



BOOK OF ABSTRACTS

First Legume Society Conference
2013: A Legume Odyssey

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Book of Abstracts

Editors:
Aleksandar Mikić
Diego Rubiales
Vuk Đorđević

International Legume Society
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2013

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Technical Editors:
Sanja Mikić and Aleksandar Mikić

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Getting the message out: grow, use, feed and eat legumes

Uncertainty analysis of trypsin inhibitor activity determination using microtiter plate method

Vesna Župunski, Zorica Nikolić, Gordana Zdjelar, Dušica Jovičić, Maja Ignjatov, Dragana Milošević, Gordana Tamindžić

Institute of Field and Vegetable Crops, Laboratory for seed testing, Novi Sad, Serbia

Measurement of proteinase inhibitor activity of legume seeds is widely used since some of these exert toxic and antinutritional effects. Standard method assay for trypsin inhibitor activity (TIA) determination is based on the hydrolysis of benzoyl-DL-arginine-p-nitroanilide (BAPA) by trypsin and includes spectrophotometric measurement of the reaction products. Assaying of trypsin inhibitor activity is usually performed using reaction mixture volumes of 4-10 ml, however, it was shown that microtitration method can also be used for TIA determination. Although the results obtained by microtitration method were consistent with those obtained by French Association for Normalization reference method (AFNOR), it was noted that any experimental error in absorbance values was increasingly magnified as the sample size was diminished. The aim of this study was to analyse measurement uncertainty of TIA determination using microtiter plate method. Assaying procedure was conducted at 37 °C on sample (soybean variety Vojvodanka), positive and negative control, which were set in four replicats in each row of the microtiter plate, with preincubation time of 10 minutes and incubation time of 30 minutes. The trypsin inhibitor activity was expressed in number of trypsin units inhibited (TUI) per miligram of sample, taking into account the fact that one trypsin unit is defined as an increase of 0.01 absorbance units at 405 nm. Large number of experiments was conducted and uncertainty analysis was obtained using uncertainty budget. Values of calculated trypsin inhibitor activity were approximately 70 TUI/mg and estimated expanded measurement uncertainty (with coverage factor $k=2$) was approximately 15%.

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In the rich world of global agriculture, diverse legumes can play key roles to develop environment-friendly production, supplying humans and animals with the products of high nutritional value.

The Legume Society was initiated in 2011 with two primary missions. One of them was to treasure the rich legume research tradition of the European Association for Grain Legume Research (AEP), with emphasis on carrying out its the triennial legume-devoted conferences. Another one is to fulfill a long-term strategy of linking together the research on all legumes worldwide, from grain and forage legumes pharmaceutical and ornamental ones and from the Old World to the Americas.

We do anticipate that the First Legume Society Conference will be a unique and genuine contribution to our common goals: to promote the legume research and all its benefits into all spheres of the society, linking science with stakeholders and decision-makers, and to demonstrate how an efficient, useful and firm network of the legume researchers of the world is possible and sustainable.

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