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Evaluation of antioxidant capasity in wild and transformed Kumato and Cherry tomatoes roots and culture media using the DPPH method

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ABSTRACT

The aim of this project was to compare the effect of Rhizobacterium rhizogenes infection, and subsequent hairy root induction, on the antioxidant capacity of in vitro root cultures derived from two tomato cultivars differing in flavonoid biosynthetic potential. Tomatoes and especially processed tomatoes are rich in lycopene, which is one of the most powerful antioxidants. Antioxidan capasity of normal (wild) roots from Kumato and Cherry seeds and transformed roots from infected cotyledons with agrobacterium was determined by the DPPH metod. The results presented in this paper indicate that the normal roots extracts and extracellular had higher antioxidants capasity than transformed roots but antioxidant capasity was higher in the extracellular than in the roots extract. Standard deviation was higher in extracellular than roots extract. This experiment was valuable because antioxidants protect human body against oxidative stress which can cause many diseases such as diabetes and accelerate the aging process of the organism.

***Keywords*:** antioxidant capasity, tomatoes roots, DPPH metod

1. INTRODUCTION

Natural antioxidants like vitamin C, lycopene or coenzyme Q10 are typically added in the production of foodstuffs and cosmetics. They ensure proper taste, colour, shelf-life of a food products and added to cosmetics, delays the formation of wrinkles and slows skin's

aging process. Antioxidants at low concentration are capable of stabilizing or deactivating free radicals before they attack cells. Free radicalls have unpaired electron, which causes tchem to seek out and still electrons from other substances in order to neutralize tchemselves. This initially stabilizes the free radical but generates another in the process. The main task of antioxidants is to protect the human body against oxidative stress. Oxidative stress means unbalance between pro-oxidants and antioxidants mechanisms. Free radicals cause damage to cell membranes, as well as DNA and proteins included in the enzymes. In the case of severe shortage of antioxidants prolonged oxidative stress may contribute to the development of many diseases, such as heart disease, strokes, cancer, diabetes and accelerate the aging proces of the organism. Free radicals can come from sun radiation, cigarete smoke, stress and are created in everyday activity in human body. Maine sources of antioxidants are nuts, green tea, broccoli etc.

2. MATERIAL AND METHODS

Kumato and Cherry seeds were disinfected twice using NaClO (Domestos) for 20 minutes or 15 minutes. Next the seeds where put to the tubes which contained MS medium with vitamins, sugar casein hydrolysete and NAA in the case of normal roots in liquid culture.

Table 1. Quantity of the extra components added in medium for the preparation of 0.5l medium.

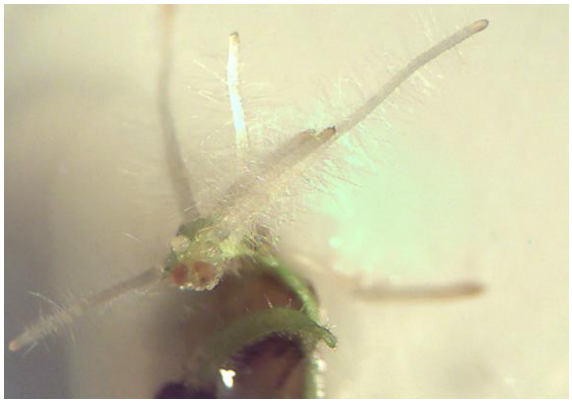
Name of the component	Used quantity
MS medium	1.0825g
Vitamins	0.25ml
Sugar	7.5g
Casein hydrolysete	62.5mg
NAA	0.125mg
Water	250ml

To all flashes were added 25 millilitres of medium.
To 21 tubes seeds where put, which:

- 6 tubes of Kumato variety treated by NaClO for 25 min.
- 5 tubes of Kumato variety treated b NaClO for 15 min.
- 5 tubes Cherry variety treated by NaClO for 25 min.
- 5 tubes Cherry treated by NaClO for 15 min.

Two Cherry tomatoes treted by NaClO for 25 min. of all samples do not germinated

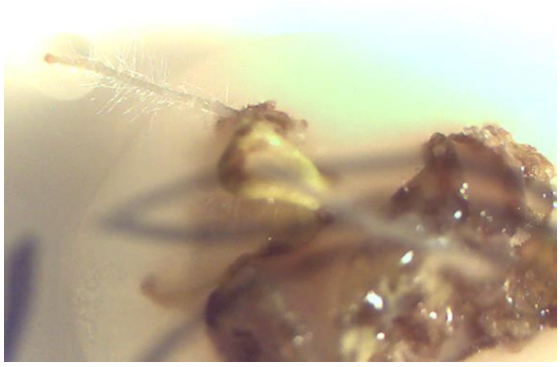
Normal (wild) roots, under aseptic conditions in a chamber, where cut and put to the tubes containe 15ml of MS with NAA and next time put to the tubes with M2. Cotyledons where cut, infected with agrobacterium using syringes, needles and put to the petrie dishes containes MS with higher concentration of auxins than cytokinins. The cells multiply and form a callus and the callus sprout the roots. Transformed roots receved from cotyledons were put to the 15 ml liquid of medium with 150 µl of antibiotics, without NAA to have opportunity to show if they are able to growth without NAA. Transformed roots where kept in dim light for one month.



Picture 1. Roots obtained from Kumato cotyledons

Picture 2. Roots obtained from Kumato cotyledons

Pictures 3. Roots obtained from Cherry cotyledons



Pictures 4. shows roots obtained from Cherry cotyledons

The roots were evaluated according to the classification:

0-zero response,

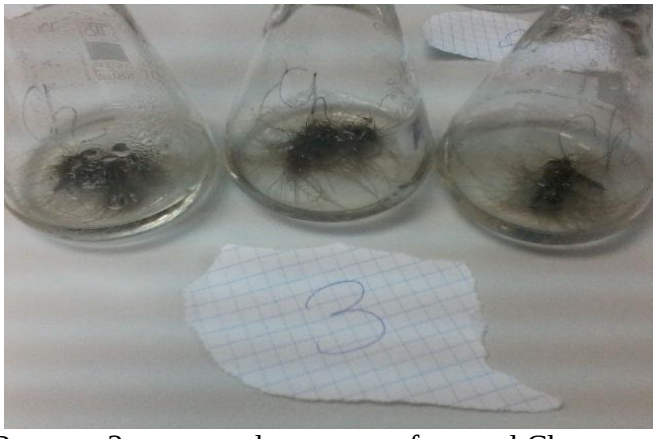
1-small responce,

2-medium response,

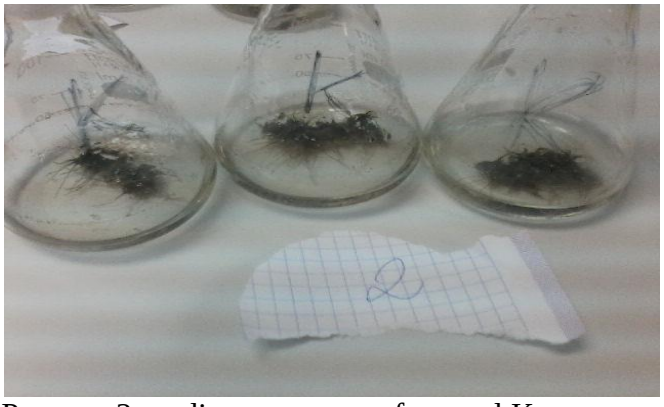
3-very good responce

Results of normal roots growt in cherry variety show very good response (3), one small response (1) and zero response (0)

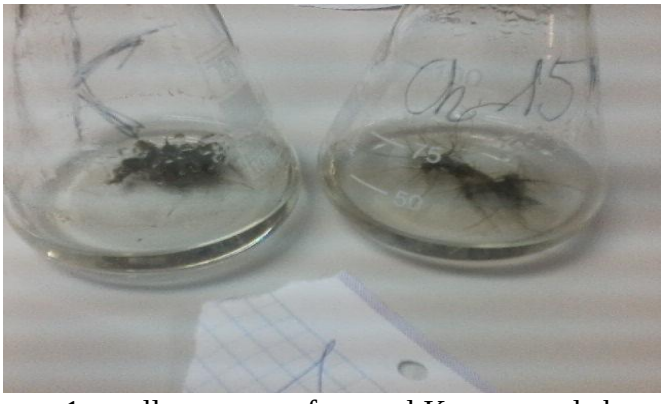
In normal Kumato variety three roots culture show medium response (2), one a small response (1) and one zero response (0).



Picture 5. Presents 3-very good responce of normal Cherry tomatoes roots



Picture 6. Presents 2-medium responce of normal Kumato tomatoes roots



Picture 7. Presents 1-small responce of normal Kumato and cherry tomatoe roots

Picture 8. Presents 0-zero responce of normal Kumato and Cherry tomatoe roots

Picture 9. Presents responce of transformed roots

Only one kumato was took to farther experience because two others where too small, as it is shown on the picture.

Table 2. The weight of transformed and normal roots

Variety	Weight
Transformed Cherry 1	354,3mg
Transformed Cherry 2	231,2mg
Transformed Kumato	93,3mg
Normal Cherry 1	621,1mg
Normal Cherry 2	466,1mg

Normal Kumato 1	340,9mg
Normal Kumato 2	400,00mg

To find antioxidant capsity in the roots extract the roots were crushed and was added 5 ml of metanol (the roots were covered by methanol). Sonication process take 30 min at 30 °C and 37 000 Hz. One thousand microliters of roots extract was centrifuge in 15 000 rcf for 10min at 6 °C to obtaine clear supernatant. 400 microliters of DPPH and 50 microliters of supernatant, obtained from crushed roots witch metanol, was added to ependorfs. Next this liquid has been kept in dark for 1h.150 microliters of DPPH plus 50 microliters of sampel volume were added to 7 holes in macroplate of spectrophotometer machine. In the case of control,which was added to eighth of the holes, was added methanol and DPPH. Extracellular in normal medium had 15 millilitres, in transformed 20 millilitres. To find antioxidant capasity in the extracellular, one thousand microliters of medium in which were roots were centrifuge in 15 000 rcf for 10 min at 6 °C to obtaine clear supernatant.400microliters of DPPH and 50microliters of supernatant was added in each ependorfs. Next this liquid has been kept in dark for 1h.150microliters of DPPH plus 50 microliters sampel volume were added to macroplate of spectrophotometer machine. In the case of control was added methanol and DPPH.

3. CONCLUSIONS

Antioxidant capasity in the case of extracellular and roots extract was the most higher in normal Cherry roots and the lowest in transformed Kumato roots. Antioxidant capasity was higher in the extracellular than in the roots extract. Standard deviation is higher in extracellular than roots extract

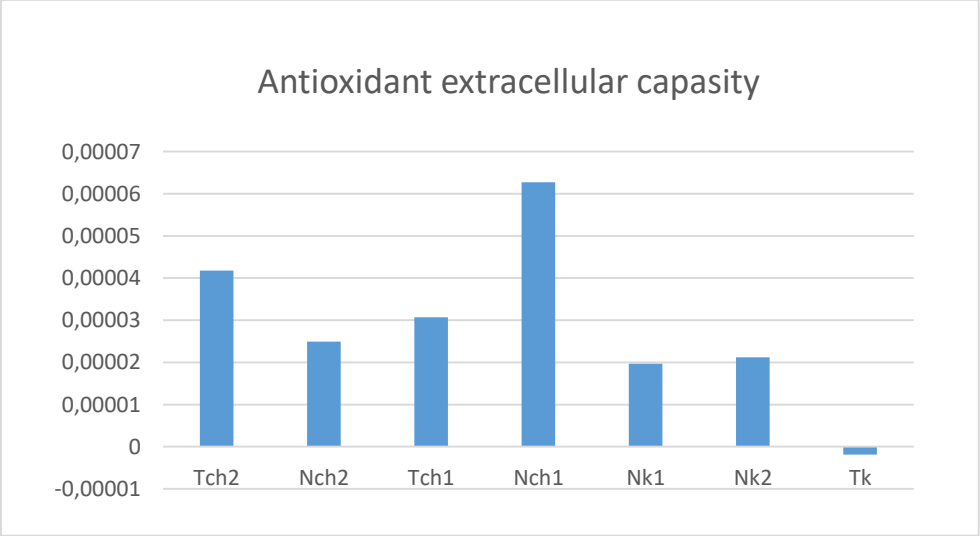


Figure 1. Antioxidant extracellular capasity

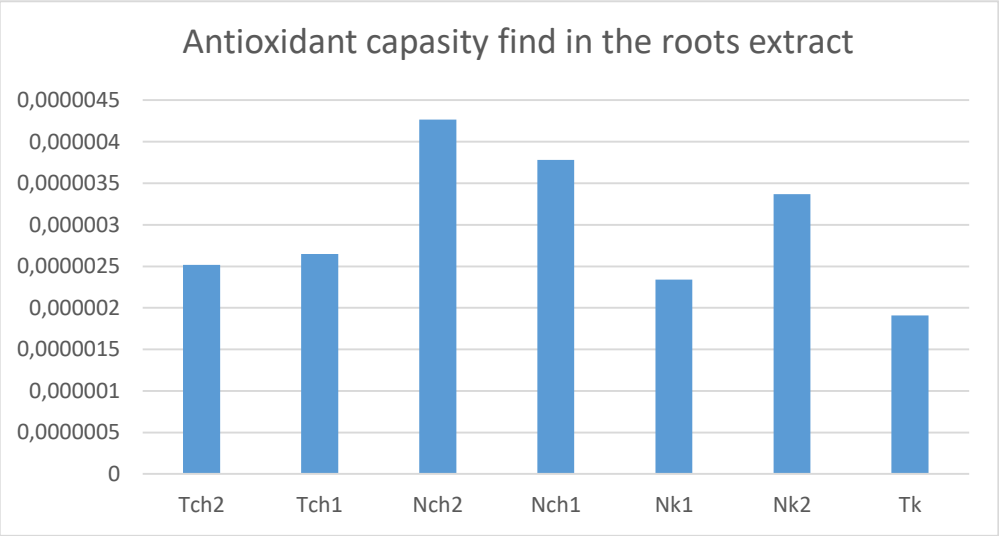


Figure 2. Antioxidant capasity find in the roots extract

Table 3. Standard deviation of extracellular

Transformed Cherry	0,025785267
Normal Cherry	0,026999074
Normal Kumato	0,039588245
Transformed Kumato	0,016334167

Table 4. Standard deviation of roots extract

Transformed Cherry	0,035467861
Normal Cherry	0,019623625
Normal Kumato	0,010133443
Transformed Kumato	0,019304015

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