# Effect of creatine and creatine lysinate *on in vitro* cultivation of *Stevia rebaudiana* Bertoni and *Leontopodium alpinum* Cass.

**36th European Peptide Symposium and 12th International Peptide Symposium**
Aug 28 – Sept 2, 2022
Barcelona, Spain
**36th EPS/12th IPS**

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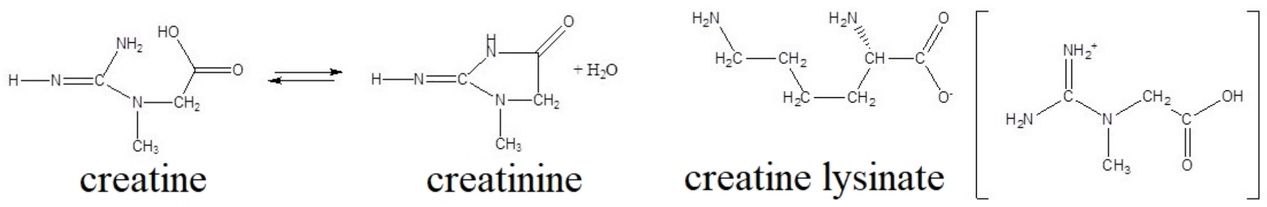
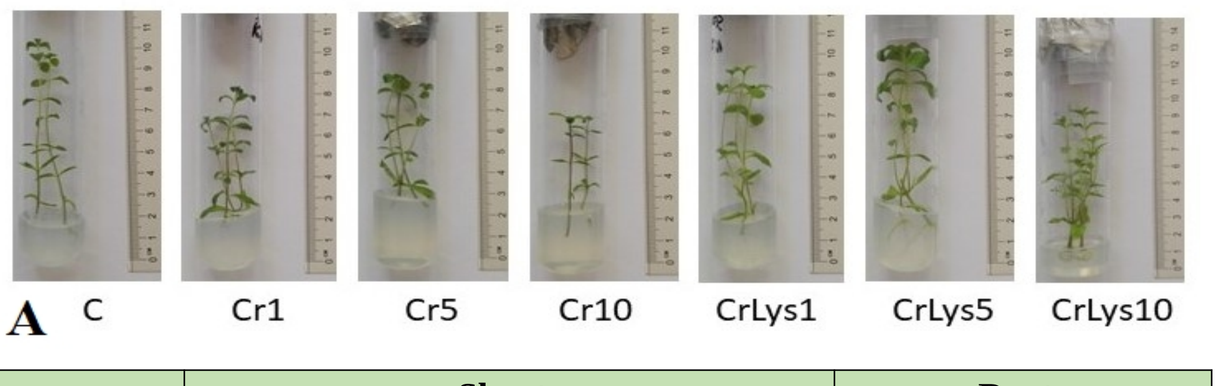
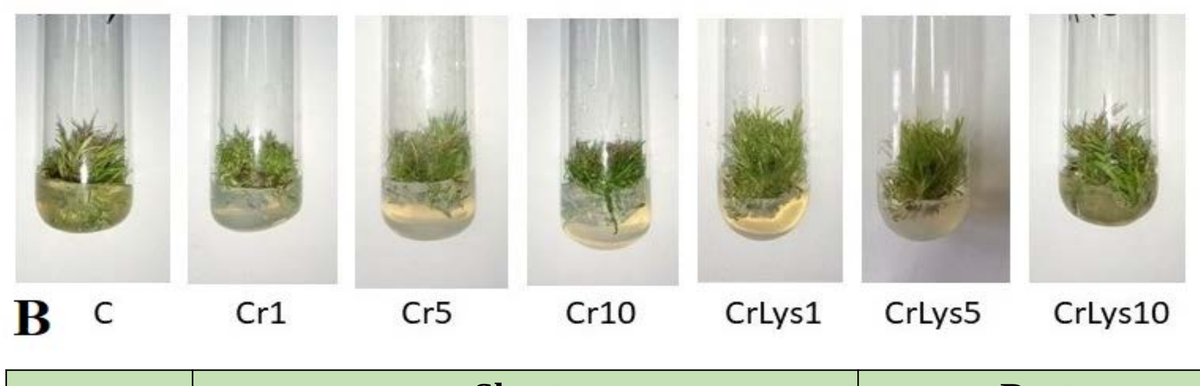
**BACKGROUND:** In recent years, fertilizer combinations not containing nitrate for culture cultivating are widely sought. Amino acids provide plant cells with nitrogen that is more easily assimilated than inorganic nitrogen sources. Therefore several α-amino acids have been studied as additives to the nutrient medium of some plants. Except for nitrogen supply, these amino acids have shown an effect on other physiological processes in plants. Creatinine is a non-proteinogenic *nitrogenous* rich amino acid, which beneficial effect on the growth of wheat seedlings have been reported. Widely distributed and easily obtained by arginine, glycine, and methionine, it plays an important role in the metabolism of proteins in animals' organisms. As in an aqueous solution, creatine is converted to creatinine, to be stabilized it is transformed to salts with L-Lysine.

**OBJECTIVE:** The present study demonstrates the effect of creatine and creatine lysinate added to MS nutrient medium on *Stevia rebaudiana* Bertoni and *Leontopodium alpinum* Cass micropropagation as well as their effect on enzyme and non-enzyme antioxidant activity of stevia and edelweiss extracts. To our knowledge, this is the first report in which these two amino acids have been studied for their effects on *in vitro* cultivation of any plant species.




**PLANT MATERIAL AND TREATMENTS:** *Stevia rebaudiana* (**A**) and *Leontopodium alpinum* (**B**) stem explants were cultured on MS media supplemented with different concentration (1, 5 and 10 mg/l) of creatine (Cr) and creatine lysinate (CrLys) at 22±2ºC and 16 h photoperiod for 4 weeks



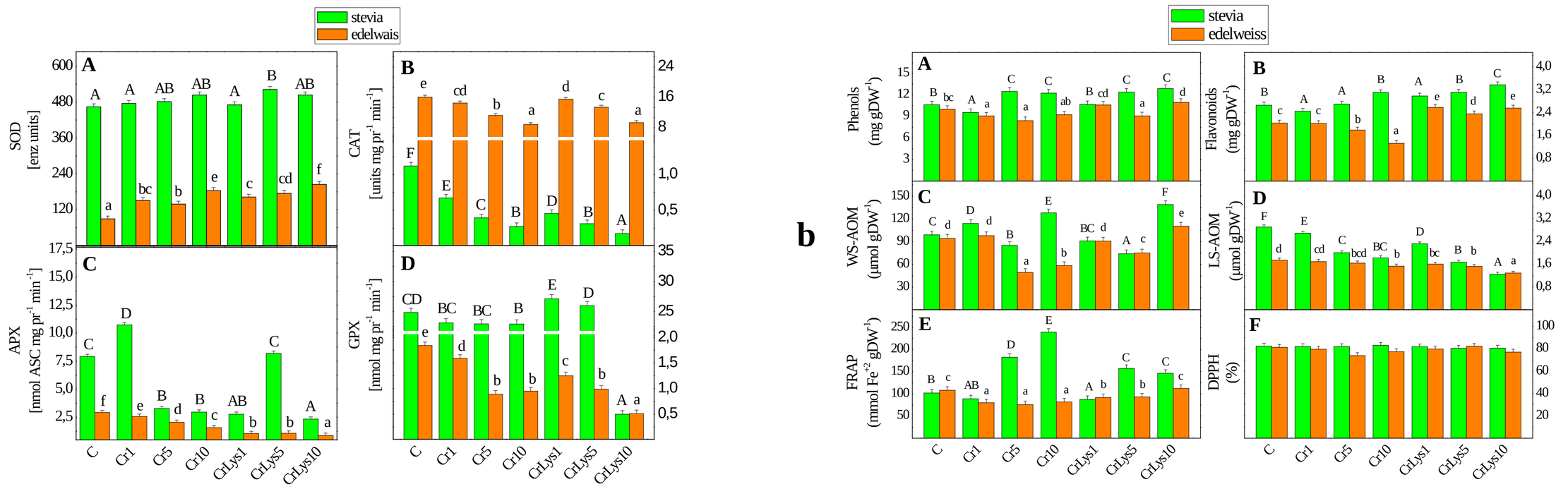

| Treatment | Shoots | | | Roots | |
|---|---|---|---|---|---|
| | number/explant | height, cm | FW, mg | length, cm | FW, mg |
| **Control** | 1.65±0.13 | 8.95±0.35 | 0.164±0.01 | 1.34±0.10 | 0.04±0.008 |
| **Cr1** | 1.55±0.15 | 7.55±0.37 | 0.158±0.01 | 1.22±0.16 | 0.04±0.009 |
| **Cr5** | 1.75±0.17 | 7.50±0.38 | 0.149±0.02 | 1.44±0.19 | 0.05±0.009 |
| **Cr10** | 1.65±0.14 | 6.29±0.44 | 0.128±0.01 | 1.45±0.14 | 0.03±0.002 |
| **CrLys1** | 1.80±0.18 | 9.13±0.34 | 0.166±0.01 | 1.48±0.17 | 0.05±0.009 |
| **CrLys5** | 2.40±0.18 | 10.04±0.39 | 0.185±0.02 | 1.76±0.18 | 0.06±0.004 |
| **CrLys10** | 2.00±0.16 | 8.37±0.33 | 0.172±0.01 | 1.99±0.19 | 0.07±0.006 |

**a**

| Treatment | Shoots | | | Roots | |
|---|---|---|---|---|---|
| | number/explant | height, cm | FW, mg | length, cm | FW, mg |
| **Control** | 24.50±1.05 | 2.44±0.21 | 0.686±0.09 | 1.91±0.13 | 0.040±0.04 |
| **Cr1** | 23.00±1.29 | 2.31±0.21 | 0.532±0.01 | 1.24±0.17 | 0.019±0.01 |
| **Cr5** | 20.65±1.46 | 2.35±0.20 | 0.588±0.05 | 1.62±0.11 | 0.031±0.03 |
| **Cr10** | 19.55±1.15 | 2.17±0.27 | 0.512±0.06 | 1.64±0.04 | 0.059±0.03 |
| **CrLys1** | 19.05±1.22 | 3.61±0.34 | 1.07±0.10 | 1.94±0.17 | 0.043±0.02 |
| **CrLys5** | 18.20±1.28 | 3.22±0.33 | 0.943±0.09 | 2.62±0.17 | 0.083±0.05 |
| **CrLys10** | 17.05±1.32 | 3.51±0.54 | 0.876±0.10 | 2.77±0.31 | 0.092±0.04 |

**b**

Effect of different concentrations (1, 5 and 10 mg/l) of creatine and creatine lysinate on *in vitro* growth of *Stevia rebaudiana* (**a**) and *Leontopodium alpinum* (**b**)







The activity of antioxidant enzymes (superoxide dismutase – SOD, catalase – CAT, ascorbate peroxidase – APX and guaiacol peroxidase – GPX) (**a**), and antioxidant potential and content of metabolites with antioxidant power (**b**) in the *in vitro* propagated *Stevia rebaudiana* and *Leontopodium alpinum* plantlets treated with creatine or creatine lysinate (at a concentration of 1, 5 and 10 mg/l)

**ACKNOWLEDGMENTS:** This study was conducted with financial support from National Science Fund at the Bulgarian Ministry of Education and Science, Project КП-06-Н56/8 12.11.21.

**CONCLUSION:** The creatine and creatine lysinate in MS nutrient medium affected differently the growth of *in vitro* plantlets and the activity of some antioxidant enzymes of *Stevia rebaudiana* and *Leontopodium alpinum*. Adding the creatine lysinate at MS medium influenced better *S. rebaudiana* and *L. alpinum* shoot and root growth than creatine. The higher activities of the antioxidant enzymes (SOD, APX and GPO), as well as higher content of flavonoids and phenols, were measured in stevia compared to edelweiss. However, future research should be done in order to clarify the role of these amino acids.