
Tolerance Data 2012 En Francais Torrent



Total RNA was isolated from Scots pine hypocotyls, roots, stems, and needles using Spectrum Plant Total RNA Kit (Sigma) following the manufacturers instructions. The mRNA concentration and quality was determined using NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific Inc.) and Bioanalyzer 2100 (Agilent Technologies Inc.), respectively. RNA library preparation and subsequent sequencing were performed at SciLifeLab (Stockholm, Sweden). Strand-specific RNA libraries for sequencing were prepared with TruSeq Stranded mRNA Sample prep kit of 96 dual indexes (Illumina) according to the manufacturers instructions except for the following changes: the protocols were automated in Agilent NGS workstation (Agilent Technologies) using purification steps as described in Lundin et al. (2010) and Borgstrom et al. (2011). Clonal clusters were generated using cBot (Illumina) and sequenced on HiSeq2500 (Illumina) according to manufacturers instructions. Bcl to Fastq conversion was performed with bcl2Fastq v1.8.3 from the CASAVA software suite. The quality scale was Sanger/phred33/Illumina 1.8+. The obtained data was deposited to the ENA and is accessible under the accession number RJEB19683. Quality of the raw data was assessed with the FASTQC tool and data was trimmed of sequencing adapters. Reads were sorted, and filtered to remove low-quality bases (i.e., bases with a Phred-scaled quality score of Q

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In addition to hypocotyl and seedling elongation responses, we recorded visual estimates of the water status of the needle group, radicle length and number of additional leaves in the area covered by the shade cloth, and hypothesized that these should be the biomass and shade tolerance markers. The biomass markers were normalized as the stem length in relation to the number of plants in the sample for both needle groups in order to distinguish between plants with different number of needles. The visual estimates of the water status of the needle group was estimated as “green”, “brown” or “spotted”, together with estimates of the intensity of the browning. The radicle length and number of additional leaves were used as estimates of biomass. “Green” was used as a baseline in order to judge the intensity of the browning by adding one point for each increment of 100% in the severity of the browning. Considering that the needle group covered a minimum of 7% of the leaf, the radicle length and the additional leaves, normalized to leaf size, were used as proxies of biomass accumulation. The visual estimates of the water status of the needle group were normalized as 1, 2 or 3 for “green”, “brown”, and “spotted”, respectively. The visual estimates of the intensity of the browning were also normalized as 1, 2 or 3 for “none”, “mild”, and “severe”,

respectively. The number of plants in the sample was used to normalize the height data. Among the indexes that indicate water status, we only used the visual estimates of the intensity of the browning, since the previous analyses showed its high correlation with the normalized radicle length as a biomass proxy. However, other visual estimates of the water status such as the intensity of the green color could be used as proxies as indicated in previous studies (Danielsen et al. 2003 ; Vila-Nova et al. 2011 ; Alcamo et al. 2013). 5ec8ef588b

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