Comparison of endogenous cytokinins, ABA and metabolites during female cone bud differentiation in low and high cone-producing genotypes of lodgepole pine

Kong, Lisheng; von Aderkas, Patrick; Owen, Stacey J.; Wagner, Tia; Abrams, Suzanne R.

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4 **Comparison of endogenous cytokinins, ABA and metabolites during female cone bud**

5 **differentiation in low and high cone-producing genotypes of lodgepole pine**

6 Lisheng Kong[1,4,]*, Patrick von Aderkas[1], Stacey J. Owen[2], Tia Wagner[3], Suzanne R. Abrams[2]

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8 [1] Centre for Forest Biology, Department of Biology, University of Victoria, 3800 Finnerty Rd.,

9 Victoria, BC, Canada V8W 3N5. [2] Plant Biotechnology Institute, National Research Council

10 of Canada, 110 Gymnasium Place, Saskatoon, SK, Canada S7N 0W9. [3] Vernon Seed

11 Orchard Company, 6555 Bench Row Road, Vernon, BC, Canada, V1H 1G2. [4] Warnell

12 School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602 USA

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16 * Lisheng Kong (Corresponding author)

17 Address: Centre for Forest Biology, Department of Biology, University of Victoria, 3800

18 Finnerty Rd., Victoria, BC, Canada V8W 3N5

19 Tel: 1-(250)-721- 8926

20 Fax: 1-(250)-721-6611

21 E-mail: lkong@uvic.ca; lkong@warnell.uga.edu

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24 **Running title:** Endogenous hormones and cone yield in lodgepole pine

25

26   **Abstract**

27   In lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.), cone bud initiation

28   within long-shoot buds varies according to genotype. We chose to study hormone profiles of

29   two genotypes that differed significantly in cone yield. Phytohormone profiles were

30   established by high performance liquid chromatography-electrospray ionization tandem mass

31   spectrometry (HPLC-ESI-MS/MS) in multiple reaction monitoring (MRM) mode with samples

32   collected from genotypes 299 and 233, the typically high and low cone producers. Generally,

33   concentrations of *trans*-zeatin-*O*-glucoside were higher in genotype 299, whereas

34   dihydrozeatin concentrations were higher in genotype 233. Both isopentenyl adenine and

35   isopentenyl adenosine were present at higher concentrations in genotype 233. The ratio of total

36   quantifiable zeatin (Z)-type cytokinins to isopentenyl (iP)-type cytokinins was approximate

37   three-fold higher in genotype 299 during female cone bud differentiation. In genotype 299,

38   ABA concentration was significantly lower than in genotype 233 on the first sampling date,

39   while the phaseic acid concentration was lower consistently throughout the period investigated.

40   Dihydrophaseic acid was present in low concentrations in most samples of genotype 233, but

41   was not quantifiable in genotype 299. Our study reveals that long-shoot buds of the high cone-

42   producing genotype had higher ratios of Z-type cytokinins to iP-type cytokinins than were

43   found in the low cone-producing genotype. High cone-producing buds also contained less

44   ABA, phaseic acid and dihydrophaseic acid during female cone bud differentiation.

45

46 **Keywords:** ABA, cytokinins, female cone yield, genotype, hormone metabolites, lodgepole

47 pine.

48

49 *Abbreviations***:** high performance liquid chromatography-electrospray ionization tandem mass

50 spectrometry, HPLC-ESI-MS/MS; multiple reaction monitoring, MRM; gibberellin, GA;

51 abscisic acid, ABA; phaseic acid, PA; dihydrophaseic acid, DPA; 7'-hydroxy ABA, 7'-OH

52 ABA; *neo*phaseic acid, *neo*PA; abscisic acid glucose ester, ABA-GE; *trans*-zeatin, *t*-Z; *trans*-

53 zeatin riboside, *t*-ZR; *trans*-zeatin-*O*-glucoside, *t*-Z-*O*-Glu; dihydrozeatin, dhZ; dihydrozeatin

54 riboside, dhZR; isopentenyl, iP; isopentenyl adenine, 2iP; isopentenyl adenosine, iPA.

55
56

Introduction

In pines, both reproductive buds and vegetative buds are initiated along long shoots. In the specific case of female cone bud differentiation in lodgepole pine, initiation is begun in summer and is completed by fall. A further two years are required for these female cones to mature and produce seed (O'Reilly and Owens 1987; 1988). Although many abiotic and biotic factors affect cone productivity, genotypic effects are relatively strong (Longman 1983; Philippe et al. 2006). This is noticeable in seed orchards. Certain genotypes consistently exhibit extremes in productivity. This undermines the purpose of a seed orchard, which is to produce as much seed as possible. Part of this inefficiency lies in the original selection of genotypes. It was not on the basis of seed production, but for fast growth, high wood quality, pest or disease resistance. Consequently, one reason for low seed productivity is low female bud initiation due to genotype.

To overcome genotype-related limitations in cone production, intervention in bud hormone physiology has proven useful. In both angiosperms (Fiehn et al. 2000) and gymnosperms (Kong et al. 2009), different genotypes exhibit unique metabolic and hormone profiles. In Douglas-fir, correlations between female cone yields and endogenous phytohormone levels of shoot buds have been shown (Kong et al. 2009). Studies of endogenous phytohormone levels during cone initiation can provide strategies to overcome cone yield limitations. Most commonly, plant growth regulators (PGRs), such as gibberellins (GAs), are directly applied. Occasionally, cytokinins and even indole-acetic acid have also been used to improve productivity. These plant growth regulators alter concentrations of

78 endogenous phytohormones and boost cone productivity (McMullan 1980; Bonnet-Masimbert

79 and Zaerr 1987; Pilate et al. 1990; Kong et al. 2008).

80   Cytokinins have been shown to play an important role during the reproductive process

81 (Imbault et al. 1988; Morris et al. 1990; Corbesier et al. 2003; Wakushima 2004). Recent

82 studies with *Arabidopsis thaliana* indicate that cytokinin receptors may have different affinities

83 for particular cytokinins, such as zeatin (Z)-type and isopentenyl (iP)-type cytokinins (Spíchal

84 et al. 2004; Romanov et al. 2006). Furthermore, the differential compartmentalization of

85 cytokinins may play a role in long-distance signalling (Corbesier et al. 2003; Hirose et al.

86 2008). In conifers, attention has been paid to the ratio of Z-type to iP-type cytokinins, but this

87 has been mainly in the context of tree ageing or vigour (Valdés et al. 2002; 2003; 2007) and

88 not in reproduction.

89   Abscisic acid (ABA) is involved in the regulation of many physiological processes

90 including the response of plants to environmental stresses (Bravo et al. 1998; Kumar et al.

91 2008). Abscisic acid negatively modulates the effect of other plant hormones, such as GAs

92 (Tompsett 1977) and auxin (Weiss and Ori 2007). This is of interest in conifers, because

93 exogenously applied GA has been proven to be an effective stimulant of cone formation (Ross

94 1983; Pharis and King 1985; Smith and Greenwood 1995; Kong et al. 2008). The main ABA

95 catabolic pathway in many higher plants is ABA oxidation with its end products of phaseic

96 acid (PA) and dihydrophaseic acid (DPA), but in conifers such as Douglas-fir, the dominant

97 product of ABA catabolism in long-shoots is ABA glucose ester (ABA-GE) (Kong et al. 2008;

98 2009).

99   The purpose of this research was to investigate changes, during female cone bud

100   differentiation, in endogenous cytokinins and ABA as well as some of their metabolites with

101   two genotypes that differed in cone yield. Lodgepole pine (*Pinus contorta* Dougl. ex Loud. var.

102   *latifolia* Engelm.), an important forest species in western North America, was used. Seed

103   improvement programs have been created in this species consequently. Many of these trees are

104   genetically well-characterized. Hormone analyses of samples were completed by high

105   performance liquid chromatography-electrospray ionization tandem mass spectrometry

106   (HPLC-ESI-MS/MS) in multiple reaction monitoring (MRM) mode (Chiwocha et al. 2003;

107   2005). This method has a number of advantages: multiple compounds can be quantified from

108   the same sample and the need to process separate samples for each class of compounds is

109   eliminated.

110

111   **Material and Methods**

112   *Genotype selection*

113   Genotypes 233 and 299 were selected from an established seed orchard of 15-year-old trees

114   owned by Vernon Seed Orchard Company, located in Vernon, British Columbia (50°13′N,

115   119°19′W). The selection procedure was based on a ranking according to productivity. Cone

116   yields for the three previous years were pooled for six ramets of each genotype. Significantly

117   different ($P < 0.05$) genotypes were chosen.

118

119   *Sample collection, processing and storage*

6

120   Samples were collected five times during female cone bud differentiation between the end of

121   July and mid-October, 2006. Depending on the size of the bud, ten to 20 long-shoot buds were

122   collected from each ramet per sampling time. After collection, the buds were kept frozen at -

123   20 °C for 2 to 3 d before they were lyophilized in a freeze-drier for 48 hrs. The resulting dry

124   samples were sealed in plastic bags and stored at - 20 °C.

125

126   *Measurement of moisture content*

127   Fresh weight and dry weight of the samples were used to derive moisture content according to

128   the following formula:

129
130
131    $$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$
132
133
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136
137   *Analysis of hormones and their metabolites*
138
139   Chemicals: Pure hormone standards, used in calibration curve and quality control solutions,

140   were obtained as follows: dihydrophaseic acid (DPA), abscisic acid glucose ester (ABA-GE),

141   phaseic acid (PA), 7'-hydroxy ABA (7'-OH ABA) and *neo*phaseic acid (*neo*PA) from the Plant

142   Biotechnology Institute of the National Research Council of Canada (PBI-NRC, Saskatoon, SK,

143   Canada); IAA, indole-3-acetic acid aspartate (IAA-Asp), indole-3-acetic acid glutamate (IAA-

144   Glu), ABA, *trans*-zeatin (*t*-Z), *trans*-zeatin riboside (*t*-ZR), isopentenyl adenosine (iPA), and

145   isopentenyl adenine (2iP) from Sigma-Aldrich (Oakville, ON, Canada); dihydrozeatin (dhZ),

7

146 dihydrozeatin riboside (dhZR), and *trans*-zeatin-*O*-glucoside (*t-Z-O*-Glu) from Olchemim Ltd.

147 (Olomouc, Czech Republic); GA_1, GA_3, GA_4, and GA_7 from Prof. Lewis Mander (Australian

148 National University, Canberra, Australia). Bulk amounts of the deuterated forms of the

149 hormones, used as internal standards, were obtained as follows: d_3-DPA, d_5-ABA-GE, d_3-PA,

150 d_4-7'-OH ABA, d_3- *neo*PA, d_4-ABA, d_3-IAA-Asp, and d_3-IAA-Glu from PBI-NRC (Saskatoon,

151 SK, Canada); d_5-IAA, d_3-dhZ, d_3-dhZR, d_5-*t-Z-O*-Glu, d_6-iPA, and d_6-2iP from Olchemim Ltd.

152 (Olomouc, Czech Republic); d_2-GA_1, and d_2-GA_4 from Prof. Lewis Mander (Australian

153 National University, Canberra, Australia). Bulk amounts of the deuterated forms of selected

154 hormones which were used as recovery standards, namely d_6-ABA and d_2-ABA-GE, were

155 obtained from PBI-NRC. Preparations of ABA and ABA metabolite standards were described

156 by Abrams et al. (2003) and Zaharia et al. (2005).

157

158 *Extraction, purification and quantification by HPLC-ESI-MS/MS:*

159 Extraction and purification steps were carried out as in Kong et al. (2008). The procedure used

160 for quantification of multiple hormones, including abscisic acid and its metabolites, was a

161 modification of Chiwocha et al. (2003; 2005). To the spectrum of 18 hormones originally

162 quantified in these papers, including IAA, IAA-Asp, ABA, 7′-OH ABA, PA, DPA, ABA-GE,

163 GA_1 (*m/z* 347>259), GA_3, GA_4 (*m/z* 331>243), GA_7, *t*-Z, *t*-ZR, dhZ, dhZR, *t-Z-O*-Glu, 2iP, and

164 iPA, were added an additional two; namely IAA-Glu (*m/z* 303>146) and *neo*PA (*m/z* 279>205).

165 Samples were injected onto a Genesis C18 HPLC column (100 × 2.1 mm, 4 μm,

166 Chromatographic Specialties, Brockville, ON, Canada) and separated by a gradient elution of

167 water against an increasing percentage of acetonitrile and methanol plus 0.04% acetic acid.

168 Calibration curves were generated from the MRM signals obtained from standard solutions

169 using the ratio of the chromatographic peak area for each analyte to that of the corresponding

170 internal standard, as described by Ross et al. (2004). Quality control samples, internal standard

171 blanks, and solvent blanks were also prepared and analyzed along with each batch of tissue

172 samples. The concentrations of IAA, IAA-Asp and IAA-Glu were generally below quantifiable

173 limits of <61 ng g^{-1} DW, <58 ng g^{-1} DW, and <58 ng g^{-1} DW, respectively. All GAs analyzed

174 in this study were also below quantifiable levels of <473 ng g^{-1} DW for GA$_1$, <238 ng g^{-1} DW

175 for GA$_3$, <116 ng g^{-1} DW for GA$_4$, and <116 ng g^{-1} DW for GA$_7$. As a consequence IAA, IAA

176 metabolites and GAs are not included in the results.

177

178 *Experimental design and statistical analysis*

179 Six ramets were used as replicates for female cone yield evaluation in each genotype, whereas

180 3 ramets per genotype were used as replicates for hormone analysis. Data were subjected to

181 one-way analysis of variance (ANOVA) using Minitab statistical software (Minitab, State

182 College, Pennsylvania, USA). The variance was analyzed by Tukey's significant difference

183 with the level of significance set to $P < 0.05$. For analysis purposes, concentrations below

184 quantifiable levels were treated as zeros. Significance differences in overall patterns or at each

185 sampling date have been indicated in the text.

186

187

Results

189 *Genotype performance in cone production*

190 Cone yield was significantly different ($P < 0.05$) between the genotypes. The mean value of

191 female cone yield per tree of genotype 299 was 3.5 times higher than that of genotype 233

192 (Table 1).

193

194 *Dry weight and moisture content*

195 The dry weight of long-shoot buds increased as the season advanced (Fig. 1). Overall, no

196 difference in dry weight existed between these two genotypes (F= 1.79, $P = 0.16$). Moisture

197 content of the bud decreased consistently in both of the genotypes over the growth period (Fig.

198 2). No significant difference (F = 0, $P = 0.951$) was found between genotypes 299 and 233.

199 Although the dry weight per bud in genotype 299 was slightly higher than that of genotype 233

200 at weeks 4 and 10, these differences were not significant (F = 4.15, $P = 0.069$ and F = 3.76, $P =$

201 0.081).

202

203 *Cytokinins and metabolites*

204 *Zeatin (Z)-type cytokinins:* The concentration of *t*-ZR was highest at the beginning of August

205 (week 0), reaching 109 ng g^{-1} DW and 90 ng g^{-1} DW in genotypes 299 and 233, respectively

206 (Fig. 3). Concentrations then declined as the season advanced. The average concentration of *t*-

207 ZR in genotype 299 samples was slightly higher than that in genotype 233 until week 6.

208 Concentrations of *t*-ZR in genotype 299 samples then dropped below quantifiable levels. There

209　was no significant difference (F = 0.04, P = 0.836) between genotypes 299 and 233 in the

210　overall pattern of t-ZR concentration changes. In genotype 233, the concentration of dhZ

211　remained fairly steady until week 4 when it dropped below quantifiable levels (Table 2). In

212　contrast, dhZ levels were below quantifiable levels in all genotype 299 samples at all sampling

213　dates. In genotype 299, the concentration of t-Z-O-Glu peaked at week 4 before progressively

214　declining. At week 10, its concentration dropped below quantifiable levels (Table 2). Genotype

215　233's t-Z-O-Glu levels were below quantifiable levels throughout. In both genotypes,

216　concentrations of dhZR and t-Z were generally below quantifiable levels.

217

218　*Isopentenyl (iP)-type cytokinins:* A significant difference (F = 10.94, P = 0.003) existed in the

219　overall pattern of iPA concentration changes between the two genotypes. The concentration of

220　2iP in genotype 233 peaked at week 2, declined at week 4 and remained unchanged throughout

221　the subsequent sampling dates (Table 2). In genotype 299 samples, 2iP was only quantifiable at

222　week 10.　The concentration of iPA in genotype 233 stayed relatively constant throughout the

223　time period studied, varying between 11 and 14 ng g^{-1} DW (Fig. 4). The concentrations of iPA

224　were significantly (P < 0.05) lower in the samples of genotype 299 than in genotype 233 at

225　weeks 0, 6 and 10 (Fig. 4). In genotype 299, the highest concentration of iPA (13 ng g^{-1} DW)

226　was observed at week 4.

227

228　*The ratio of Z-type to iP-type cytokinins: S*ignificant difference (P < 0.05) existed between

229　these two genotypes in the ratios of Z-type cytokinins (dhZ, t-Z-O-Glu, t-ZR, dhZR) to iP-type

230   cytokinins (2iP, iPA). This ratio was approximately 3-fold higher in genotype 299 than in

231   genotype 233 during the first four weeks (Fig. 5). It then decreased as the season advanced.

232

233   *Abscisic acid*

234   Concentrations of ABA were significantly lower in genotype 299 than in genotype 233 only at

235   the first sampling date (F = 14.49, P = 0.019) (Fig. 6). Afterwards, no significant differences

236   were noted between these genotypes. The overall pattern of a continuous increase in ABA

237   concentration was observed for both genotypes (F = 2.22, P = 0.147).

238

239   *ABA metabolites*

240   Concentrations of ABA-GE were initially 343 ng g^{-1} DW and 408 ng g^{-1} DW in long-shoot

241   samples of genotypes 299 and 233, respectively. As with ABA, the concentration of ABA-GE

242   in both genotypes continued to increase as the season progressed (Fig. 7). The overall pattern

243   of ABA-GE concentration change showed little difference (F = 0.36, P = 0.551) between the

244   two genotypes. Generally, the PA concentration was higher in genotype 233 than in genotype

245   299 (Fig. 8). The initial concentration of PA was 84 ng g^{-1} DW in genotype 233, and it

246   remained fairly consistent thereafter. Phaseic acid was only quantifiable at low concentrations

247   in genotype 299 during the first four weeks, ranging from 21 to 46 ng g^{-1} DW. The overall

248   pattern of PA concentration changes between these two genotypes was significantly different

249   (F = 49.03, P < 0.001). Dihydrophaseic acid was only quantifiable in the samples of genotype

250   233, with concentrations ranging from 9 ng g^{-1} DW to 21 ng g^{-1} DW at all time points except

251 that of week 6, where the level was below the limit of quantification (data not shown).

252 Dihydrophaseic acid was below quantifiable levels in all genotype 299 samples. Neither 7′-OH

253 ABA nor *neo*PA was quantifiable in either of the genotypes.

254

255 **Discussion**

256   The ratio of Z-type to iP-type cytokinins in lodgepole pine long-shoot buds differed

257 between genotypes of high and low cone yield. This supports previous studies in which

258 cytokinins were shown to regulate bud differentiation and shoot development in Norway

259 spruce (Bollmark et al. 1995; Chen et al. 1996) and radiata pine (Zhang et al. 2001; 2003). In

260 angiosperms, cytokinin receptors have different affinities for Z-type and iP-type cytokinins

261 (Spíchal et al. 2004; Romanov et al. 2006). Both cell fate and organ formation have been

262 associated with local concentration gradients of Z-type and iP-type cytokinins (Frugis et al.

263 2001). These hormones may also act as long-distance signals since Z-type cytokinins exist

264 predominantly in xylem sap whereas phloem sap mainly contains iP-type cytokinins (Corbesier

265 et al. 2003; Hirose et al. 2008).

266   There is some evidence that Z-type cytokinins may favour female cone bud

267 differentiation. The largest differences in the ratio of Z-type to iP-type cytokinins were seen

268 from the middle of summer to early September. In terms of development, this is no longer the

269 bud initiation stage, but corresponds to the early stages of differentiation. These ratio

270 differences disappeared by late in the growing season. In our study, the absolute amounts of Z-

271 type cytokinins were higher than iP-type cytokinins in the higher cone producer. Our results for

272   lodgepole pine accord with those of Morris et al. (1990) for Douglas-fir, in which

273   concentrations of Z-type cytokinins were also higher than iP-type cytokinins in female cone

274   buds and vegetative buds, but not male cone buds. Z-type cytokinins are derived from iP-type

275   compounds and not vice versa (Kakimoto 2003; Sakakibara 2006). Thus, the lower

276   concentrations of 2iP and iPA in the genotype with better cone production indicates a higher

277   capability for Z-type cytokinin synthesis. *Trans*-zeatin riboside and iPA were the major

278   cytokinins in both of the genotypes, whereas *t-Z-O*-Glu was only quantifiable in genotype 299,

279   a good cone producer. These three compounds were also found in Douglas-fir shoots with

280   differentiating cone buds (Kong et al. 2008; 2009).

281   Abscisic acid metabolism differed between the two genotypes. The ABA oxidation

282   pathway, which leads to DPA and PA, was more active in the low cone-producing genotype.

283   Kong and von Aderkas (2007) reported ABA utilization was genotype-dependent during

284   conifer somatic embryogenesis; genotypes that responded to ABA supplementation during

285   maturation converted more ABA into PA and DPA. Concentrations of ABA in developing

286   buds are also sensitive to physiological intervention. When trees are subjected to cone

287   induction treatments, such as GA injection, ABA concentration decreases (Kong et al. 2008).

288   Abscisic acid metabolism may also vary by the type of bud. In *Pinus tabulaeformis*, Bao and

289   Zheng (2005) found much higher ABA concentrations in female-sterile trees than in fertile

290   trees.

291   Cytokinins and ABA may play a role in regulating female cone differentiation but, to

292   date, studies are few and evidence is scant and mixed. In Douglas-fir, the lowest ABA

293   concentration was observed when bud primordia began to form (Kong et al. 2008; 2009). In

294   *Pinus tabulaeformis*, high levels of ABA have been correlated with female gametophyte

295   abortion in a female-sterile genotype (Bao and Zheng 2005). In other higher plants, cytokinin

296   levels increased during flowering induction (Lejeune et al. 1994; Corbesier et al. 2003). Higher

297   cytokinin concentrations were found in female gametophytes than in male gametophytes in

298   *Blechnum spicant* (Menéndez et al. 2009).

299   We have shown that a genotype that characteristically had high cone yield also had

300   much higher ratios of Z-type to iP-type cytokinins compared with a low cone yield genotype.

301   Most of the pronounced changes occurred before week 6. This period corresponds to female

302   cone bud differentiation on the basis of our previous structural study of lodgepole pine long-

303   shoot buds (von Aderkas et al. 2007). In addition, the dramatic increases of bud dry weight

304   between weeks 2 and 6 also indicate fast growth during cone bud differentiation and

305   development. Comparison of bud dry weight and moisture content between the high and low

306   yielding genotypes does not indicate a difference in the health of these trees. The buds in both

307   types grew equally well. The difference in cytokinin metabolism should be further investigated,

308   as this pathway may provide opportunities in developing new strategies for cone induction. For

309   example, cone bud gender in pines is known to be developmentally sensitive to exogenously

310   applied cytokinins (Wakushima 2004, Kong et al. 2011). To date, only adenine type cytokinins

311   have been used, e.g. 6-benzylaminopurine, with other more stable phenylurea types (e.g.

312   thidiazuron) untried. Cytokinins would appear to offer some yet unexplored possibilities in

313   female cone induction (Kong et al. 2011).

325

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Figure legends

448

Figure 1 Changes in dry weight of long-shoot buds during female cone differentiation in

genotype 299 (open circle) and genotype 233 (solid circle). Sample collection started on

August 1, Mean ± SE, n > 30.

Figure 2 Changes in moisture content of long-shoot buds during female cone differentiation in

genotype 299 (open circle) and genotype 233 (solid circle). Sample collection started on

August 1, Mean ± SE, n > 30.

Figure 3 Concentration of t-ZR in long-shoot buds during female cone differentiation in

genotypes 299 (open circle) and 233 (solid circle). Sample collection started on August 1,

Mean ± SE, n=3.

458 **Figure 4** Concentration of iPA in long-shoot buds during female cone differentiation in

459 genotypes 299 (open circle) and 233 (solid circle). Sample collection started on August 1,

460 Mean ± SE, n=3. Asterisk (*) indicates a significant difference ($P < 0.05$) between genotypes

461 at the individual time point.

462 **Figure 5** Ratio of Z-type cytokinins to iP-type cytokinins in long-shoot buds during female

463 cone differentiation in genotypes 299 (open circle) and 233 (solid circle). Sample collection

464 started on August 1, Mean, n=3. Asterisk (*) indicates a significant difference ($P < 0.05$)

465 between genotypes at the individual time point.

466 **Figure 6** Concentration of ABA in long-shoot buds during female cone differentiation in

467 genotypes 299 (open circle) and 233 (solid circle). Sample collection started on August 1,

468 Mean ± SE, n=3. Asterisk (*) indicates a significant difference ($P < 0.05$) between genotypes

469 at the individual time point.

470 **Figure 7** Concentration of ABA-GE in long-shoot buds during female cone differentiation in

471 genotypes 299 (open circle) and 233 (solid circle). Sample collection started on August 1,

472 Mean ± SE, n=3.

473 **Figure 8** Concentration of PA in long-shoot buds during female cone differentiation in

474 genotypes 299 (open circle) and 233 (solid circle). Sample collection started on August 1,

475 Mean ± SE, n=3. Asterisk (*) indicates a significant difference ($P < 0.05$) between genotypes

476 at the individual time point.

Table 1 Female cone production per ramet in lodgepole pine genotypes 299 and 233. Cone yield data was collected by Vernon seed orchard company (VSOC) during a three-year period before sampling. Mean ± SE, n = 6.

Genotype 299		Genotype 233	
Ramet	**Cone yield**	**Ramet**	**Cone yield**
BB91	267 ± 22	AA89	83 ± 8
P91	267 ± 44	BB63	38 ± 7
T67	208 ± 22	O93	60 ± 21
U103	300 ± 52	S66	108 ± 33
Y99	217 ± 22	S79	58 ± 8
O100	142 ± 22	V64	47 ± 3
Total	233 ± 17	**Total**	66 ± 8

Table 2 Concentrations (ng g^{-1} DW) of cytokinins in long-shoot buds during female cone differentiation in genotypes 233 and 299. Sample collection started on August 1, Mean ± SE, n=3. NQ stands for not quantifiable.

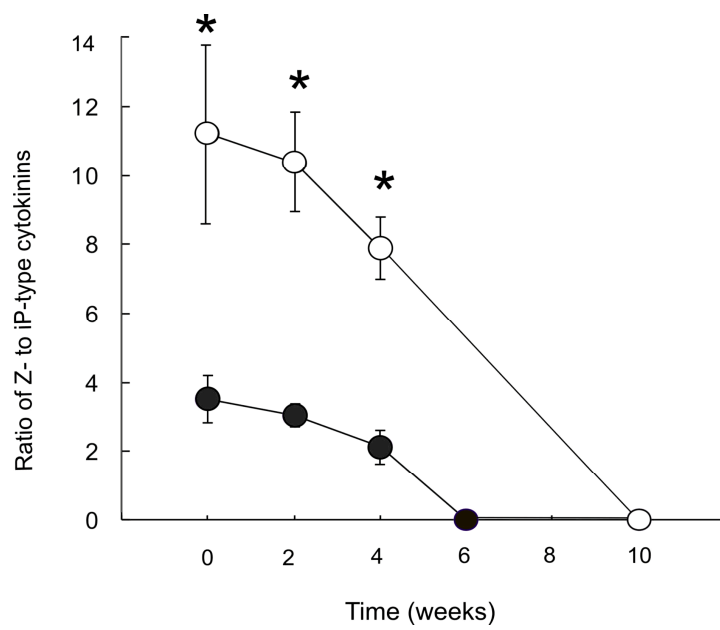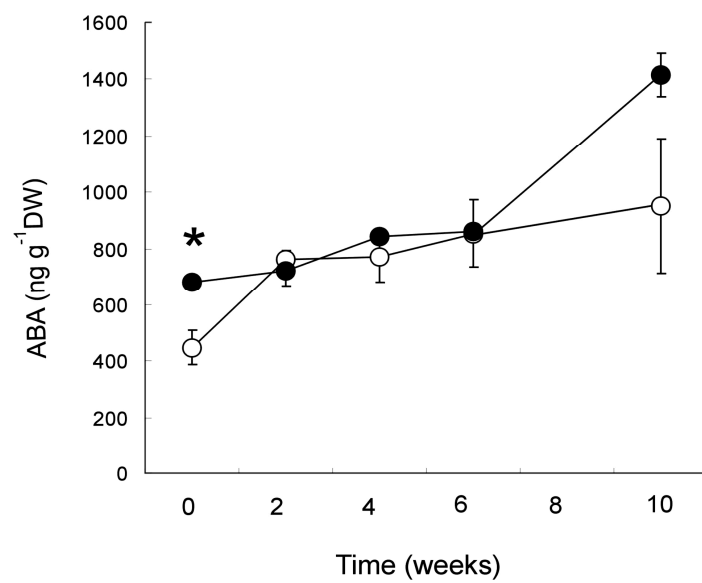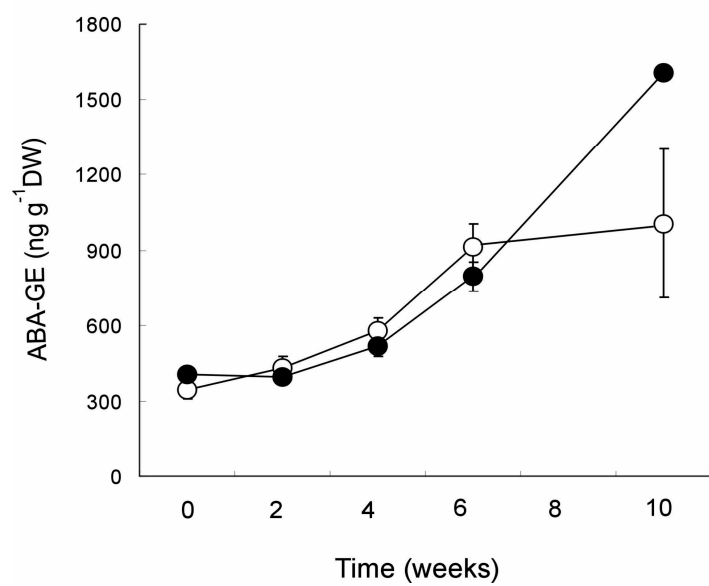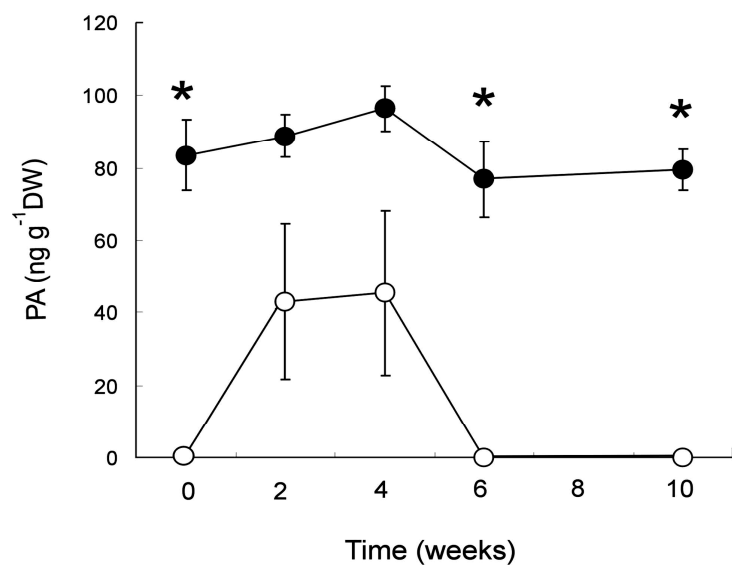| Week	dhZ		*t*-**Z**-*O*-**Glu**		2iP	
	233	**299**	**233**	**299**	**233**	**299**
0	12 ± 2	NQ	NQ	7± 3	15.3 ± 0.1	NQ
2	10± 2	NQ	NQ	7 ±4	18± 2	NQ
4	10± 1	NQ	NQ	10.1± 0.4	14± 2	NQ
6	NQ	NQ	NQ	7 ± 34	14 ±1	NQ
10	NQ	NQ	NQ	NQ	13 ± 1	6 ± 3

Figures
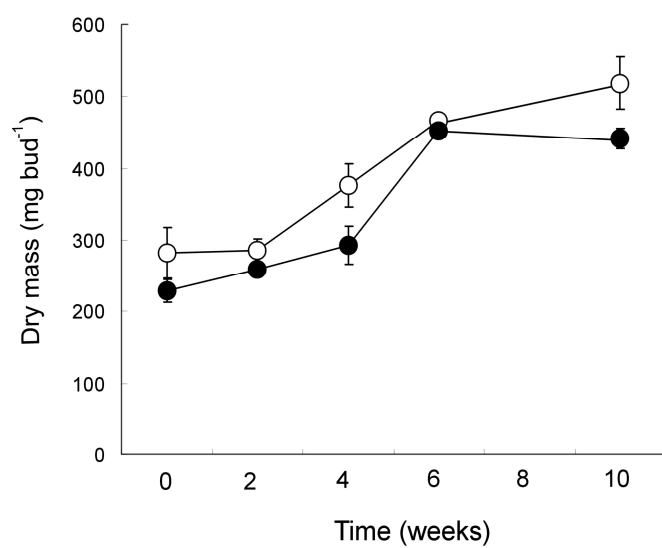
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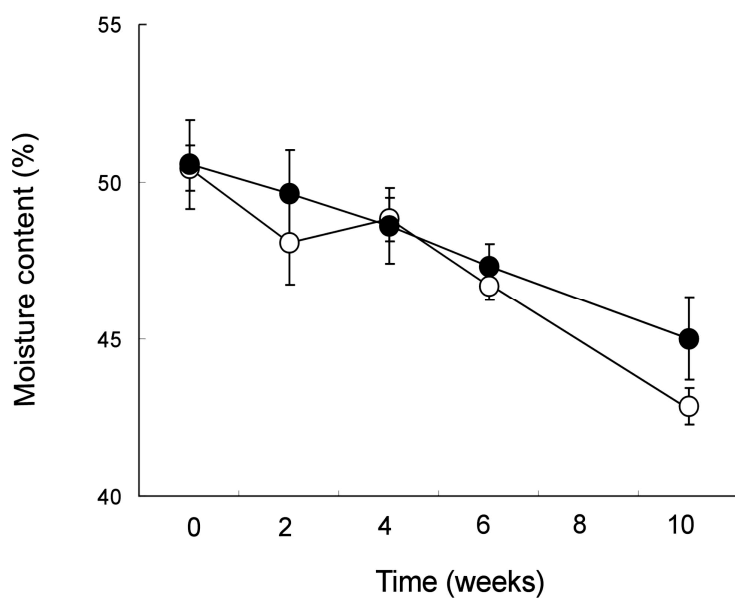
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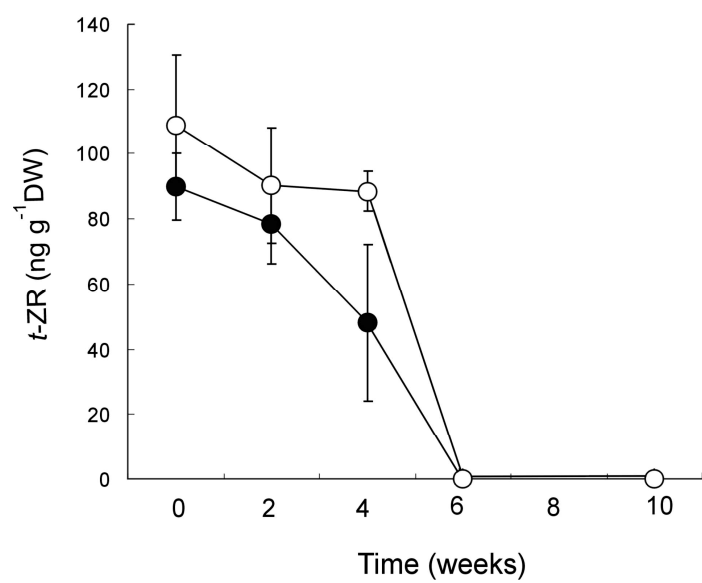
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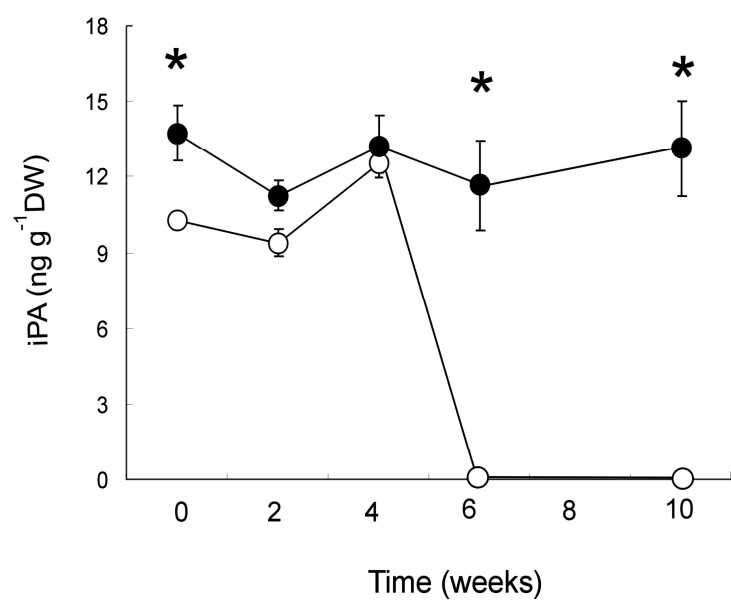
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