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Povrće, krompir, ukrasne, aromatične i lekovite vrste

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ZBORNIK RADOVA

PROCEEDINGS

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In vivo and in vitro content of capsaicin in pepper (*Capsicum annuum* L.)

Liljana R. KOLEVA-GUDEVA[1], Mirko M. SPASENOSKI[2],
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Abstract: From all groups of biological active – secondary metabolites, in the species of genus *Capsicum* the most importance have the alkaloids capsaicinoides, which are present only in the cultivars of genus *Capsicum*, and only they are responsible for the pungent of pepper. From all capsaicinoides only two compounds with 80-90% are responsible for the pungent of pepper, and they are capsaicin and dihidrocapsaicin.

The purpose of our examination was to evaluate the total content of capsaicin *in vivo* and *in vitro* in pepper (*Capsicum annuum* L.). The content of capsaicin was detected in *in vivo* fruits of nine different in pungent varieties of pepper and in the *in vitro* cultures of shoots, callus and cotyledons of Kurtovska Kapija and Golden Medal varieties. It is conclude that capsaicin has been syntheses in *in vitro* condition and researches about the plant growth regulators effect on its synthesis are done.

The results from the *in vitro* content of capsaicin have not only fundamental but also and applicative character and could be with large contribution for following exploitation and application of capsaicin in the medicine, pharmacy biotechnology, plant physiology and biochemistry and in the agriculture.

Key words: capsaicin, pepper (*Capsicum annuum* L.), plant tissue culture.

Introduction

Capsaicin, N-(4-hydroxy-3-metoxy benzyl)-8-methilnon-trans-6-anamid, is strong and stable crystal alkaloid, it is unchangeable at cold or warm, and keeps the original strength after warm or freeze exposing for a long period of time. Even it has no colour, taste and smell, capsaicin is one of the hottest chilly known substances, and according to ***De Witt,*** (1999), human palate can register it in 1: 17 000 000 dilutes.

Capsaicinoides are simple phenolic amides, that can be present only in samples from genus Capsicum, and they are responsible for the chilly taste (***Govindarajn,*** 1986). According to ***Suzuki, et al.*** (1956), capsaicinoides synthesizes is reaction of vanillin amine dehydratation, made of phenyl alanin in phenyl propion cycle or made from lipid acids with branched chain, synthesized form valin, leucin and izoleucin Capsaicinoides are complexes of related components, benzilanin derivates, and the general five representatives are: capsaicin - 69% represented in the group of capsaicinoides; dihydrocapsaicin - 22% represented in the group of capsaicinoides; nordihydrocapsaicin - 7% represented in the group of capsaicinoides; homocapsaicin - 1% represented in the group of capsaicinoides and homohydrocapsaicin - 1% represented in the group of capsaicinoides.

According to the content of capsaicin, ***Govindarajn,*** (1986), gives the following classification of peppers: varieties with sweet taste content 0,1-0,2%, middle chilly 0,2-0,4%, chilly 0,4-0,6% and hot chilly varieties 0,6-1,0% even to 1,4% capsaicinoides. ***Todd,*** (1958), exposed that the content of capsaicin in commercial chilly peppers is about 0.08% to 0,8 % in fresh mass.

Material and methods

The content of capsaicin was detected in *in vivo* fruits of nine different in pungent varieties of pepper: Feferona (long, hot type), Slatko Luta (long, middle hot type), Vezena Luta (long, hot type), Sivrija (long, sweet type), Golden Medal (sweet spice type), Kurtovska Kapija (sweet spice type), California Wonder (bell, sweet type), Rotund (tomato-shaped, sweet type), and Feherozon (wax-bell, sweet type).

From the *in vitro* pepper cultures, the content of capsaicin was detected in shoots culture, callus culture from shoots and cotyledons and in cotyledons culture of Kurtovska Kapija and Golden Medal varieties. Researches about the plant growth regulators effect on capsaicin *in vitro* synthesis were done, with the following seven different combinations: MS + 5,0 mg/l KIN; MS + 5,0 mg/l BAP; MS + 5,0 mg/l ZEA; MS + 5,0 mg/l 2,4-D; MS + 0,5 mg/l NAA+5,0 mg/l BAP; MS + 0,5 mg/l NAA+10,0 mg/l KIN; MS + 1,0 mg/l IAA+10,0 mg/l BAP.

Initial explants (apical buds and cotyledons) of pepper were isolated from aseptically grown seedlings, and they were cultivated on MS (***Murashige and Skoog,*** 1962) medium, containing phytohormones with above mentioned combinations and concentrations. All cultures were held at primary growth room under illumination of 50 $\mu mol \cdot m^{2} \cdot s^{-1}$, photoperiod 16 h light / 8 h dark, 25±1°C temperature and relative humidity of 80%.

In vivo and *in vitro* plant material was dried on room temperature 6-7 days. The rest of the moisture was corrected with dehidratation on the samples in the thermostat to the constant weight.

The extraction of the capsaicin from the dry plan material was done with 96% ethanol in the water bath on 40°C temperature for the time of 5 hours. The ethanol extract of capsaicin was obtained with water vacuum filtration and was appropriate dilute for measurements.

The absorbance of the total capsaicin was detected with spectrophotometer UV-VIS on the 281 nm wavelength (Figure 1 and 2).

Results and discussion

The results from the estimation of the content of the capsaicin in *in vivo* pepper fruits, shows up that the pungent varieties (Feferona, Slatko Luta and Vezana Luta) have the highest level of capsaicin. The sweet varieties (Sivrija, Golden Medal and Kurtovska Kapija) was with minor content of capsaicin and the bell varieties (California Wonder, Rotund and Fehefozon) shows up much less capsaicin that the other varieties. The highest value of capsaicin was present on the pungent variety Feferona (901,27±51,80** μg/g), the lower value on the bell variety Feherozon (205,76±93,69** μg/g). The content of capsaicin in the Feferona is almost 4,5 times more than the Feherozon variety (Table1).

Examinating the regeneration abilities of varieties Kurtovska Kapija and Golden Medal in *iv vitro* conditions from the apical buds and cotyledons, it was conclude that capsaicin has been synthesis in *in vitro* conditions. Therefore the researches about the plant growth regulators effect on capsaicin synthesis in callus, shoots and cotyledons pepper culture were done.

In the shoot culture the both varieties Kurtovska Kapija and Golden Medal was able for capsaicin syntesis, the control group (without hormonal treatment) contain less capsaicin than the each examinee hormonal treatment (Table 2).

The analysis of the content of capsaicin in the callus pepper culture shows up that they are also able for capsaicin biosynthesis, which was intensive by the different phytohormones (Table 3 and 4).

The best favorites for capsaicin synthesis in callus culture are the citokinines, for the both varieties. For the Kurtovska Kapija variety BAP has the bigger effect, for the Golden Medal variety ZEA has the best influence.

Even in the cotyledon culture the capsaicin biosynthesis occurs in the alternative pathway (Table 5).

In contrast to shoot and callus culture in the cotyledons culture the auxin 2,4D has the bigger stimulation effect (Kurtovska Kapija 513,77±7,26** μg/g; Golden Medal 439,8027±75,73** μg/g). The other examinee plant growth regulators have the similar influence on the capsaicin synthesis and from the combinations auxin/citokinin the combination IAA/BAP was the best, also in the cotyledons culture.

The biosynthesis of capsaicin in *in vitro* pepper culture is remarkable and occurs by the alternative pathway, so in the shoot culture the biosynthesis is higher (213-992 μg/g), a little less in the culture of cotyledons (189-753 μg/g) and in the callus culture the synthesis of capsaicin is lower (96-740 μg/g).

Conclusion

The research results about capsaicin content at *in vivo* crops in nine different varieties of pepper are in order with the world estimates for pepper hotness at the species from genus *Capsicum*. That means that by taste and by the capsaicin content examined varieties really belong in the propriety group. The content of capsaicin in the hot varieties is from 618- 901 μg/g; in sweet varieties from 271-532 μg/g; and in bell pepper varieties from 201-234 μg/g capsaicin /g fresh mass.

Nowadays, the science researches are concentrate at examine *in vitro* production of some second metabolites in the species from genus capsicum. In this view, the use of the method at plant cells and tissues cultures is directed in the way of biosynthesis increase, of the second metabolites at *in vitro* conditions, ***Sasson*** (1991).

Our research has shown that the capsaicin synthesis at *in vitro* conditions has been existed, and every different plant growth regulator has a different stimulation of its biosynthesis (table 2, 3, 4 and 5). Evidently, in the shoot culture the capsaicin synthesis is the biggest, some lower is in the cotyledon culture and the smallest is in the callus culture.

Cytokinines stimulate the capsaicin content at *in vitro* conditions, but BAP and ZEA have the biggest influence. From tha combinations aukxin/citokinin, IAA/BAP has the biggest influence on the capsaicin biosynthesis (256-753 μg/g).

The results from our researches would be of great importance for further researches and as for using of this natural alkaloid in plant biochemistry and physiology, as well as for its use in medicine, pharmacy, technology and agriculture.

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References

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De Witt, D. (2000): Creams, Sprays, Gels, Stics, Powders and Compounds a Capsaicin Update, 2000, The Healing Powers of Peppers.

Govindarajan, V.S. (1986): Capsicum - production, technology, chemistry and quality - Part III. Chemistry of the color, aroma and pungency stimuli, CRC, Critical Review in Food Science and Nutrition 24 (3): 254-355.

Govindarajan, V.S. (1985): Capsicum - production, technology, chemistry and quality - Part II. Processed products, standards world production and trade, CRC, Critical Review in Food Science and Nutrition, 23 (3): 207-288.

Govindarajan, V.S. (1985): *Capsicum* - production, technology, chemistry and quality - Part I. Botany, cultivation and primary processing, CRC, Critical *Review* in Food Science and Nutrition, 22 (2): 109-176.

Todd, P.H. Jr. (1958): Detection of foreign pungent compounds, Food Technology, 58: 168-470.

Sasson A., (1991): Production of useful biochemicals by higher-plant cell cultures: biotechnological and economic aspects. CHIEAM, Options Mediterraneennes, 14: 59-74.

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