1   Title:

2   Phytohormone inhibitor treatments phenocopy brassinosteroid and gibberellin dwarf

3   mutant interactions in maize.

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26 **Abstract**

27 Phytohormone biosynthesis produces metabolites with profound effects on plant growth

28 and development. Modulation of hormone levels during developmental events, in

29 response to the environment, by genetic polymorphism, or by chemical application can

30 reveal the plant processes most responsive to a phytohormone. In many cases, chemical

31 inhibitors are applied and the levels of specific phytohormones are measured to determine

32 if, and which, phytohormone is affected by a molecule. In many cases, the sensitivity of

33 biochemical testing has determined multiple pathways affected by a single inhibitor.

34 Genetic studies are not subject to this problem, and a wealth of data about the

35 morphological impacts of hormone biosynthetic inhibition has accumulated through the

36 study of enzyme mutants. We previously identified a complex interplay between

37 brassinosteroid (BR) and gibberellin (GA) in maize, where the interdependence of the

38 two differs dependent on the developmental context. We found that: GA is required for

39 loss of BR to induce retained pistils in the tassel florets (POPIT); BR is required for the

40 loss of GA to induce tiller outgrowth; BR and GA are additive for plant height; BR has

41 no effect on the induction of anther retention in ear florets of GA mutants. In this work,

42 we sought to assess the specificity of three triazole inhibitors of cytochrome P450s by

43 determining their abilities to recapitulate the phenotype of double mutants. The GA

44 biosynthetic inhibitors uniconazole (UCZ) and paclobutrazol (PAC) were applied to the

45 BR biosynthetic mutant *na2* and all double mutant phenotypes were recovered in the

46 UCZ treatment. PAC was unable to suppress the retention of pistils in the tassels of *nana*

47 *plant2* (*na2*) mutant plants. The BR biosynthetic inhibitor propiconazole (PCZ)

48 suppressed tiller outgrowth in the GA biosynthetic mutant *dwarf5* (*d5*). All treatments

49 were additive with genetic mutants for effects on plant height. Due to additional

50 measurements done here but not in previous studies of the double mutants, we detected

51 new interactions between GA and BR biosynthesis affecting plastochron index and tassel

52 branching. These experiments, a refinement of our previous model, and a discussion of

53 the extension of this type of work are presented.

54

Introduction

       Phytohormones control plant growth and development at very low concentrations. The entire life cycle of plants are influenced by the availability and amount of these metabolites. The number of identified classes of phytohormones currently includes auxins, cytokinins (CK), abscisic acid (ABA), jasmonic acid, salicylic acid, strigolactones, brassinosteroids (BR), and gibberellins (GA).[1–3] The biosynthesis, transport, signaling, and responses to these phytohormones have been intensely studied in many species, including *Zea mays* (maize). Both biochemistry and genetic studies identified genes encoding phytohormone biosynthetic steps and mutants blocking hormone biosynthesis in many genetic model systems including *Pisum sativum* (pea), *Solanum lycopersicum* (tomato), *Arabidopsis thaliana* (Arabidopsis), and maize.[4–8] Despite these studies, the molecular identities of many steps are as yet unidentified. Furthermore, the roles of phytohormones in processes that do not occur in established genetic models are less well explored. Biochemical inhibitors, particularly the triazoles, have elucidated physiological effects of these phytohormones in plant systems that lack the expansive genetic resources of species with vibrant genetics research communities like Arabidopsis and maize. The utility of inhibitor treatments for determining the roles of phytohormones in plant processes is determined by the specificity of the biochemical inhibitors. A test of this specificity is available if we determine the concordance between phenotypes induced by reducing hormone biosynthesis via genetic ablation of biosynthetic enzymes and the phenotypes observed after chemical inhibition.

       A number of classical maize dwarf mutants are now demonstrated to encode GA and BR biosynthetic genes. Many mutants of maize affecting steps in gibberellin biosynthesis have been identified, including *ent*-copalyl synthase (*anther ear1*), *ent*-kaurene synthase (*dwarf5; d5*), CYP88A3 (*d3*), and GA3oxidase (*d1*).[9–13] In addition to reduced stature, GA biosynthetic mutants of maize exhibit retained anthers in the normally pistillate ear florets, increased tiller outgrowth, shortened and broadened leaves, and decreased primary tassel branching.[10] Maize mutants in three steps of BR biosynthesis are known, including disruptions of a Δ24-sterol reductase (*nana plant2; na2*), 5α-steroid reductase (*na1*), and CYP85A1/BR-6-oxidase (*brassinosteroid deficient1; brd1*).[8,14–17] In addition to dwarfism, the maize BR biosynthetic mutants have

86   presence of pistils in the tassel flowers (POPIT) and reduced tiller branch outgrowth.

87   Genetic interactions between BR and GA biosynthetic mutants were developmentally

88   specific and varied. Mutants in BR and GA were additive for plant height, indicating

89   independent effects on this trait. BR mutants were epistatic to GA mutants for tiller

90   outgrowth but GA mutants were epistatic to BR mutants for POPIT.

91   Specific and effective inhibition of GA and BR biosynthesis should recapitulate

92   the phenotypes of these three differing genetic interactions seen in the mutants.

93   Interpretation of inhibitor studies requires confidence in the specificity and amplitude of

94   inhibition caused by chemical treatment. Inhibitors of plant hormone biosynthesis are

95   widely used as plant growth regulators in industry and basic science to modify plant

96   metabolism and phenotype. Triazole PGRs are widely used, cheap to produce, and

97   effective on both plants and fungi. The chemical structures of three triazole inhibitors

98   containing the 1,2,4-triazole ring used in this study are shown in Figure 1. PGR

99   specificity within plants is known to be poor with wide impacts across P450s (Figure 2).

100   It is currently unknown how wide these impacts actually are.[18]

101   Propiconazole (PCZ) was first identified as a fungal growth inhibitor that blocked

102   the C-14 demethylation of lanosterol (Figure 1A).[19,20] Propiconazole is a specific and

103   potent inhibitor of BR biosynthesis, as evidenced by the reversal of growth inhibition by

104   the co-application of BR.[21] PCZ binds the CYP90D1 of Arabidopsis (Figure 2A) but it is

105   unknown whether PCZ also binds other P450s in BR biosynthesis such as

106   CYP90B1/DWF4, CYP90C1/ROT3, or CYP90A1/CPD.[22] The related BR biosynthesis

107   inhibitor, brassinazole, effectively binds to CYP90B1 and the effectiveness of these two

108   inhibitors may stem from an ability to simultaneously inhibit multiple CYP90/CYP85 BR

109   biosynthetic enzymes. Co-treatment of Arabidopsis with epi-brassinolide reversed the

110   phenotypic effects of both brassinazole and PCZ, suggesting that the effects of these

111   triazoles were predominantly due to BR inhibition.[21,23,24] PCZ is also used as a fungicide

112   in agriculture due to inhibition of ergosterol biosynthesis. It is possible that PCZ also

113   affects structural sterol biosynthesis in plants. To date, no experiments have been

114   conducted on PCZ and enzymes in other metabolic pathways to determine effects on

115   other phytohormones or sterol biosynthesis.

116   Paclobutrazol (PAC) was also first identified as a fungicide (Figure 1B).[25] In

117   fungi, it inhibits C-14 demethylation, similar to PCZ. In barley and celery cell cultures it

118   was shown to inhibit the 14α-demethylase CYP51 required for the conversion of

119   obtusifoliol to $\Delta^{8\text{-}14}$sterol (Figure 2A).[26–28] Supplementation of cell cultures with

120   stigmasterol at high concentrations, or a mix of cholesterol and stigmasterol, reversed the

121   growth retardation that resulted from PAC treatment of celery cell cultures. Ratios of

122   stigmasterol to sitosterol, a 22-desaturation carried out by CYP710A, were also altered in

123   PAC treated cells suggesting that this P450 may also be a target. The BR precursor

124   campesterol was also reduced by PAC treatment, and it may be that PAC achieves

125   growth retardation by affecting structural sterols as well as BR levels.[27] PAC is also

126   known to reduce GA levels by inhibiting the *ent*-kaurene oxidase/CYP701 in plants

127   (Figure 2B).[18] Hedden & Graebe[29] showed the percent conversion of the CYP701 targets,

128   *ent*-kaurene, *ent*-kaurenol, and *ent*-kaurenal, into later intermediates was greatly inhibited

129   by 1 µM (2RS, 3RS)-PAC in a cell-free system from *Cucurbita maxima* endosperm. The

130   different enantiomers of PAC have demonstrated differential effects on plants as

131   compared to fungi. The 2R-3R diastereoisomer of PAC affected sterol biosynthesis in

132   fungi but had no effect on plant height at 170.2 µM while the 2S 3S diastereoisomer

133   dramatically reduced plant height at 34.0 µM.[25] A number of other downstream effects

134   have been documented in PAC-treated plants including reduced accumulation of 1-

135   aminocyclopropane-1-carboxylic acid (ACC) and ethylene production in water-stressed

136   apple seedlings, however the mode of action is unknown.[30]

137   The uniconazole (UCZ) structure differs from PAC by one desaturation leading to

138   a double bond (Figure 1C) and has been shown to inhibit GA biosynthesis by affecting

139   the same enzymes (Figure 2B).[18,31,32] However, the height of a GA_3-insensitive DELLA

140   mutant in *Helianthus annuus* (sunflower) was further reduced by UCZ-treatment.[33] UCZ

141   has also been shown to affect structural sterol levels in *Oryza sativa* (rice) and pea

142   (Figure 2A).[34,35] In addition, UCZ treatment reduced castasterone accumulation in pea

143   and blocked BR-induced tracheary element differentiation in *Zinnia elegans* L.

144   mesophyll cells.[36,37] Whether these phenotypes result from BR-inhibition, the dependence

145   of BR-induced tracheary element differentiation on GA, or some other impact is

146   unknown. Recently, it has been shown that UCZ affects ABA catabolism by inhibiting

147   CYP707A in Arabidopsis and tobacco cell cultures (Figure 2D).[38–40] UCZ also affects

148   cytokinin biosynthesis; however, there are conflicting reports depending on the species

149   tested. In Arabidopsis it inhibited trans-Zeatin accumulation (Figure 2C) but UCZ

150   treatment in rice and *Glycine max* showed higher levels of trans-Zeatin.[41–43] Izumi et al[41]

151   showed that ethylene levels were higher but that ABA was unaffected in UCZ-treated

152   rice. Of the three PGRs used in this study, UCZ has been the most studied. The

153   conflicting reports on hormonal levels in different species highlights the necessity of

154   studying the specificity of PGRs within the species being tested due to potential

155   differences in uptake, transport, or binding affinity by potential target P450s.[18]

156   We sought to test the model for BR and GA interaction in maize derived from

157   genetics experiments.[17] Given the multiple interactions between the mutants it is less

158   likely that the same effects, and direction of interaction, will be generated by non-specific

159   growth retardation or general toxicity of a chemical treatment. In addition, using the

160   genetics experiments as orthogonal data confirming the utility of these inhibitors'

161   specificity in maize would permit stronger interpretation of inhibitor studies across the

162   grasses, including species without the genetic resources of maize. Orthogonal data, if

163   confirmatory, will also bolster our confidence in the interpretation of previous genetic

164   interactions between BR and GA discovered in maize.[17]

165   We treated the *na2-1* BR- and *d5* GA-biosynthetic mutants with each of the three

166   triazole inhibitors described above and measured phenotypes known to respond to BR

167   and GA reduction. All three inhibitors were additive with the two mutants for plant

168   height. UCZ had the strongest impact on plant height. Consistent with the expectation for

169   reduced BR biosynthesis, PCZ enhanced the POPIT phenotype of BR mutants but was

170   unable to induce POPIT in wild-type siblings or *d5* mutants at the concentrations

171   employed. PCZ application phenocopied the BR-GA interaction and suppressed the

172   outgrowth of tillers in *d5* mutants. Just like GA dwarfs, PAC treatment induced tillering

173   in wild-type plants. Unlike GA dwarfs, PAC treatment did not rescue the POPIT

174   phenotype of BR mutants nor did it induce anthers in ear florets. Only UCZ recapitulated

175   all GA dwarf phenotypes including the effects on floral organ persistence and interactions

176   with loss of BR biosynthesis, as UCZ treatment induced anther-ear in wild type and *na2-*

177   *1*.

Results

Effect of hormone biosynthetic inhibitors on *na2-1* and wild-type siblings

As described previously,[17] *na2-1* mutants overall height and organ lengths were shorter, lower leaves were more upright, mutants flowered later, and a majority of plants had POPIT. Mock-treated wild type and *na2-1* are shown in Figure 3 along with a PCZ-treated wild-type plant. PCZ treated wild types strongly resembled mock treated *na2-1* plants (Figure 3A-C). Treatment of *na2-1* plants with PCZ displayed an enhanced phenotype, indicating synergy between the loss of *na2* function and the effects of PCZ. Organs and internodes were shorter in PCZ-treated plants than in mock-treated plants for both *na2-1* and wild-type siblings (Table 1 and Figure 4A-B). PCZ treatment had a dramatic effect on *na2-1* mutant plant height indicating either bioactive BR accumulation in the mutant or non-specific inhibition of other growth-promoting metabolites by PCZ application. Primary tassel branch number was 10 fold lower in PCZ treated *na2-1* than in treated wild-type siblings, whereas mutants and wild types were indistinguishable in the mock treated samples (Table 1). Tassel length was also non-additively affected by PCZ application to *na2-1* mutants, causing a decrease by 40% in wild type and 80% in *na2-1* mutants. Just as *na2-1* mutants flowered later than wild types, PCZ treatment extended days to flowering in wild types. PCZ treatment of the mutants further enhanced this delay (Table 1). Yet, in neither comparisons between *na2-1* and wild type nor in PCZ treatments did the number of leaves per plant change. Taken together, PCZ reproduced all but the POPIT phenotype in wild-type treated plants and enhanced all loss-of-BR phenotypes in the *na2-1* mutant, including increased POPIT penetrance.

As expected if PAC primarily acts via the inhibition of GA biosynthesis, all organs and overall plant height were reduced by PAC treatment in both *na2-1* and wild-type siblings (Table 1 and Figure 4A,C). Treatment of wild-type siblings with PAC resulted in a significant increase in tiller outgrowth similar to the GA biosynthetic mutant *d5*. The mock-treated *na2-1* mutants did not tiller and *na2-1* suppressed the tiller outgrowth affected by PAC-treatment. Thus, PAC treatment phenocopied the *na2-1/d5* double mutants, where *na2* was required for tillering induced by a loss of GA. Unlike *na2-1/d5* double mutants, PAC did not suppress POPIT in *na2-1* mutants indicating that PAC treatment was insufficient to cause all of the changes in plant growth affected by

209   genetic loss of GA biosynthesis. Similar to the phenotypes of *d5* mutants,[17] PAC

210   treatment of wild-type siblings caused the lower leaf angle to become more upright.

211   Contrastingly, the upper leaf angles of wild-type siblings went the opposite direction and

212   were less upright following PAC treatment. As was the case for tillering, *na2-1*

213   suppressed the effects of PAC on leaf angle and no discernable difference between mock

214   and PAC treatments were observed. Similar to PCZ, primary tassel branch numbers of

215   *na2-1* plants were reduced by PAC treatments. Flowering time was increased by PAC

216   treatment of both *na2-1* and wild types. Surprisingly, PAC treatment reduced the number

217   of leaves before the top ear and the total number of leaves per plant in *na2-1* but not wild-

218   type siblings. This demonstrates that PAC reduced the plastochron index in *na2-1*

219   mutants. For all phenotypes except suppression of POPIT and induction of anther-ear,

220   PAC recapitulated the phenotypes observed in *d5* and *d1* mutants as well as the genetic

221   interactions of *d5* and *na2*.

222   Treatment with UCZ reproduced all of the effects of a loss of GA biosynthesis

223   and the genetic interactions between *d5* and *na2*. The effect of UCZ on organ length and

224   plant height of *na2-1* and wild-type siblings was similar to what was observed in *na2-*

225   *1/d5* double mutants, PAC, and PCZ treatments (Table 1 and Figure 4A, D). Consistent

226   with a strong inhibition of GA biosynthesis, UCZ caused ears of both *na2-1* and wild-

227   type siblings to exhibit florets with persistent anthers. Similar to *d5* mutants and PAC

228   treatment, UCZ treated wild types profusely tillered. Similar to the *na2-1/d5* double

229   mutants, and consistent with the genetic BR and GA interactions, *na2-1* strongly

230   suppressed UCZ-induced tillering. UCZ treatment reduced the frequency of POPIT in

231   *na2-1* by over two fold, similar to the suppression of POPIT in *na2-1/d5* double mutants,

232   but this difference was not statistically significant from mock treated *na2-1* mutants.

233   UCZ treatments had a synergistic effect on leaf width in combination with *na2-1* and

234   caused *na2-1* leaves to become narrower. UCZ resulted in a dramatic decrease in primary

235   tassel branch number, similar to the other inhibitors. UCZ treatment delayed the number

236   of days before tassel emergence in both mutants and wild-type siblings. Similar to PAC

237   treatment, both the number of leaves before the top ear and the total number of leaves per

238   plant were dramatically reduced in *na2-1*, but not wild-type siblings, by UCZ treatment.

239   Thus, for UCZ treatments this extreme delay without additional production of leaves

240   resulted in an increase in plastochron index in *na2-1* UCZ treated plants. Taken together,

241   UCZ treatments reproduced all of the phenotypes present in GA biosynthetic mutants and

242   consistently affected the same interactions with loss of BR biosynthesis observed in *na2-*

243   *1/d5* double mutants.

244

245   **Effect of hormone biosynthetic inhibitors on *d5* and wild-type siblings.**

246   Similar to what was found previously,[17] *d5* mutants flowered later, were shorter, had

247   shorter organs, exhibited persistent anthers in the ear florets, had reduced primary tassel

248   branch numbers, and tillered profusely. Shown in Figure 5 are mock-treated wild-type

249   and *d5* plants compared to PAC-treated and UCZ-treated wild-type siblings. Similar to

250   what was observed for the wild-type siblings of *na2-1*, PAC treatment largely

251   recapitulated the phenotypes affected by loss of function mutants in GA biosynthesis

252   (Figure 5A-C). At the concentrations employed, one out of sixteen wild types exhibited

253   anther-ear and the reduction in tassel length was less in PAC treated wild types than in

254   mock-treated *d5* mutants. PAC further reduced the height and organ lengths of *d5* plants

255   (Table 2 and Figure 6A, C). Similar to the effects of PAC on primary tassel branch

256   number in *na2-1* mutants, PAC reduced primary tassel branch number to zero in *d5*. The

257   *d5* mutants had greater leaf widths than wild-type controls. PAC treatment increased leaf

258   widths of wild types but PAC treated *d5* mutants had narrower leaves than mock treated

259   mutants. The *d5* mutants had the same number of nodes and, unlike PAC-treated *na2-1,*

260   treatment of *d5* mutants with PAC did not affect node number. PAC treatment delayed

261   flowering in wild-type siblings and further delayed flowering in *d5* mutants as compared

262   to mock treatments. Thus, a delay without increased leaf numbers suggests an increase in

263   plastochron index, though not as dramatic as the synergistic effects of inhibitor treatment

264   in *na2-1* mutants. All PAC treated phenotypes were consistent with a loss of GA, but the

265   low frequency of anther-ear, similar to the lack of suppression of POPIT in *na2-1*,

266   suggest that at this concentration PAC is a weak GA inhibitor in maize.

267   As was observed in applications of UCZ to *na2-1* and wild types, application of

268   UCZ to *d5* and wild-type siblings recapitulated all loss-of-GA phenotypes (Figure 5A-B,

269   D). Application of UCZ decreased plant height and the lengths of all organs and

270   internodes for both *d5* and wild-type siblings (Table 2 and Figure 6A, D). Similar to the

271   effects of UCZ on *na2-1*, UCZ narrowed both the upper and lower leaves of *d5* mutants.

272   Unlike PAC treatment, UCZ induced anthers in the ear florets of all wild-type siblings,

273   consistent with it effectively inhibiting GA biosynthesis at the concentration employed.

274   UCZ substantially reduced primary tassel branch number in *d5* mutants, just as it had for

275   *na2-1*. While not affecting an increase in the number of leaves produced per plants, UCZ

276   treatment increased days to tassel emergence for both *d5* and wild-type siblings. Thus,

277   UCZ also appears to increase flowering time though no synergism affecting node number

278   was observed between *d5* and UCZ, consistent with the mode of action affecting the

279   same pathway.

280   The BR inhibitor PCZ reduced overall plant height, internode lengths, and the

281   lengths of all measured organs for both *d5* and wild-type siblings (Table 2 and Figure

282   6A-B). Primary tassel branching decreased dramatically in both PCZ treated *d5* mutants

283   and their wild-type siblings. PCZ application at this concentration did not reproduce the

284   *na2-1* effect on POPIT in the wild-type controls and as expected from the *d5* suppression

285   of POPIT in *na2-1/d5* double mutants no POPIT was observed in PCZ treated *d5* plants.[17]

286   Consistent with the inability of *na2-1* to suppress anther-ear in *d5*,[17] PCZ treatment failed

287   to suppress persistence of anthers in ear florets of *d5* mutants. The *d5* mutants exhibited

288   increased tillering, which we previously demonstrated was dependent on BR.[17] PCZ

289   treatment suppressed tiller outgrowth induced by the loss of *d5*, just as *na2-1* suppressed

290   tiller outgrowth in *na2-1/d5* double mutants.[17] PCZ treatment increased the angle of the

291   upper leaf in *d5* mutants and the angle of the lower leaf in wild types. Days to tassel

292   emergence were greater in PCZ treated *d5* and wild-type siblings consistent with the

293   flowering delay in double mutants and PAC treatment of *na2-1* plants. Thus, PCZ

294   treatment of *d5* mutants and wild-type siblings delayed flowering, but no discernable

295   difference was observed in the total number of nodes. Different from treatment of *na2-1*

296   treated with PAC, synergism was not detected when *d5* was combined with PCZ

297   treatment at this concentration for effect of total node number.

298

299   **Discussion**

300   We report here the effects of PCZ, PAC, and UCZ treatment on the phenotypes of

301   *na2-1*, *d5*, and wild-type siblings at maturity. PCZ-treatment of wild-type plants was able

302 to recapitulate the phenotype of *na2-1*, except for the POPIT phenotype (Table 1 and

303 Figure 3B-C). However, PCZ at twice the concentration (500 µM) used in our study

304 induced POPIT in wild-type siblings of the *na1-1* mutant, which was in a unknown

305 mixed-parentage *Mu*-active genetic background.[8] Off-target and non-specific effects of

306 inhibitors are a greater concern as concentrations go up and we sought to minimize these

307 with lower concentrations. PCZ-treatment did decrease plant height and increase POPIT

308 in *na2-1* (Table 1). These results, and our unpublished observations that *brd1* mutants

309 and *na1/na2* double mutants are more severely dwarfed than *na1* or *na2* single mutants

310 suggests that disruption of this gene may not be a complete knock-out of brassinosteroid

311 biosynthesis. Alternative bioactive BRs can accumulate in rice brassinosteroid

312 biosynthetic mutants due to the existence of a bypass pathway to C29/28 and C27

313 bioactive BRs and the same alternative BR pathway may exist in maize.[44] Targeted

314 metabolic analysis of these alternative BRs in the maize mutants is required to determine

315 if this is the case.

316 UCZ treatment to wild-type plants was able to phenocopy *d5* mock-treated plants

317 and UCZ treated *na2-1* plants displayed all the interactions predicted for loss of both GA

318 and BR biosynthesis from *na2-1/d5* double mutant studies (Table 1 and 2). UCZ reduced

319 plant height, promoted tiller outgrowth, induced anther persistence in the ear, and

320 suppressed the *na2-1* mutant dependent POPIT (Table 1 and 2). These phenotypic results

321 are consistent with UCZ affecting maize growth via specific inhibition of GA

322 biosynthesis at the concentrations employed. However, the suppression of tiller formation

323 induced by a loss of *na2* was the least effective of all treatment and genetic combinations

324 observed (Table 1). Our experiments do not rule out inhibition of P450 enzymes outside

325 of GA biosynthesis. Indeed it was established that UCZ inhibits ABA catabolism in

326 Arabidopsis at concentrations lower than employed here and that specific inhibition of

327 recombinant CYP707A occurs in with an IC50 of 68nM (Figure 2D).[45] This reaction

328 contributes to the formation of phaseic acid which has been demonstrated to have

329 additional biological activities of its own (Figure 2D).[46,47] The perfect agreement of the

330 developmental phenotypes caused by UCZ and the GA mutants was somewhat surprising

331 in light of these additional modes of action (Figure 2A-D). It is possible that the weak

332 suppression of tiller formation by *na2-1* in UCZ treatments may result from inhibition of

333   other P450s. It is formally possible, though we have no evidence indicating this is the

334   case, that UCZ might inhibit P450s in strigolactone biosynthesis or that ABA might

335   enhance tillering in maize. The simplest interpretation of our results is that UCZ affects

336   these changes in development via inhibition of GA, for which there is ample evidence.[18]

337   Further research, particularly work comprehensively evaluating the binding and catalytic

338   inhibition by these compounds on isolated or recombinant P450s, as was done for ABA

339   catabolism and UCZ, is needed to test these hypotheses and establish the specificity of all

340   triazole inhibitors.

341   PAC treatment was unable to phenocopy all of the effects of GA deficient

342   mutants in maize. PAC treatment of wild type and *na2-1* did not promote anther retention

343   in the ear florets nor did it suppress POPIT in *na2-1* plants (Table 1). This contrasts with

344   both UCZ treatments and our previous genetic results.[17] The concentration of PAC used

345   was the same as UCZ, but it may be that PAC is a weaker inhibitor as has been

346   demonstrated in sunflower, *Impatiens wallerana*, *Salvia splendens*, *Tagetes erecta* L.,

347   and *Petunia hybrid*.[48,49] If PAC is effectively inhibiting GA biosynthesis, this would

348   contradict maize double mutant analyses where GA biosynthetic mutants were epistatic

349   to BR biosynthetic mutants for the expression of POPIT.[17] More likely the discordance

350   between PAC and genetic ablation resulted from incomplete inhibition of GA

351   biosynthesis by our repeated root drenches with 60 µM PAC. The effects of treatment on

352   height and stimulation of tiller outgrowth in wild-type plants, and suppression of

353   outgrowth by *na2-1*, matched the effects of reduced GA biosynthesis (Table 1 and 2).

354   The inability of PAC treatment to induce anther-ear in any genotype, except for *d5*, or

355   suppress POPIT in *na2-1* may be due to the concentration used, unspecific inhibition of

356   other pathways besides GA biosynthesis (Figure 2), or combination of the two. The

357   observation that PAC growth arrest can be reversed by application of sterols raises the

358   possibility that PAC achieves some height reduction by affecting both BR and GA

359   levels.[27] If BR biosynthesis is inhibited by PAC treatment this could increase the

360   penetrance of POPIT in the *na2-1* mutant, as was observed in PCZ treatment, and require

361   greater reduction in GA to prevent floral organ persistence. However, the lack of anthers

362   in ear florets in PAC treatments, which were observed in all maize GA mutants,

363   suggested that PAC might simply be a weaker GA biosynthesis inhibitor.

364   One unexpected finding was the effect of GA biosynthesis on tassel branch

365   numbers. The *d5* mutants had fewer primary tassel branches than wild-type siblings but

366   *na2-1* mutant primary tassel branch numbers were unaffected in both the results of this

367   study and our previous work (Table1 & Table 2).[17] Treatment of wild-type plants with

368   PAC or UCZ also reduced primary tassel branch numbers. Unlike the *na2-1* mutants,

369   PCZ treatment reduced tassel branch number, however the interpretation of these results

370   is complicated by the potential for genetic background effects. The wild-type background

371   of the *d5* mutants, but not the wild-type siblings of *na2-1*, had fewer primary tassel

372   branches following PCZ treatment. The *na2-1* mutants did not have fewer branches in

373   mock treatments but PCZ treatment of mutants did reduce tassel branch numbers. The

374   inability of PCZ to reduce tassel branching in *na2-1* wild-type siblings casts some doubt

375   into whether the full tassel branching phenotype of *na2-1* might be different in the

376   genetic background of our *d5* mutants. Residual BR biosynthetic activity in all *na2* alleles

377   is suggested by the stronger phenotype of *brd1* mutants and *na1/na2* double mutants

378   (Dilkes and Best, data not shown) and it may be that the residual BRs are sufficient to

379   maintain normal tassel branching in *na2-1* mutants.[16] Alternatively, PCZ might have

380   effects on regulators of tassel branching encoded by P450s outside of BR biosynthesis.

381   All triazoles affected branching in *na2-1* and *d5* mutants. In our previous work,

382   increasing GA by exogenous application to *na2-1*, *d5*, and wild type growing apices did

383   not alter tassel branch numbers.[17] Additional work determining the physiological basis of

384   triazole application on tassel branching may uncover as yet unknown impacts of GA in

385   tassel architecture, or implicate triazoles in the inhibition of other branch-promoting

386   signals.

387   We repeatedly treated plants with PGRs and this continuous treatment was

388   required to observe the dramatic decrease of plant height. This requirement suggests that

389   maize can catabolize or inactivate these PGRs over the course of a few days. Reduction

390   in the concentrations of bioactive PGRs should permit the biosynthesis of phytohormones

391   to return and promote growth in responsive cells. We ceased treatment at tassel

392   emergence during the elongation of the uppermost internodes and the lengths of these

393   internodes were not as affected in our treated plants (Figure 4). We propose that PGR

394   inactivation rather than phytohormone-independent growth underlies these differences in

395   internode responses.

396   Days to tassel emergence was significantly longer for *na2-1* and *d5* (Tables 1 and

397   2) compared to their wild-type siblings similar to our earlier work.[17] Wild-type siblings

398   treated with PCZ, PAC, or UCZ had greater days to tassel emergence than controls.

399   Every mutant-triazole combination further increased days to tassel emergence. When the

400   *na2-1* mutant was combined with PAC or UCZ treatment, this resulted in a synthetic

401   interaction producing fewer nodes even though plants took 2-3 weeks longer to flower.

402   The plastochron index is the rate of lateral organ initiation from the meristem. We

403   quantified total number of nodes and time to tassel emergence, so it is possible that we

404   could overestimate the effect of these treatments as decreased elongation may delay

405   emergence by a few days. However, the average increase of days to tassel emergence of

406   *na2-1* or *d5* treated with any one inhibitor was an average of 19.0 days or 13.4 days,

407   respectively. These results could be due to synergistic effects between BR and GA or

408   inhibition of another cytochrome P450 by the triazoles. Overexpression of the maize

409   *plastochron1* gene, which encodes cytochrome P450 CYP78A1, increases leaf number

410   but decreased plastochron index.[50] Loss of function of CYP78A1 exhibited slower leaf

411   elongation, but interpretation is limited by the presence of multiple CYP78A paralogs in

412   the maize genome.

413   The ability to combine chemical and genetic inhibition of phytohormone

414   biosynthesis to assess interactions and combinatorial effects is limited only by resource

415   availability. The discovery of compounds with additional modes of action would enable

416   work on the effects of all phytohormones on plant development. An additional triazole

417   was identified by Ito et al[51] that inhibits strigolactone (SL) biosynthesis. This inhibitor,

418   TIS13, effectively reduced SL levels in roots and root exudates of rice and increased tiller

419   outgrowth of the second tiller. In these tests, PAC and UCZ had no effect on SL levels in

420   root or root exudates, eliminating off-target inhibition of SL biosynthesis as a possible

421   side-effect of PAC and UCZ treatments in rice. SLs control tiller outgrowth in maize.[52] If

422   SL also affects tassel branching, we would expect treatment of maize with TIS13 or

423   GR24, a synthetic SL, to cause a decrease or increase of primary tassel branching,

424   respectively. It may be that loss of GA affects tillering and reduced tassel branching via

425   SL accumulation or signaling. A similar study to this one, using TIS3 application to GA

426   and BR biosynthetic mutants of maize as well as UCZ, PCZ, and PAC treatment of the

427   *carotenoid cleavage dioxygenase8* mutant of maize should provide a test of this

428   hypothesis.[52] Similarly, identification of specific inhibitors of cytochrome P450s in other

429   metabolic pathways would permit combinatorial assessment with available mutants to

430   explore metabolite function in maize.

431   In conclusion, PCZ treatment of wild-type siblings largely phenocopied *na2-1*

432   mutants and PAC or UCZ treatment predominantly phenocopied *d5* mutants. PAC did

433   not affect sexual organ persistence, unlike GA biosynthetic mutants or UCZ treatment.

434   There was no strong evidence of non-specific growth inhibition by these three triazole

435   inhibitors in maize. Unexpectedly, primary tassel branch number was reduced in *na2-1* or

436   *d5* treated with PCZ, PAC, or UCZ. Thus, either all three triazoles another pathway

437   controlling tassel branching or BR and GA biosynthetic pathways interact to affect tassel

438   branching. Plastochron index was synergistically increased by PCZ, PAC, or UCZ

439   treatment of both *na2-1* and *d5*. The mechanism for this is unknown, and we did not

440   measure flowering time in *na2-1*/*d5* double mutants in our previous study.[17] The

441   similarity of the effects on plant height, tiller outgrowth, and floral organ persistence in

442   the mutant analyses and inhibitor treatments were consistent with the genetic model

443   proposed previously.[17] The concordance of phenotypes increased the confidence of

444   interpretation for UCZ and PCZ as GA and BR inhibitors, respectively. Using these

445   inhibitors in the greenhouse via simple repeated pot drench will allow for rapid

446   assessment of phytohormone function, even in the absence of genetic resources.

447   Confidence in the interpretation of phenotypes resulting from inhibitor application should

448   encourage exploration of BR and GA effects on growth and development in grass species

449   such as *Sorghum bicolor*, *Panicum virgatum*, *Saccharum officinarum*, *Miscanthus*

450   *sinensis*, and *Setaria virdis*.

451

Materials and Methods

Plant growth and genetic materials

Maize was planted in the winter of 2013 at the Purdue Horticulture Plant Growth Facility in 2 gallon pots with 2:1 mixture of Turface MVP:peat germinating mix and irrigated with fertilizer (MiracleGro Exel at 200ppm). Supplemental light was provided by sodium lamps for a L:D cycle of 16:8 with target temperatures of 27 °C Day 21°C Nighttime. All experiments were planted as complete randomized blocks, controlling for genotype and treatment. Maize genotypes *na2-1*, *d5*, and their respective wild types were described previously.[17] Both mutants are recovered from segregating families. As such all comparisons are between mutants and wild-type siblings.

Inhibitor treatments

All inhibitors were supplied to plants as a soil drench. Inhibitors were dissolved in methanol and added to irrigant to a final concentration of 0.15% methanol, 250 µM propiconazole (94% purity; ORICO Global, Zhuhai, China), 60 µM uniconazole (95% purity; Seven Continent, Zhangjiagang, China), or 60 µM paclobutrazol (0.4%; formulated as *Bonzi*, Syngenta, Basel, Switzerland). Inhibitor concentrations were chosen based on soilless media inhibition of efficacy and to closely phenocopy respective genetic mutants.[53] A mock treatment consisting of 0.15% methanol was used as the control for all experiments. Plants were treated first at 10 days after germination (DAG) and on every subsequent 3rd or 4th day until tassel emergence. At the time of tassel emergence, treatments were stopped.

Phenotypic measurements and statistical analysis

In total, 256 plants were phenotyped for an array of growth and developmental parameters. Plant height was measured as the distance from the soil to the uppermost leaf collar. The width at the widest point and the length from the leaf collar to the tip of the blade was determined for the second uppermost leaf (one below the flag leaf) and the leaf below the uppermost ear. These are described as the upper leaf and lower leaf in the tables. Leaf angle was measured as the difference from a 90° projection from the stem and the abaxial side of the leaf; greater values indicate a more upright leaf. The number of nodes on each plant, the length of each node, the node of the uppermost ear, the number of tassel branches, and number of tillers per plant were all recorded at maturity

483   after stripping the plant of leaves. Tassels were inspected for POPIT and given a binary

484   yes/no score. Ears were inspected for anthers and given a binary yes/no score. Flowering

485   time was measured as DAG to visible tassel emergence from the leaf whorl. Statistical

486   analysis of differences between genotypes and between treatments for continuously

487   variable traits were performed by ANOVA with post-hoc pairwise tests by the Holm-

488   Sidak method implemented in Daniel's XL Toolbox add-in (ver. 7.2.6,

489   http://xltoolbox.sourceforge.net) for Microsoft Excel (Redmond, WA). For the binary

490   traits, differences were tested by Fisher's exact tests using a Bonferroni correction for

491   multiple tests that sets a corrected $p<0.05$ at the nominal $p<0.002$ as implemented in the

492   Excel add-in Real Statistics Resource Pack (ver. 3.3.1, http://real-statistics.com).[54]

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Figure 1. Structures of chemicals used in this study.

Chemical structure of (A) propiconazole, (B) paclobutrazol, and (C) uniconazole.

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Figure 2. All three plant growth regulators are known to affect more than one

pathway. (A) Brassinosteroid, (B) gibberellin, (C) cytokinin, and (D) ABA metabolic

pathways. Metabolites are indicated in black text and enzymes by black arrows with

respective names in grey text. Functionally characterized enzymes in maize are indicated

in parentheses. Previously identified enzymes inhibited by PCZ (yellow), PAC (purple),

and UCZ (green) are indicated by colored ovals.

515

Figure 3. Effects of *na2-1* or PCZ on maize plant architecture. Mock treated (A) wild-

type plant and (B) *na2-1* plant. (C) PCZ-treated wild-type plant.

518

Figure 4. Effects of plant growth regulators and genotype on internode length. Mean

internode lengths in cm and SD of (A) mock-treated, (B) PCZ-treated, (C) PAC-treated,

and (D) UCZ-treated *na2-1* and wild-type siblings.

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Figure 5. Effects of *d5*, PAC, and UCZ on maize plant architecture. Mock treated (A)

wild-type plant and (B) *d5* plant. (C) PAC-treated and (D) UCZ-treated wild-type plant.

525

Figure 6. Effects of plant growth regulators and genotype on internode length. Mean

internode lengths in cm and SD of (A) mock-treated, (B) PCZ-treated, (C) PAC-treated,

and (D) UCZ-treated *d5* and wild-type siblings.

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Table 1. Morphometric analysis of *na2-1* and heterozygous wild-type siblings treated with PCZ, PAC, and UCZ.

	Mock +/na2-1	na2-1/na2-1	+PCZ +/na2-1	na2-1/na2-1	+PAC +/na2-1	na2-1/na2-1	+UCZ +/na2-1	na2-1/na2-1
n	18	14	17	9	15	17	17	13
plant height[a]	175.9 ±22.1a	30.6 ±4.8b	24.5 ±6.3c	7.1 ±2.0d	39.4 ±11.9e	9.7 ±2.8f	18.2 ±5.3g	6.5 ±2.2d
days to tassel[a]	57.8 ±3.6a	67.9 ±2.8b	64.8 ±5.5b	86.6 ±13.9cd	67.3 ±4.6b	80.9 ±7.1c	80.2 ±5.1c	93.3 ±9.2d
tillers per plant[a]	0.3 ±0.7a	0 ±0a	0 ±0a	0 ±0a	3.1 ±0.7b	0.1 ±0.2a	4.5 ±0.8c	1.0 ±1.0a
plants with POPIT[b]	0 (0%)a	9 (64%)bc	0 (0%)a	9 (100%)b	0 (0%)a	13 (76.5%)bc	0 (0%)a	3 (23%)ac
plants with anthers in ears[b]	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a	17 (100%)b	13 (100%)b
tassel branches[a]	14.6 ±4.1a	12.4 ±4.7a	12.5 ±4.3a	1.1 ±1.5b	3.8 ±1.7c	0.7 ±1.4b	0.2 ±1.0b	0 ±0b
total nodes[a]	15.7 ±1.6a	14.9 ±1.2ab	15.9 ±1.2a	13.9 ±1.8abc	15.7 ±1.6a	13.1 ±1.2c	16.1 ±2.1a	13.4 ±1.5bc
node of top ear[a]	10.6 ±1.2a	10.2 ±1.5ab	10.5 ±1.4a	10.1 ±1.8ab	11.3 ±1.7a	8.9 ±1.3b	12.0 ±2.0a	8.9 ±1.4b
tassel length[a]	50.3 ±5.2a	28.4 ±5.9bc	31.3 ±6.6c	4.9 ±1.7d	39.8 ±7.3e	13.3 ±6.1f	22.5 ±7.1b	8.8 ±4.5df
angle of upper leaf[a]	45.0 ±27.0ab	65.7 ±17.4a	64.1 ±17.9a	89.4 ±1.4c	26.0 ±16.6b	62.9 ±24.4ac	54.1 ±22.7a	59.6 ±15.6a
length of upper leaf[a]	48.3 ±11.5a	21.6 ±11.9bc	20.0 ±8.4bc	13.1 ±4.0cd	25.3 ±7.2b	11.1±3.3d	13.5±3.5cd	5.7±1.0e
width of upper leaf[a]	5.9 ±1.1a	4.3 ±2.0abc	3.6 ±1.3bc	2.5 ±0.6cd	5.9 ±1.6a	3.4 ±1.3cd	4.8 ±1.3ab	2.2 ±0.7d
angle of lower leaf[a]	44.2 ±10.2a	64.6 ±10.3b	75.6 ±6.1c	67.2 ±4.4b	61.3 ±10.6b	59.4 ±15.1b	62.9 ±12.8b	59.6 ±16.0b
length of lower leaf[a]	99.8 ±9.3a	59.5 ±7.6b	56.9 ±8.4bc	18.4 ±2.9d	51.1 ±4.5c	26.3 ±5.9e	32.1 ±7.9e	16.4 ±6.2d
width of lower leaf[a]	8.7 ±1.0a	10.1 ±1.0a	9.1 ±1.6a	4.7 ±0.9b	11.4 ±1.1c	8.3 ±2.1ad	9.9 ±2.1a	6.5 ±1.4d

532
533 [a]Data are presented as means with SD. Lowercase letters indicate connecting letter report as determined by ANOVA with post-hoc
534 analysis using the Holm-Sidak algorithm with P < 0.05. [b]Number of plants with tassel seeds or anthers in ear florets, with the
535 percentage of plants in parentheses. Lowercase letters indicate connecting letter report as determined by Fisher's exact test with *P* <
536 0.01.
537

538 Table 2. Morphometric analysis of *d5* treated with PCZ, PAC, and UCZ.

539

	Mock		+PCZ		+PAC		+UCZ	
	+/d5	d5/d5	+/d5	d5/d5	+/d5	d5/d5	+/d5	d5/d5
n	16	16	16	16	16	16	16	16
plant height[a]	196.3±17.8a	33.8±7.3b	26.4±5.2c	12.2±2.7d	45.5±12.8e	10.1±1.9f	26.4±7.1c	6.5±1.3g
days to tassel[a]	52.5±4.4a	63.3±4.0bc	58.3±5.8d	72.9±5.6e	60.0±5.5bd	78.8±6.0e	66.4±4.5c	78.3±13.5e
tillers per plant[a]	0.3±0.4ab	3.1±1.4cd	0±0a	0.6±0.6b	2.9±1.2c	3.1±1.5c	5.2±2.3d	3.9±1.7cd
plants with POPIT[b]	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a	0 (0%)a
plants with anthers in ears[b]	0 (0%)a	16 (100%)b	0 (0%)a	15 (94%)b	1 (6%)a	16 (100%)b	16 (100%)b	16 (100%)b
tassel branches[a]	23.9±6.1a	8.9±4.1b	15.8±7.8c	3.8±3.0d	5.1±4.7d	0±0e	1.6±1.5f	0±0e
total nodes[a]	14.1±1.2a	14.4±2.1a	14.2±0.8a	14.9±2.0a	14.7±1.2a	15.0±2.3a	14.9±1.7a	13.9±2.2a
node of top ear[a]	8.6±1.3a	8.5±1.3a	9.6±1.1a	9.3±1.8a	9.8±0.8a	8.4±2.3a	9.5±2.0a	7.8±2.5a
tassel length[a]	63.3±4.7a	30.5±2.1b	41.2±12.9c	21.4±3.7d	55.5±7.0e	15.1±4.4f	37.3±6.9c	10.8±3.4g
angle of upper leaf[a]	52.0±23.2abc	39.7±16.3a	49.7±17.2ab	74.4±7.7d	46.3±14.2a	66.9±16.9bcd	51.6±25.0abc	72.5±12.9cd
length of upper leaf[a]	53.2±7.4a	28.9±6.5bc	29.4±10.8bc	19.6±5.2d	34.5±6.2b	10.0±2.5e	22.6±6.2cd	7.0±2.3f
width of upper leaf[a]	7.2±1.0abc	7.7±1.6ab	5.9±1.5c	6.5±1.3bc	8.0±1.0a	4.0±1.3d	6.2±1.3bc	3.6±1.0d

angle of lower leaf[a]	50.0±10.0a	63.8±6.5b	70.6±8.1b	71.6±8.3b	68.1±6.8b	69.7±7.6b	68.1±7.0b	68.8±11.2b
length of lower leaf[a]	107.9±8.7a	56.5±8.0b	57.3±9.4b	34.7±7.2c	52.3±9.3b	16.3±2.0d	34.4±7.1c	11.9±1.7e
width of lower leaf[a]	7.8±0.8a	10.0±1.3bc	9.8±0.8bc	10.2±1.4bc	10.6±0.9c	7.3±1.0a	9.2±1.4b	5.9±1.1d

540   [a]Data are presented as means with SD. Lowercase letters indicate connecting letter report as determined by ANOVA with post-hoc
541   analysis using the Holm-Sidak algorithm with P < 0.05. [b]Number of plants with tassel seeds or anthers in ear florets, with the
542   percentage of plants in parentheses. Lowercase letters indicate connecting letter report as determined by Fisher's exact test with $P <$
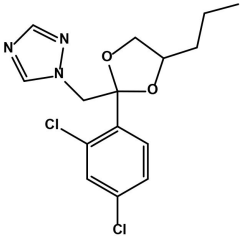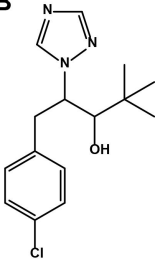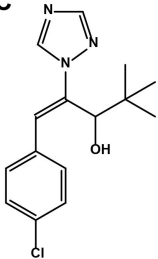543   0.01.

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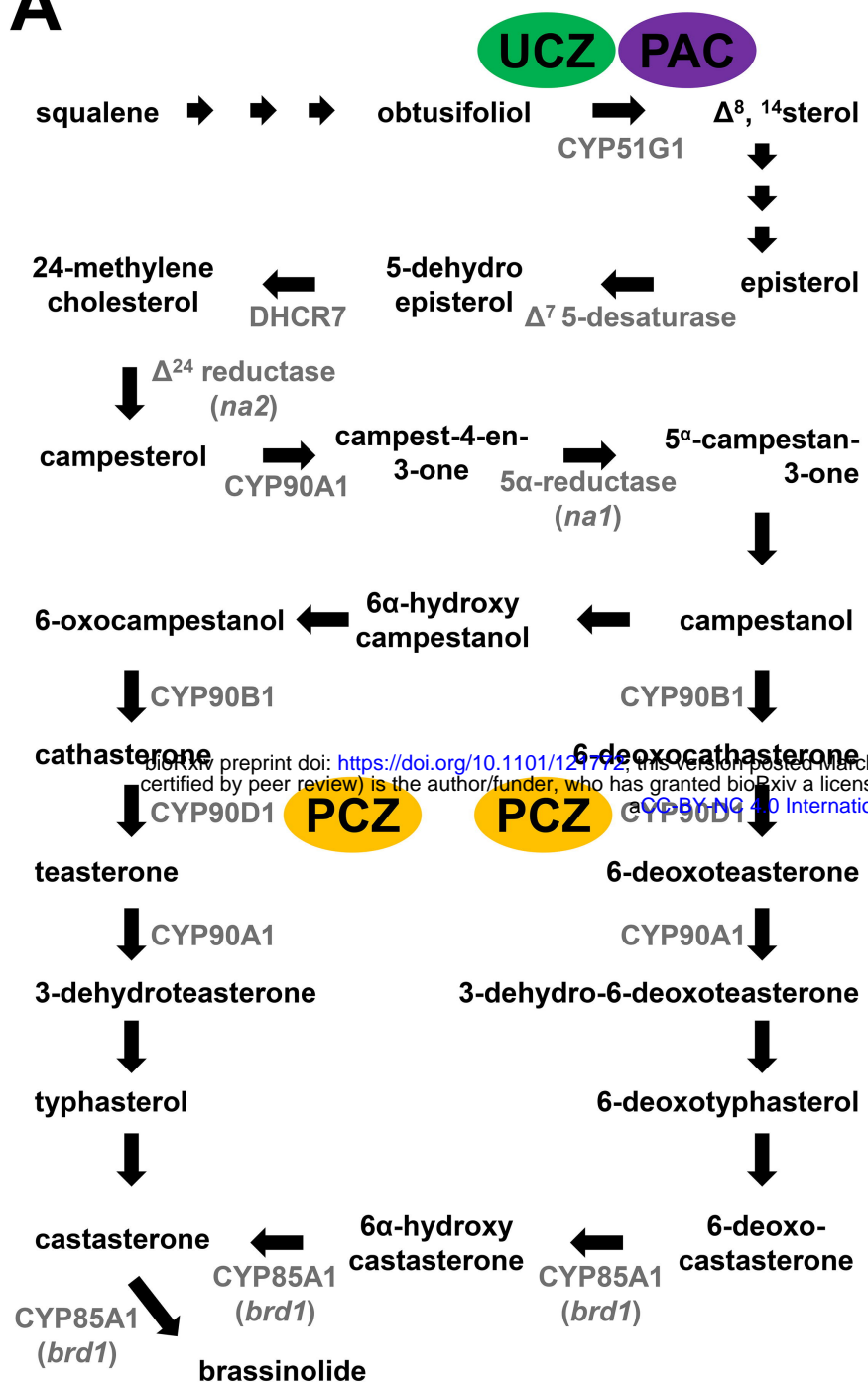607   25.   Sugavanam B. Diastereoisomers and enantiomers of paclobutrazol: Their

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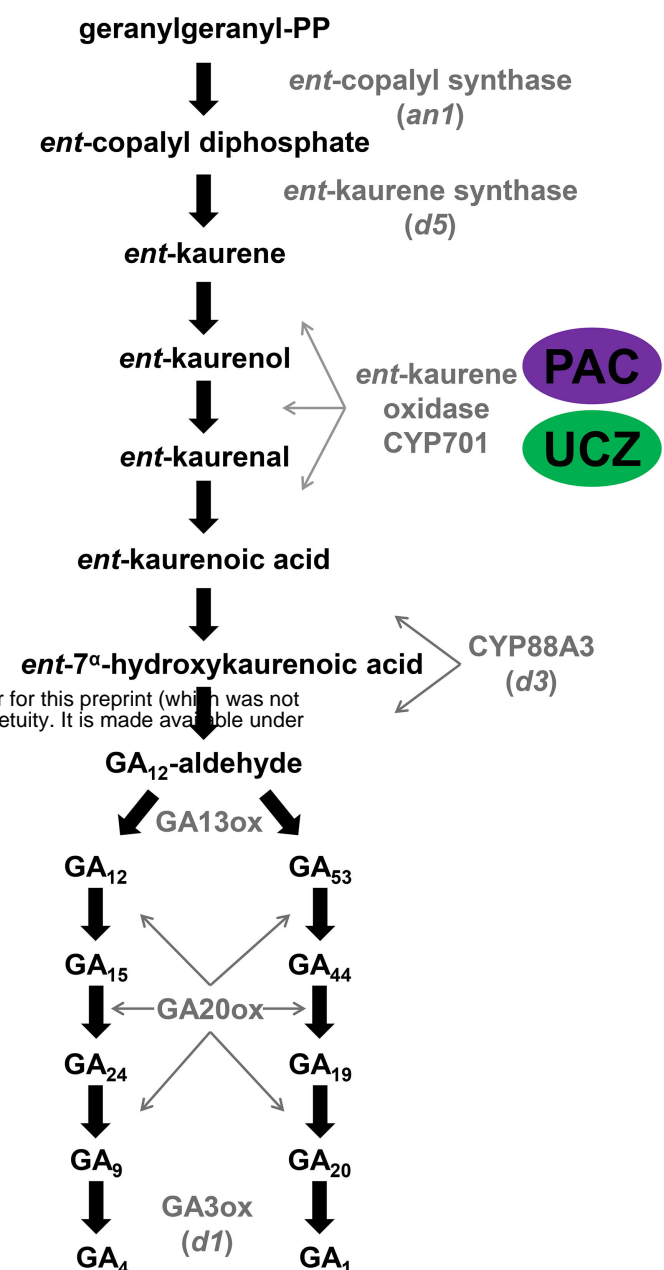
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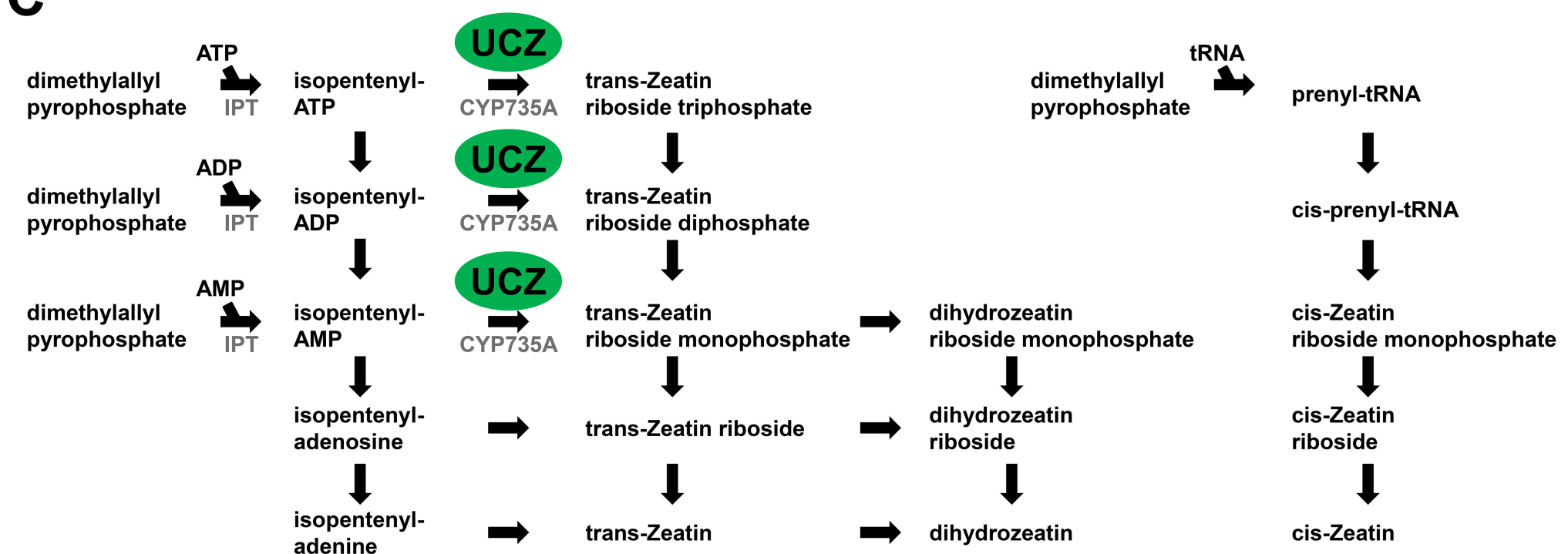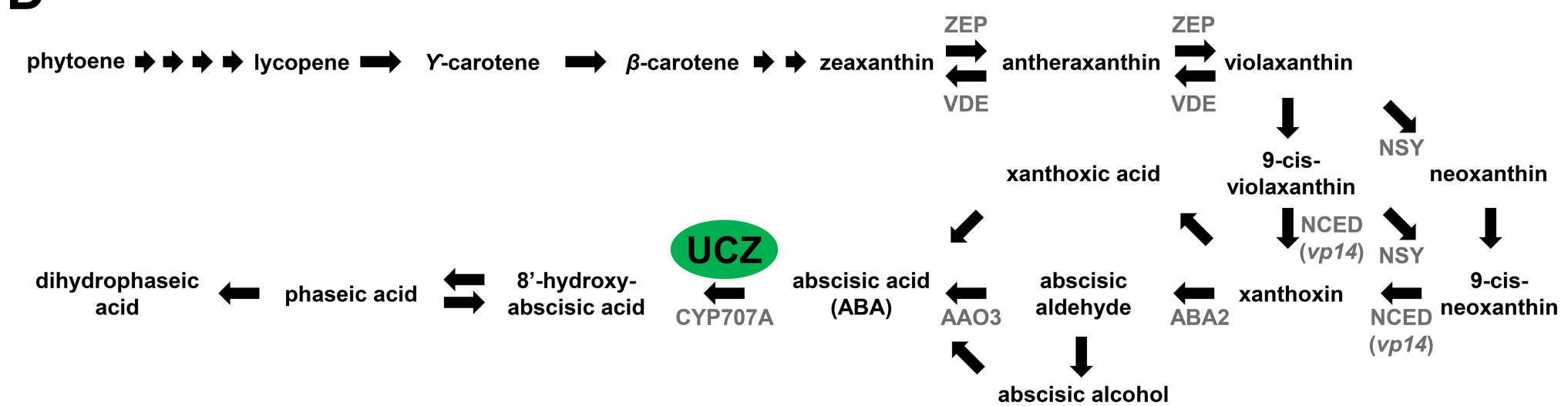698   2010.

A

B

C

A

squalene ➡ ➡ ➡ obtusifoliol ➡ Δ8, 14sterol

UCZ **PAC**

CYP51G1

Δ8, 14sterol ⬇⬇ epislerol

24-methylene cholesterol ⬅ 5-dehydro episterol ⬅ epislerol

DHCR7   Δ7 5-desaturase

Δ24 reductase (*na2*) ⬇

campesterol ➡ campest-4-en-3-one ➡ 5α-campestan-3-one

CYP90A1   5α-reductase (*na1*)

5α-campestan-3-one ⬇

6-oxocampestanol ⬅ 6α-hydroxy campestanol ⬅ campestanol

CYP90B1 ⬇   CYP90B1 ⬇

cathasterone   6-deoxocathasterone

CYP90D1 ⬇ **PCZ**   **PCZ** ⬇ CYP90D1

teasterone   6-deoxoteasterone

CYP90A1 ⬇   CYP90A1 ⬇

3-dehydroteasterone   3-dehydro-6-deoxoteasterone

⬇   ⬇

typhasterol   6-deoxotyphasterol

⬇   ⬇

castasterone ⬅ 6α-hydroxy castasterone ⬅ 6-deoxo-castasterone

CYP85A1 (*brd1*)   CYP85A1 (*brd1*)

CYP85A1 (*brd1*) ⬇

brassinolide

B

geranylgeranyl-PP

⬇ *ent*-copalyl synthase (*an1*)

ent-copalyl diphosphate

⬇ *ent*-kaurene synthase (*d5*)

ent-kaurene

⬇

ent-kaurenol

⬇ ⟷ *ent*-kaurene oxidase CYP701 **PAC** **UCZ**

ent-kaurenal

⬇

ent-kaurenoic acid

⬇

ent-7α-hydroxykaurenoic acid ⟷ CYP88A3 (*d3*)

⬇

GA12-aldehyde

⬇ GA13ox ⬇

GA12   GA53

⬇   ⬇

GA15 ⟷ GA20ox ⟷ GA44

⬇   ⬇

GA24   GA19

⬇   ⬇

GA9   GA20

⬇ GA3ox (*d1*)   ⬇

GA4   GA1

C

dimethylallyl pyrophosphate ➡ isopentenyl-ATP ➡ trans-Zeatin riboside triphosphate

ATP   **UCZ**
IPT   CYP735A

dimethylallyl pyrophosphate ➡ isopentenyl-ADP ➡ trans-Zeatin riboside diphosphate

ADP   **UCZ**
IPT   CYP735A

dimethylallyl pyrophosphate ➡ isopentenyl-AMP ➡ trans-Zeatin riboside monophosphate ➡ dihydrozeatin riboside monophosphate

AMP   **UCZ**
IPT   CYP735A

isopentenyl-adenosine ➡ trans-Zeatin riboside ➡ dihydrozeatin riboside

isopentenyl-adenine ➡ trans-Zeatin ➡ dihydrozeatin

dimethylallyl pyrophosphate ➡ prenyl-tRNA

tRNA

⬇

cis-prenyl-tRNA

⬇

cis-Zeatin riboside monophosphate

⬇

cis-Zeatin riboside

⬇

cis-Zeatin

D

phytoene ➡ ➡ ➡ lycopene ➡ *γ*-carotene ➡ *β*-carotene ➡ zeaxanthin

zeaxanthin ⟷ antheraxanthin ⟷ violaxanthin

ZEP   ZEP
VDE   VDE

antheraxanthin ⬇   violaxanthin ⬇ ➡ NSY ➡ neoxanthin

xanthoxic acid   9-cis-violaxanthin

⬇ NCED (*vp14*)

9-cis-violaxanthin ⬇ NCED (*vp14*)

neoxanthin ⬇ NSY

dihydrophaseic acid ⬅ phaseic acid ⬅ 8'-hydroxy-abscisic acid ⟷ abscisic acid (ABA) ⬅ abscisic aldehyde ⬅ xanthoxin ⬅ 9-cis-neoxanthin

UCZ   AAO3   ABA2   NCED (*vp14*)
CYP707A

abscisic alcohol