## *In vitro* antibacterial and cytotoxicity activities and NO inhibitory effect associated with selenium and sulfur nutrition and further changes in primary metabolism in green and red lettuce

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Selenium (Se) plays an important role for many organisms as an essential trace element nutrient. Its biological effects comprise mainly incorporation into the structure of selenoproteins, which are involved in cell activation, proliferation and differentiation in innate and adaptive immunity. Additionally, Se demonstrates antioxidant and anticancer potential at optimal doses. Furthermore, sulfur (S)-containing compounds have considerable promise regarding antimicrobial and anticancer drug development, in addition to the area of green and ecofriendly pesticides. Nitric oxide (NO) is known as a pro-inflammatory mediator associated with various physiological processes and plays an important role in the immune response.

Regarding primary metabolites, water-soluble sugars and organic acids affect the taste and other organoleptic properties of fruits and vegetables such as color, flavor and aroma. The aim of the present study was to investigate the influence of Se and S enrichment on antibacterial and cytotoxicity activities, NO inhibition and further changes in primary metabolism in multi-leaf green (V1) and red (V2) lettuce. The plants were treated with three levels of Se via foliar application in the presence of three S levels in the nutrient solution under greenhouse conditions. The authors tested the hypothesis that the 'crosstalk' between Se and S can affect antibacterial activity, cytotoxicity and NO inhibition in lettuce plants under Se and S enrichment. Additionally, Se and S have a unique effect on soluble sugars and organic acids biosynthesis.

The results indicated that extracts of the treated green and red multi-leaf lettuce were not cytotoxic to Vero kidney cells at the highest concentration tested of 1 mg/mL. The acetone extract of red lettuce had antibacterial activity against Pseudomonas aeruginosa with minimum inhibitory concentration (MIC) of 0.156 and 0.625  $\mu$ g/mL under S2/Se1 and S2/Se2 treatments, respectively. Extracts of both previous treatments were active against Staphylococcus aureus and Escherichia coli with MIC = 1.25  $\mu$ g/mL. As with antibacterial activity, the acetone extract of green (V1) and red (V2) lettuce treated with higher S (S2) under Se-limiting condition produced the best NO inhibition with IC50 = 97  $\mu$ g/mL. Moreover, the red cultivar (V3) exhibited NO inhibition with IC50 = 113 and 130  $\mu$ g/mL in response to S1/Se2 and S2/Se2 treatments, respectively.

Under higher S and Se-limiting condition in the green and red multi-leaf lettuce, the levels of glucose and fructose increased therein to 125.0±0.36 and 94.8±1.6 mg g-1 DM, respectively, whereas in the red lettuce subjected to higher S and low Se (Se1)

fertilization, the glucose level significantly increased to 89.3±0.37 mg g-1 DM. Under higher S and Se conditions (Se2/S2) glucose level in green and red lettuce enhanced dramatically (80.8±0.25 and 75.4±1.4 mg g-1 DM, respectively) in comparison to Se0/S0, Se1/S1, Se2/S1 treatments, but did not show significant changes between both cultivars.

The elevated Se and S treatments (Se2/S2) accumulated more fructose in red lettuce (66.1±0.6 mg g-1 DM) than the green one (53.9±1.5 mg g-1 DM). Apart from watersoluble sugars malic acid accumulated in red lettuce under Se and S treatments, however its concentration remained unaltered among all treatments. While in green lettuce a synergistic interaction between Se and S revealed a dramatic decline in malic acid level from (15.3 ±0.86 mg g-1 DM) under Se and S-limiting condition to 11.0±0.74 and 6.64±0.43 mg g-1 DM in response to Se1/S1 and Se2/S2 treatments, respectively.

Collectively, the findings indicate that Se and S enrichment evoked antibacterial and NO inhibitory activities. Additionally, Se/S crosstalk showed both antagonistic and synergistic effects on the biosynthesis of soluble sugars and organic acids and have great effects on the final nutritional value and quality of lettuce plants.

## References

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55. Jahrestagung der Deutschen Gesellschaft für Qualitätsforschung (Pflanzliche Nahrungsmittel) e.V. (DGQ)



22./23. März 2022 Universität Hohenheim (Online-Veranstaltung)