting the fact that both from a functional and gravimetric point of view (Fig. 7, 8, 9) the weight of the root system is dominat and is higher than the rest of the plant weight.

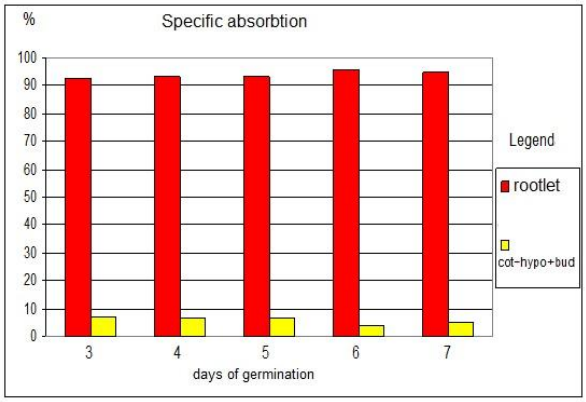

Fig. 7. Comparative results regarding *specific absorption* (mg/g/2h) of the neutral red vital stain in the rootlets and plantlet of common bean *(Phaseolus vulgaris)*in the first seven days of germination expressed as percentage values relative to the absorption and accumulation of the vital stain, values considered to be 100%. (abbreviations: cot – cotyledons; hypo – hypocotyl; bud – budlet).

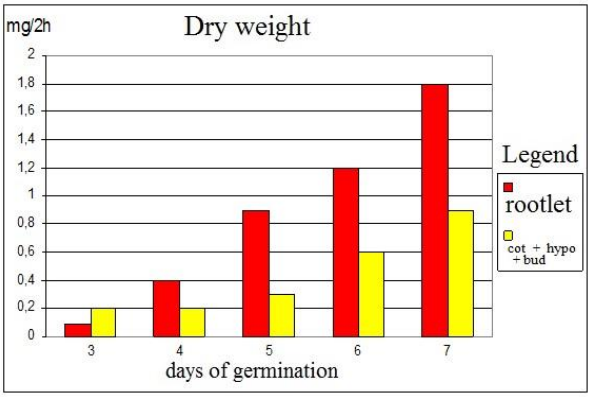

Fig. 8. Comparative results regarding the variation of *dry weight* (mg) of the rootlets, cotyledons and the hypocotyl of the common bean plantlet (*Phaseolus vulgaris*) in the first seven days of germination (abbreviations: cot - cotyledons; hypo – hypocotyl; bud – budlet).

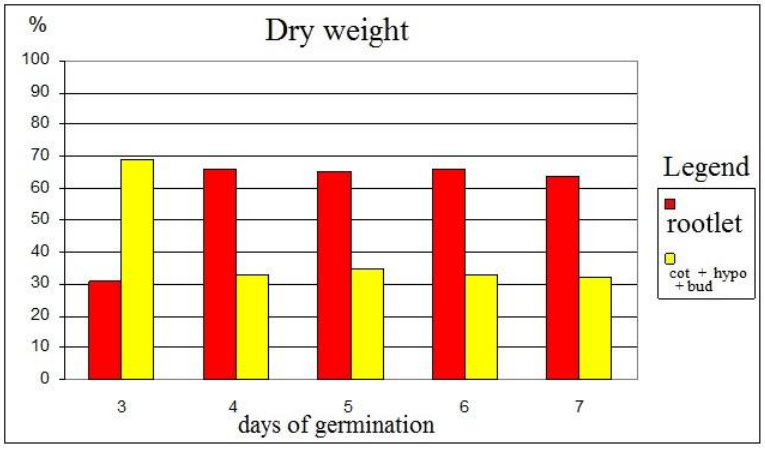

Fig. 9. Comparative results regarding the variation of the *dry weight* (mg) of the rootlets, cotyledon and hypocotyl of common bean (*Phaseolus vulgaris*) plantlets in the first seven days of germination, expressed in percentage values relative to the dry weight of the whole plantlet, considered as reference value of 100% (abbreviations: cot – cotyledons; hypo – hypocotyl; bud – budlet).

CONCLUSIONS:

The experiment regarding the absorption of neutral red vital stain by the common bean plantlets during germination has proven that there is a variability in the structure of the plantlets during germination. In a relatively short time, the embryo respectively the plantlet undergo different stage of growth and metabolic activity resulting in a variability of their absorption capacity. Such phenomenon was highlighted by the neutral red vital stain both qualitatively and quantitatively at the microscopic and macroscopic level.

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