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# Genome plasticity of Scots pine (*Pinus sylvestris* L.) under different stress conditions

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# Introduction

- Short generation time
- Low genetic diversity
- Human selection since 8500 B.C. using phenotypic traits
- High specificity to enviroment
- Molecular markers for selection are broadly used

Aegilops (wheat wild ancestor), http://www.arcad-project.org

- Long generation time & Molecular markers
- High genetic diversity
- Natural selection
- high plastisity to enviroment
- Phenotypic traits selection should be used in association with molecular markers

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# Retrotransposon variation

- mobile genetic elements
- replicative transposition
- the largest compound of plant genomes (15-90 %)
- claster formation

? rearrangement of genome (instability), - functional mutations,-somaclonal variation,- recombination process,- genome structure upkeep

- transpozitional activation observed in stress conditions

*(McClintoc, 1984, Peschke et al.1987, Grandbastien et al. 1998, Hirochika et al. 1993, Poteau et al.1994, Ramallo et al., 2008).*

Adaptive selection to larger genomes with higher retrotransposon activity?

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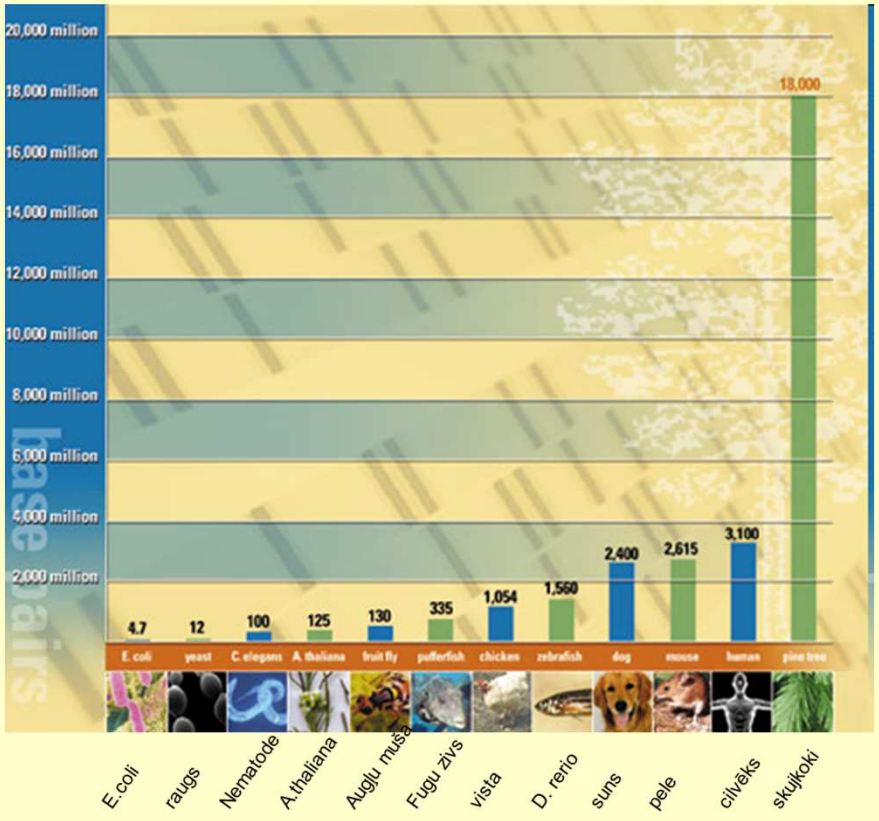
# Scots pine genome

- one of the ancient's plant groups gymnosperms (*Pinophyta*)
- *2n=24* genome size:
  - 50 pg (Grotkopp et al, 2004);
  - 42,5 (Bogunic et al., 2003);
  - 55,6 (Valkonen et al. 1994).

- 70-75 % repetitive sequences
- Pinus nigra population study of variation in genome size (Bogunic et al. 2007) 0,64 pg 2,6%.
- variation is determined by spread of different sequence repeats as satellites and retrotransposons.




*http://www.genome.duke.edu/research/highlights/environmental/forestry-genomics.php*

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# Aims of study

Identify active retrotransposons in Scots pine (*Pinus sylvestris* L.) genome and characterize its structure, distribution and transcriptional rate during different stressors in controlled conditions.

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# Brief structure of study

- ✓ Control molecular markes desighn
- ✓ Optimisation of methods;
- ✓ High temperature effects on the transcription of pine mobile genetic elements
  - Confirmation of clonal identity of the ramets;
  - Induction of stress conditions and RNA extraction;
  - Identification of mobile genetic elements;
  - Specific marker desighn;
  - Data collection and analysis.

►Biotic stress effects on transcription of pine mobile genetic elements;

►effects of Salicilic acid and ABA.

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# Brief structure of study

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► analyses of established molecular markers on the natural pine stands growing in different conditions;

► Full-size retrotransposon isolation and sequencing;

► ► Identification of retrotransposons/ classification/ structural studies / prevalence studies in the pine genome

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# Scots pine ramets and stress initiation

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Pine Woolly Aphid
(*Pineus pini*)

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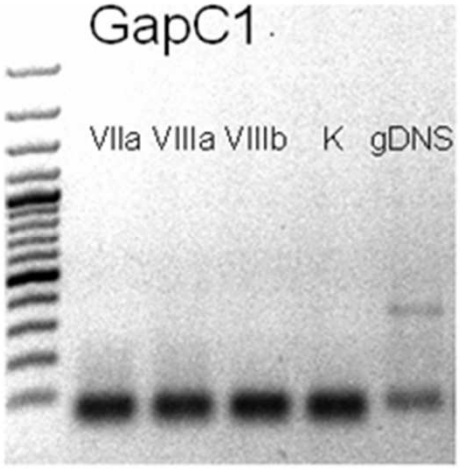
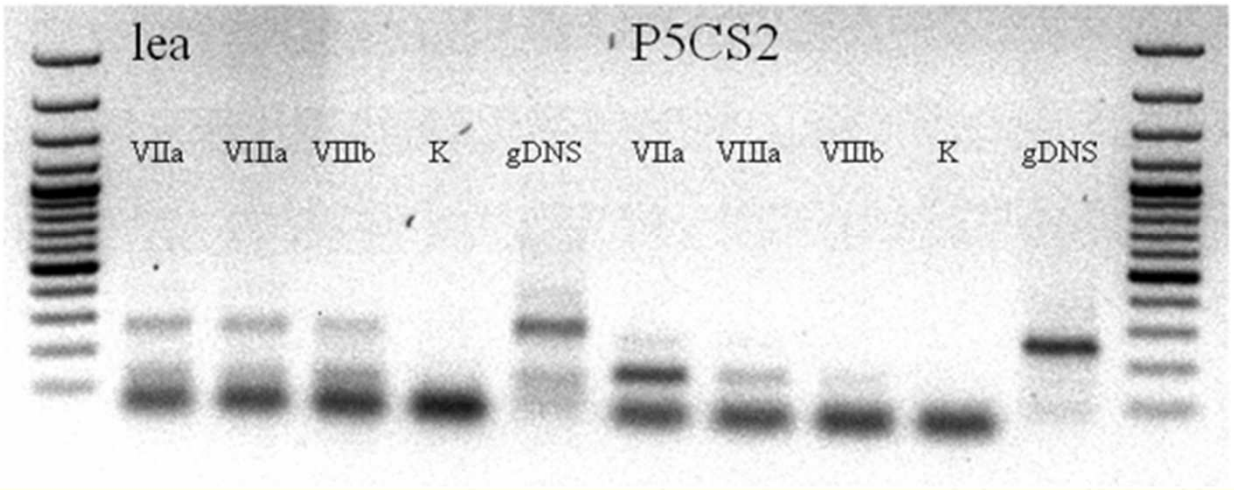
# Control molecular markes design

- dhn3 (AJ512362.1)
- abaH (FJ201653.1)
- Lea (FJ201577.1)

- P5CS2 (NM_115419.4, EF412967.1)
- GapC1 (L07501.1 Jaakola et al. 2004)
- pns (S50350.1)
- lp3 (U67135.1)



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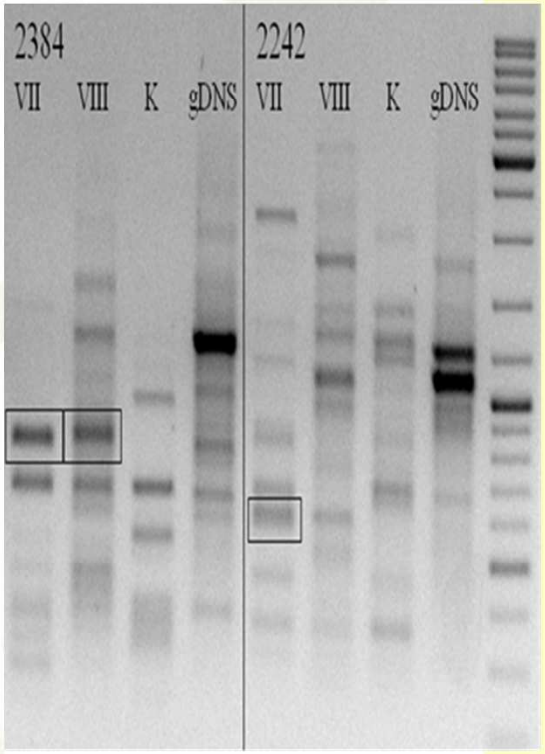
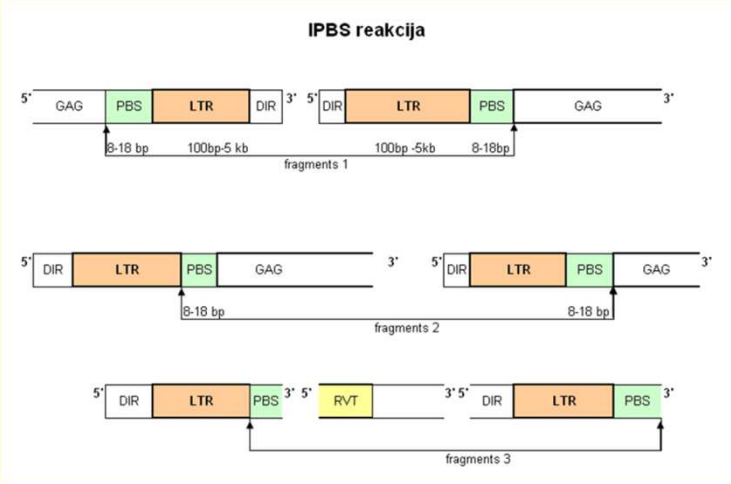
# Methods




iPBS reaction (Kalendar et.al., 2010);




Inter PBS amplification. Lanes VII and VIII show fragments amplified from cDNA samples from heat stressed trees, lane K is the cDNA sample from the control tree, lane gDNS shows amplification with genomic DNA of the same ramet, and the last lane is size marker GeneRuler DNA Ladder Mix (Fermentas). Excised fragments are indicated.

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# Classification of mobile genetic elements

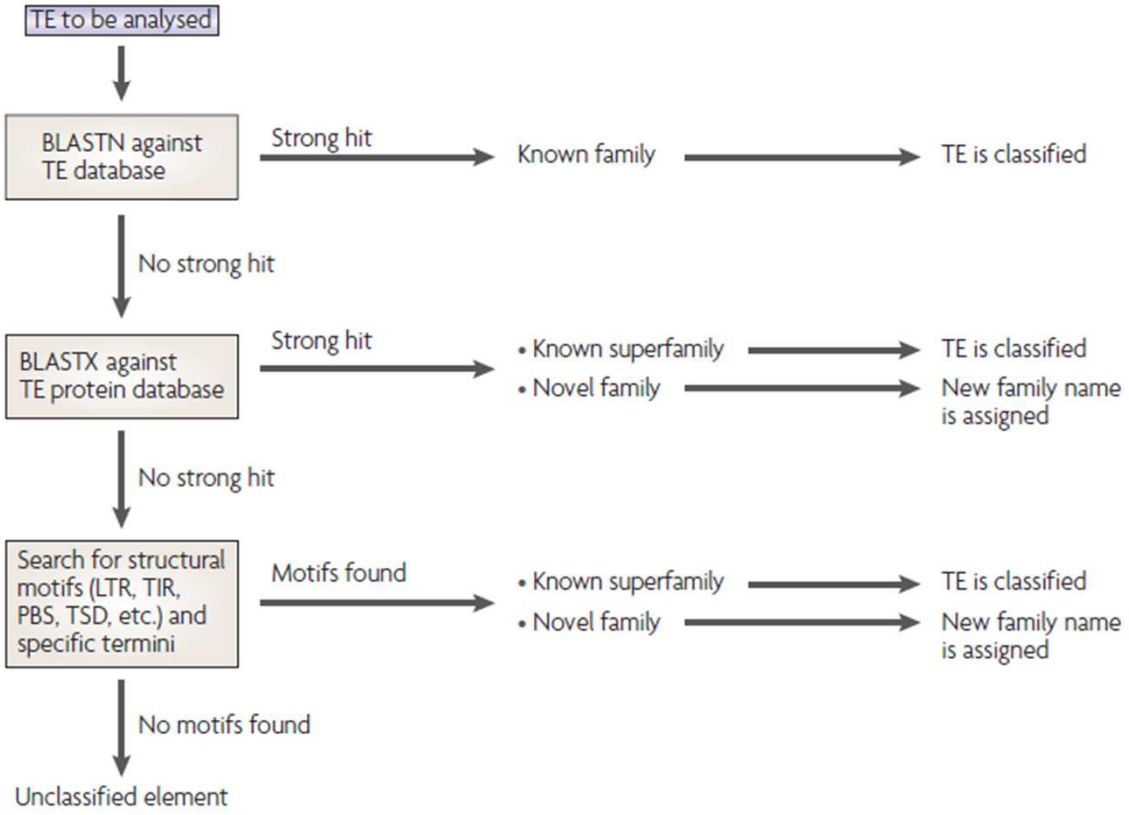


Figure 3 | **Step by step transposable element (TE) classification.** LTR, long terminal repeat; PBS, primer binding site; TIR, terminal inverted repeat; TSD, target site duplication.

*Wicker et al., 2007*

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# Sequence analyses

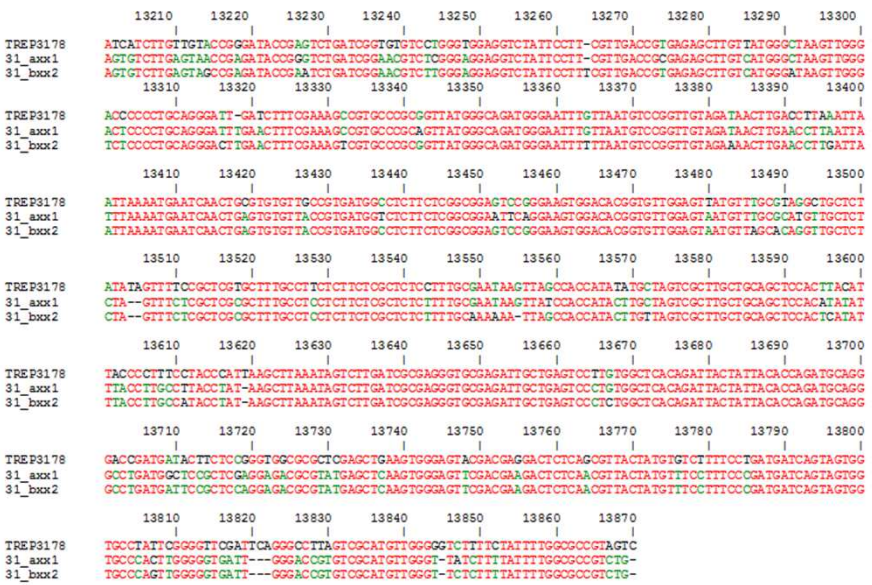
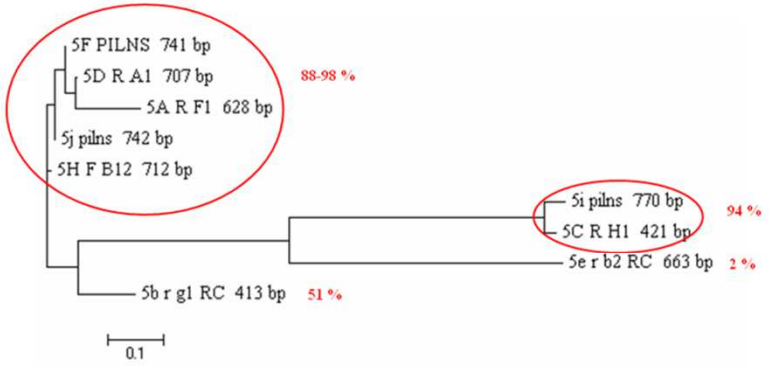



Figure 3. Multiple sequence alignment of 3.1 group fragments with LTR *Gypsy*, *Erika* TE (data base number: TREP3178) nucleotide sequences.




```
>gnl|PTREP|PTREP173 DNA transposon, TIR, CACTA,"DTC_Conan_consensus-1";
(TREP3415) ORF2 protein Length = 1124

 Score =  274 bits (701), Expect(2) = 2e-89
 Identities = 170/228 (74%), Positives = 188/228 (82%), Gaps = 2/228 (0%)
 Frame = +2

Query: 101 PRCRTWFYAHGGA-LDQKTGKISWKASLKGAKQKIIDTIEESRRGEFMPNRENDELTCAL 277
           PRCRTWFYAHGG  LD KTG +S KA L GA    ++  IEE+R G F PNRENDELT AL
Sbjct: 277 PRCRTWFYAHGGGELDPKTGNVSTKACLNGADDALLVAIEEARSGVFQPNRENDELTRAL 336

Query: 278 GNPEYQGRTRGKGVIPWYEGFSDWNDDYRSRARKKMEEEKKRKLEEEQRKQDAERLQGLE 457
           GNPE+ GRTRGKG IPWYEGFSDWN DYR+RARKK+ EEKKRK+EEEQRK D ERLQGLE
Sbjct: 337 GNPEHPGRTRGKGAIPWYEGFSDWNADYRTRARKKIAEEKKRKMEEEQRKLDYERLQGLE 396

Query: 458 ARHADLALKFRQQQQ*IDSLSQERGSQQRQQQADDRPALDSTVPSMPRSSVGSAPGD-TL 634
           A  A+LA KF++QQ+ IDSLSQ+RGSQQ QQ ADD PALD+T PSMPRSSVGSAPGD  +
Sbjct: 397 ASQAELAAKFQRQQEQIDSLSQQRGSQQLQQLADD-PALDTTAPSMPRSSVGSAPGDAVV 455

Query: 635 LDTYPVDDIIEDTNCELHSKMKNISMKVADGVAFPVTPRATYHCIPIP 778
           LD YPVDDI E+TNCELH KMKNISMKVAD VAF  +P AT+HC PIP
Sbjct: 456 LDRYPVDDITENTNCELHFKMKNISMKVADAVAFSNSPEATFHCNPIP 503
```

**Searches were done in the**

- NCBI data base (http://www.ncbi.nlm.nih.gov/BLAST/ );
- GrainGenes Triticeae Repeat Sequence Database (http://wheat.pw.usda.gov/ITMI/Repeats/blastrepeats3.html )
- Gypsy Database 2.0 (http://gydb.org/index.php/Blast ) (Llorens et al., 2011).

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# Results of similarity searches

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| Fragment | LTR elements | | Non-LTR elements | DNA transposable elements | Unclassified elements | EST | No similarity | Blastn: Score/ E-value/Positives | Blastx: Score/ E-value/Identities/ Positives |
|---|---|---|---|---|---|---|---|---|---|
| | *Gypsy* superfamily | *Copia* superfamily | LINE | CACTA | | | | | |
| 1.1 | | | | | | | ● | - | - |
| 1.2 | | ● | | | | | | - | 135/3e-34/41%/61% |
| 2.1 | ● | | | | ● | ● | | - | 55.1/1e-09/29%/43% |
| 2.2 | | | | | | ● | | - | - |
| 3.1 | ● | | | | | | | 670/0.0/88% | - |
| 3.2 | ● | | | | ● | ● | | - | 54.7/2e-09/29%/43% |
| 3.3 | ● | | | | | | | - | 53.5/3e-09/28%/47% |
| 4 | | | | ● | | | | 620/e-178/95% | 274/2e-89/74%/82% |
| 5.1 | | | ● | | | | | 56/6e-08/86% | 179/3e-47/67%/77% |
| 5.2 | | | | | | | ● | - | - |
| 5.3 | | | | | | | ● | - | - |
| 6.1 | | | | | | | ● | - | - |
| 6.2 | | | | | | ● | | - | - |
| 7.1 | | | | | | ● | | - | - |
| 7.2 | ● | | | | | ● | | - | - |
| 7.3 | ● | | | | | | | 722/0.0/93% | - |
| 8 | ● | | | | ● | ● | | - | 52.8/6e-09/31%/43% |
| 9.1 | | | | ● | | | | 680/0.0/96% | 295/4e-82/68%/76% |
| 9.2 | ● | | | | | ● | | 50.1/1e-06/83% | 89/1e-20/52%/64% |
| 9.3 | | ● | | | | | | - | - |
| 10.1 | ● | | | | | ● | | 686/0.0/90% | - |
| 10.2 | | ● | | | | | | - | 84.7/8e-19/59%/73% |

# Classification of analyzed fragment sequences according to transposable elements from the TREP and GyDB databases

| Fragment | Order, Superfamily | Name of TE | Domain | Data base | Organism |
|---|---|---|---|---|---|
| 1.2 | LTR, copia | *HORPIA2* | polyprotein | TREP, blastx | *Hordeum vulgare* |
| | LTR, copia | *BARE1* | polyprotein | TREP, blastx | *Hordeum vulgare* |
| | LTR, copia | *Oryco1-1* | INT | GyDB, cores | *Oryza sativa* |
| | LTR, copia | *Tnt-1* | INT | GyDB, cores | *Nicotiana tabacum* |
| 2.1/ 8/ 3.2 | LTR, Gypsy | *Geneva* | GAG | TREP, blastx | *Hordeum vulgare* |
| | LTR, Gypsy | *Sabrina* | polyprotein | TREP, blastx | *Triticum turgidum* |
| 3.1 | LTR, Gypsy | *Erika* | genomic | TREP, blastn | *Triticeae* |
| 3.3 | LTR, Gypsy | *Sabrina* | polyprotein | TREP, blastx | *Triticum turgidum* |
| | LTR, Gypsy | *Diaspora* | GAG | GyDB, cores | *Glycine max* |
| 4/ 9.1 | DNS, TIR, CACTA | *Conan* | pol | TREP, blastn | *Triticeae* |
| 5.1 | LINE | *Persephone* | genomic | TREP, blastn | *Hordeum vulgare* |
| | LINE | *Karin* | polyprotein | TREP, blastx | *Hordeum vulgare* |
| 7.3 | LTR, Gypsy | *Wham* | genomic | TREP, blastn | *Triticeae* |
| 9.2 | LTR, Gypsy | *Ifis* | genomic | TREP, blastn | *Triticum turgidum* |
| | LTR, Gypsy | *Carmilla* | polyprotein | TREP, blastx | *Triticum aestivum* |
| 10.1 | LTR, Gypsy | *Laura* | genomic | TREP, blastn | *Triticeae* |
| 10.2 | LTR, copia | *Maximus* | polyprotein | TREP, blastx | *Triticum aestivum* |

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# Inter Retratransposon amplification

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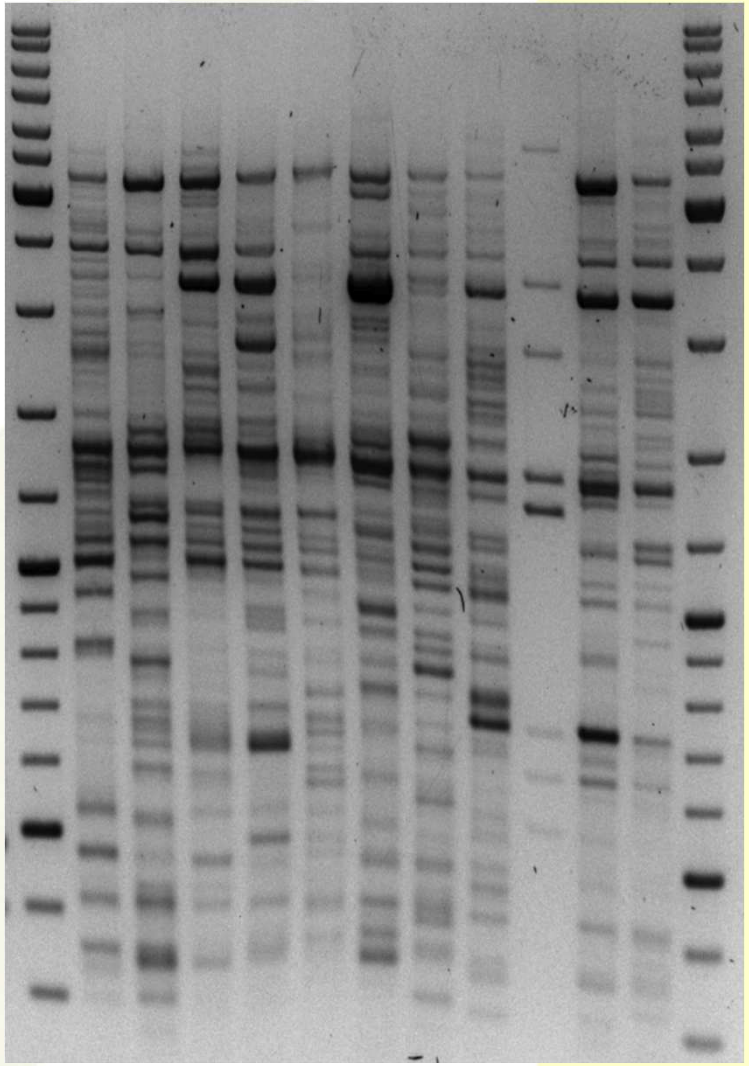

Nine newly developed Retrotransposon Markers

184 fragments were analysed

150 samples from one natural pine stand growing in highland, slope and lowland.

355-279 more fragments were found in the samples from trees growing in highland

| Stands | heat stress markers | | biotic stress markers | | Total |
|---|---|---|---|---|---|
| | Sum 40 | Average | Sum 38 | Average | |
| pop_highland | 2269 | 56,73 | 2281 | 60,03 | 4550 |
| pop_slope | 2173 | 54,56 | 2098 | 55,29 | 4271 |
| pop_lowland | 2135 | 53,59 | 2060 | 54,30 | 4195 |

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# Conclusions

- Unspecific iPBS amplification reveals significant retrotransposon variation in Scots pine genome;
- Representatives of different families of Retrotransposons are found. Three fragments could be classified to particular TE families with high probability as they showed high similarity at the nucleotide level (LTR, *Gypsy*, *Laura*; LTR, *Gypsy*, *Wham*; DNS, TIR CACTA, *Conan*; LTR, *Gypsy*, *Erica*).
- Analysed sequences are consistently transcribed under various stress conditions as analysed sequences shows similarity with EST database sequences derived from cDNA libraries obtained in various studies of stress responses.
- The presence of sequence variation indicate that the transcripts originate from different copies in the pine genome.

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# Conclusions

- One of the analysed sequences showed a similarity to a DNA transposable element *Conan*. Due to the clustering of retrotransposons within genomes, the identified sequences could be fragments or inactive elements within an active element.

- Some of the analysed sequences were similar to several known active retroelement proteins (*BARE-1*, *Tnt-1*) which could indicate that these retroelements possess transposable activity.

- These results indicate that further isolation of complete elements is needed to prove their transcriptional activation and to investigate activation of these elements in differing stress conditions.

- Due to high genetic diversity to acess diferences in natural pine stands affected by retrotransposition more molecular marker needed or we need to choose more homogeneous sample set.

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# Presentation of Results

- **Expression of retrotransposon-like sequences in Scots pine (*Pinus sylvestris*. L) in response to heat stress**. 2011. A.Voronova, Ā.Jansons, D.Ruņģis, Enviromental and Experimental Biology, in press.

- 4.2.2010.- 68. Scientific Conference of University of Latvia, Plant biology section.

- 22.-24.04.2009.- 5th International Conference “Research and Conservation of Biological Diversity in Baltic Region”, Daugavpils, Latvia (Oral presentation).

- 19.-21.03.2009.– FEBS Workshop „Adaption Potential in Plants”, Vienna, Austria (Poster presentation).

- 4.02.2009.– 67. Scientific Conference of University of Latvia, Plant biology section (Oral presentation).

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