HPLC-Analysis of Phytoalexins to differentiate various *Camelina sativa* (L.) Crtz.-cultivars

Summary

The purpose of this investigation was to differentiate 13 cultivars/breeding lines of *Camelina sativa* based on the concentration of phytoalexins after induction with an elicitor. To realize this:

1. A method was optimized to simultaneously analyze the *C. sativa* phytoalexins Camalexin and Methoxycamalexin. The extraction was accomplished with a microwave treatment in Methanol. C-18 material was used to clean the sample. The quantification of phytoalexins was performed using HPLC equipped with a fluorescence detector. Using the optimized method, simple and time saving analyses of Camalexin and Methoxycamalexin in serial investigations from fresh plant material were possible. The recovery rate was approximately 76 %.

   The optimized method was used to examine the production of phytoalexins in *C. sativa.* These examinations were necessary to create a foundation of information. Based on this foundation it was possible to differentiate the cultivars/breeding of *C . sativa.*

3. Different elicitors (*Alternaria brassicae, Botrytis cinerea,* $AgNO_3$, $CuCl_2$, BION®) were compared with regard to their applicability to induce phytoalexin production in *C. sativa* under greenhouse and climatic chamber conditions. The plant activator BION® proved to have the best potential. The application of this elicitor was uncomplicated and assimilation by the plants was good.
3. At different development stages (cotyledon stage, rosette, stem extension, flower bud development) of one breeding line from Denmark, the phytoalexin production was induced with BION® and the concentrations were analyzed. The highest concentrations were detected at the cotyledon and rosette stages. Compared to this stages the concentration of phytoalexins was approximately 50 % lower at the stem extension stage. At flower bud development stage there were no differences noted compared to the control, treated with tap water.
4. An investigation was accomplished concerning the accumulation of phytoalexins in the cultivar Bavaria at the rosette stage after treatment with BION®. Two days after treatment with BION® there was an increased concentration noted compared to the control. Eight days after treatment, an increase of phytoalexin concentration to more

than 5500 µg/kg fresh matter was observed. However, after 11 days a decrease to 3000 µg/kg fresh matter was determined. This level remained constant until the end of the investigatory period (22 days).

5. 13 *C. sativa* cultivars/breeding lines were cultivated. In the rosette stage the plants were treated with BION® and then incubated at three different temperatures. 15 days after treatment the concentration of phytoalexin (Camalexin and Methoxycamalexin) was determined. There was found a negative correlation between the temperature and the concentration of phytoalexin. The higher temperatures caused a lower amount of phytoalexins, whereas the colder temperature caused a higher amount of phytoalexin. The investigated cultivars/breeding lines showed differences regarding the concentration of Camalexin and Methoxycamalexin and the total phytoalexin. Based on the results, it was possible to differentiate the 13 *C. sativa* cultivars/breeding lines.
6. Additional investigations were carried out to determine whether other inducible bioactive compounds in *C. sativa* were present. One of the obtained fractions (contains no Camalexin or Methoxycamalexin) showed growth inhibiting bioactivity on *A. brassicae*. The bioactive compound could not be identified because the amount was too small. Furthermore, it can not be excluded that the compound is an artifact.
7. Furthermore, an inducible compound which showed no bioactivity was detected. Probably, it is a product of the indolic-synthesis-pathway.