GÖTEBORGS UNIVERSITET

Mutagenesis and transcriptome mapping in oat and characterization of high β-glucan lines

Per Sikora
Institutionen för biologi och miljövetenskap
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i växtmolekylärbiologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredag den 17 maj, 2013 kl. 10:00 i hörsalen, Institutionen för biologi och miljövetenskap, Carl Skottsbergs gata 22B, Göteborg.

ISBN: 978-91-85529-57-5

Mutagenesis and transcriptome mapping in oat and characterization of high β-glucan lines

Per Sikora
Department of Biology and Environmental Sciences, University of Gothenburg
Box 461, SE-405 30, Gothenburg, Sweden

Abstract

Oat (*Avena sativa*) is a hexaploid crop that is cultivated in Europe, North America, Russia, Australia and northern China. During recent years, oat has seen increased interest due to its potential as a functional food crop. Among beneficial health promoting bioactive molecules expressed in oat are unique antioxidants, galacto-lipids and high levels of globular proteins. Oat is also an excellent source of β-glucan (1-3;1-4 mixed link β-D glucan), a dietary fiber with blood glucose stabilizing and cholesterol lowering properties.

However, traditional breeding has hitherto had limited success in developing a high β-glucan oat. To improve the variation and precision of the breeding process and to provide a new tool for trait development in oat, an EMS-mutagenized TILLING-population of 2,500 oat lines was developed (**Paper I**). The mutation frequency was assayed by several different methods, including screening for mutations at the genetic level using the *AsCslF6* and *AsPAL1* genes as a model.

Taking advantage of the TILLING-population, seeds from 1,700 lines from the TILLING population were ground and biochemically tested for β-glucan content. Several mutated lines with dramatically increased or decreased β-glucan levels were the identified. This variation also validated the high mutation frequency in the mutagenized lines. The mutant line with the highest levels of β-glucan saw an increase of 52% compared to the starting variety. Six lines with increased and four with decreased β-glucan levels were finally selected and parameters like structure, molecular weight, solubility and localization of β-glucan in the seed kernel were analyzed. This showed that also the β-glucan quality varied greatly between mutants, suggesting that quantitative and qualitative differences may in some cases be linked (**Paper II**). The high β-glucan lines will now be used in various breeding applications as well as in studies to elucidate physiological effects of altered β-glucan structure on humans.

To increase and improve the genetic resources available in oat, a high coverage transcriptome map was developed using next generation sequencing from a diverse range of tissues and during seed development. In total, 190,261 contigs were obtained after assembly and annotated using BLAST, Interproscan and Gene Ontology. The quantitative nature of the data also allowed for expression analysis, creating an unparalleled view into the oat transcriptome. The data was then used to identify tissue-specific transcription factors and characterize the expression of β-glucan producing genes during seed development (**Paper III)**. The oat transcriptome atlas will be useful in comparative studies between oat and other cereals and also provide an important reference in future oat-genome sequencing projects.

Finally, as a complement to the transcriptome map, an oat miRNA atlas was generated by sequencing small RNA from seed development, various tissues and during abiotic stress. These microRNAs can now be used in conjunction with the transcriptome data for a further unraveling of the complex regulatory networks in oat (**Paper IV**).

Keywords: Avena sativa, oat, beta glucan, bioinformatics, mutagenesis, EMS, TILLING, transcriptomics, micro RNA, seed histology, calcofluor, fiber quality