

# Transnational Access Report

## 1. General Information

Project Acronym (ID):	CUC_GC-MS
Project Title	Identification of metabolic parameters related to flower sex expression in cucumber plants.
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## 2. Project summary (max. 250 words)

Sex determination in plants is fundamental developmental process during sexual reproduction of dioecious and monoecious plants. The mechanism which is controlling this process is not yet fully understood and numerous studies have been conducted trying to explain roles of signaling pathways involved in sex determination. Aim of this project is to gain further insight into the phenomena of sex determination and flower morphogenesis in cucumber on the metabolite level.

Cucumber sex expression is determined by the genes *F*, *m*, *a*, *gy*, and *h*. The Female (*F*) gene regulates the level of female flowers and encodes ACC-synthase (*CsACS1G*). The andromonoecious (*m*) gene controls bisexual flower and encodes another ACC-synthase (*CsACS2*). Both genes are involved in ethylene production by conversion methionine to ethylene and are correlated with female flower production. It will be interesting to detect differences in metabolites concentration which could be correlated with ethylene biosynthesis and sex expression. There are scientific reports about sugar metabolism and PCD phenomena which are correlated with sex determination. At the early stages cucumber floral primordia are bisexual and sex determination occurs through selective arrest of staminate or pistillate primordia development. To further elucidate these processes we wanted to investigate possible differences in levels of metabolites in cucumber lines differing with regard to sex that may reflect specific properties of male or female floral organs. GC-MS has not yet been used to study sex expression in cucumber and it is the first trial to search for sex-related parameters

## 3. Main achievements (max. 250 words)

Metabolite profiling using gas chromatography – mass spectrometry (GC-MS) was performed in this project to identify and quantify the relative level of metabolites between cucumber lines differing with regard to sex type.

In total, 196 metabolites were measured of which 72 are known by chemical structure. To determine the metabolites which display significant differences between the sex types, ANOVA was applied to determine specific effects on metabolism. 183 metabolites were significantly different in abundance between all 6 genotypes, among them 69 were known. Developmental stages (leaves, shoot apex, 1 -2, 3-5, 6 – 8 and 9 mm long floral buds) significantly affected the abundance of 153 metabolites, of which 58 were of known identity. In dependence of the flower type (male, hermaphrodite and female), 144 metabolites were altered 54 of which were identified. The PCA plot (see Figure below) illustrates that the most diverging metabolite profiles are found in floral buds during later growth stages when buds are 6 – 8 and 9 mm long compared to younger developmental stages 1 – 2 or 3 – 5 mm long, shoot apex or leaves. Also, clear differences are observed between male, female and hermaphrodite buds, where the

hermaphrodite flower type is intermediate in comparison to male and female. As an example, proline was identified as highly accumulating in developing flower types containing male organs. This is consistent with previous findings, showing that proline accumulates in fertile wheat anthers, but not in male sterile anthers (Kempe et al. 2013). It is suggested that proline could affect ACC to ethylene conversion (Chrominski et al. 1989). This process is essential during sex determination in cucumber. The other example is galactinol which is highly accumulated in female buds. It is suggested that galactinol acts as antioxidants and/or osmoprotectants and may lead to the increased tolerance of oxidative damage (Nishizawa et al. 2008) perhaps in female generative organ.. There are also examples of metabolites connected with sugar pathways, specific for genotypes: male (*a*) - ribonic acid and galactaric acid and female (*gy*) – glucosamine. The preliminary observation to find many of these specific changes already at the earliest analyzed time point of flower development renders metabolite profiling an important tool to study sex determination at the molecular level and to identify metabolic pathways highly relevant for sex-specific processes.

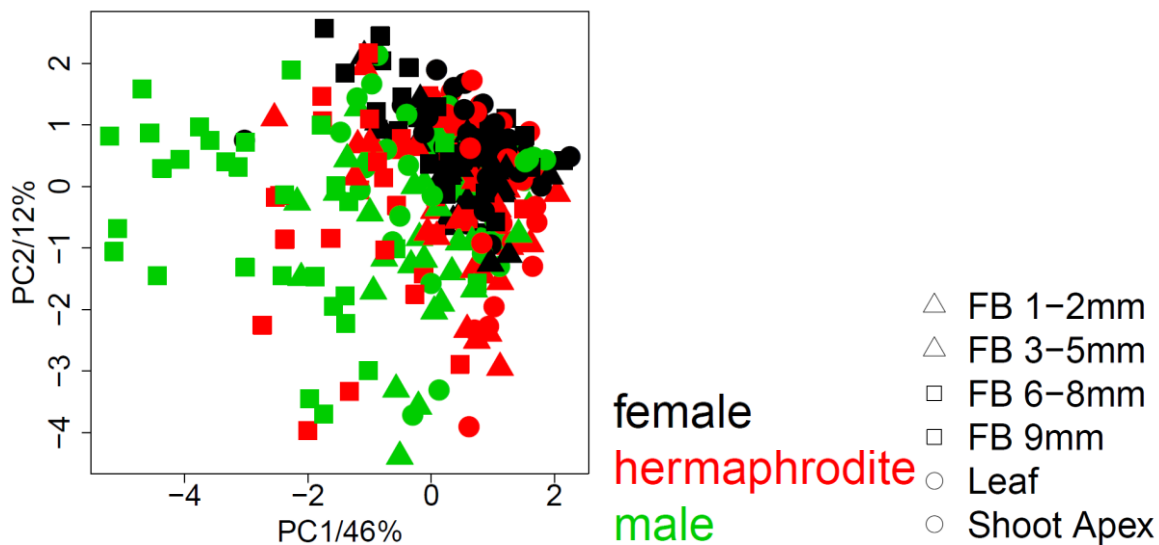


Figure 1. Principal component analysis of metabolites in the developing flower.