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Highlights

Ene reductase activity was detected in axenic plant cultures

Substrate scope suggests the activity is due to NAD(P)H-dependent flavin-independent ERs

Biocatalysts were used to transform chalcones into potentially bioactive derivatives

Plant tissue cultures as sources of new ene- and ketoreductase activities

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Abstract

While many redox enzymes are nowadays available for synthetic applications, the toolbox of ene-reductases is still limited. Consequently, the screening for these enzymes from diverse sources in the search of new biocatalyst suitable for green chemistry approaches is needed. Among 13 plant tissue cultures, *Medicago sativa* and *Tessaria absinthioides* calli, as well as *Capsicum annuum* hairy roots, were selected due to their ability to hydrogenate the C=C double bond of the model substrate 2-cyclohexene-1-one. The three axenic plant cultures showed more preference toward highly activated molecules such as nitrostyrene and maleimide rather than the classical substrates of the well-known Old Yellow Enzymes, resembling the skills of the NAD(P)H-dependent flavin-independent enzymes. When the three biocatalytic systems were applied in the reduction of chalcones, *T. absinthioides* showed high chemoselectivity toward the C=C double bond whereas the other two demonstrated abilities to biohydrogenate the C=C double bounds and the carbonyl groups in a sequential fashion.

Keywords: Ene-reductase, carbonyl reductase, chalcone, α,β-unsaturated compound, calli, hairy roots

Chemical compounds studied in this article: 2-cyclohexene-1-one (PubChem CID: 13594); 2-methyl-2-cyclopenten-1-one (PubChem CID 14266), 3-methyl-2-cyclopenten-1-one (PubChem CID: 17691); *N*-phenylmaleimide (PubChem CID: 13662); (*E*)-β-nitrostyrene (PubChem CID: 5284459); 1,3-diphenyl-2-propen-1-one (PubChem CID: 637760).

1. Introduction

Asymmetric bioreduction of activated C=C double bonds can generate up to two stereogenic centers, and therefore is one of the most widely employed strategies in chemo-enzymatic synthetic schemes for the production of enantiomerically pure compounds (Toogood *et al*., 2010). Ene-reductases (ER) catalyze the *trans*-hydrogenation of C=C double bonds conjugated with electron-withdrawing groups (EWG) such as carbonyl, nitro and ester.

ERs have been studied and classified according to their reaction mechanism, substrate profile and stereo- or enantioselectivity: (i) ERs from the Old Yellow Enzyme (OYE) family (E.C. 1.3.1.x), (ii) enoate reductase (EC 1.3.1.31), (iii) a family of medium chain

dehydrogenases/reductases (MDR) oxidoreductases (EC 1.3.1.-) and, (iv) short chain dehydrogenases/reductases (SDR) (EC 1.1.1.207-8) (Patel, 2016).

Most of the known ERs belong to the OYE family. These NAD(P)H-dependent flavin-containing enzymes can reduce activated alkenes and other functionalities such as nitrate esters, nitroglycerin, nitroaromatic explosives, and cyclic triazines (Toogood *et al*., 2012). Many homologous enzymes have been found in bacteria, yeasts, fungi and plants. A distinctive feature of OYE proteins is their promiscuous activity catalyzing the nitro group reduction of α,β-unsaturated nitroalkenes (Durchschein *et al*., 2013).

The NAD(P)H-dependent flavin-independent ERs (non-FMN ER) belonging to the SDR or MDR have also been described (Bougioukou and Stewart, 2008), but very little is known about their catalytic mechanisms (Durchschein *et al*., 2012). For example, a recent paper presents a detailed description of an ER from *Nicotiana tabacum* (NtDBR) member of the MDR superfamily, more specifically the leukotriene B4 dehydrogenase (LTD, MDR 002) subfamily (Mansell *et al*., 2013).

In recent years, some non-OYE ene-reductases have been isolated and identified from plants and mammalian cells (Gatti *et al.*, 2014). Toogood and Scrutton (2014) demonstrated that these enzymes require a higher degree of electronic activation on the substrates than OYEs. However, their ability to reduce the nitro group is completely absent, thus these enzymes might be useful for the synthesis of asymmetric nitroalkanes by chemoselective reduction of nitroalkenes.

Among the ERs, the most widely used as whole-cell biocatalysts are those belonging to fungi and bacteria due to the ease of cultivation. These enzymes have shown a broad substrate range and they have been applied successfully in several reduction processes (Winkler *et al*., 2012; Ohta *et al*., 1985; Silva *et al*., 2010). Nevertheless, it has been demonstrated that plant enzymes are also efficient biocatalysts in organic synthesis (Burda *et al*., 2009; Toogood *et al*., 2012 and ref. therein). An interesting example is the air-stable 12-oxophytodienoate reductase from tomato, used to prepare diverse enantiopure building blocks (Hall *et al*., 2008). Highly stereoselective ER activities have been reported in several plant cells, such as *Marchantia polymorpha*, *Medicago sativa, N. tabacum* and *Catharanthus roseus* (Stuermer *et al*., 2007) as well as in the plant isolated enzymes *At*DBR1, *At*OPR 1-3 and At5β-StR from *Arabidopsis thaliana* (Mano *et al.*, 2005; Costa *et al*., 2000; Burda *et al*., 2009) and, *Le*OPR-1 and *Le*OPR-3 from *Solanum lycopersicum* (Stueckler *et al.*, 2007). Recently, *Nt*DBR and two menthone dehydrogenases (MMR and MNMR from *Mentha piperita*) genes have been cloned and overexpressed in *Escherichia coli* and applied to the synthesis of (1*R*,2*S*,5*R*)-(−)-menthol and (1*S*,2*S*,5*R*)-(+)-neomenthol from pulegone (Toogood *et al.*, 2015).

In view of the uniqueness in their biosynthetic pathways and enzymatic promiscuity, plants are good candidates to be investigated in the search of new ERs in order to expand the enzyme toolbox for the reduction of activated alkenes. One of the main drawbacks of wild-type whole cells is the poor chemoselectivity of C=C versus C=O bond reduction due to the presence of ketoreductases (KRs). Nevertheless, they could be useful for alkene reduction since nicotinamide cofactors can be regenerated by cellular metabolism (Bougioukou and Stewart, 2012).

In the present work, we report the study of the ability of some plant cell cultures to reduce C=C double bonds of α,β-unsaturated compounds. With some biocatalysts in hand, we assessed their ability to hydrogenate several chalcone derivatives in order to explore their applicability in the production of bioactive compounds.

2. Material and methods

2.1. Substrates and standards

Substrates cyclohex-2-en-1-one (**1a**), cyclohexanone (**1b**), cyclohexanol (**1d**), 2-methyl-2-cyclopenten-1-one (**2a**) and 3-methyl-2-cyclopenten-1-one (**3a**) were purchased from Sigma-Aldrich Argentina S.A.

N-phenyl-maleimide (**4a**) and *N*-phenyl-succinimide (**4b**) were prepared by aldol condensation reaction with aniline and maleic or succinic anhydride, respectively, according to Fles *et al.* (2003). (*E*)-β-nitrostyrene (**5a**) was obtained by Knoevenagel condensation reaction using benzaldehyde and nitromethane (Vogel, 1996). 1,3-diphenyl-2-propen-1-one (chalcone) (**6a**) was synthesized according to standard procedures (Kohler and Chadwell, 1956). The A ring deuterated derivative of chalcone (**7a**) was prepared as previously reported (Ardanaz *et al.*, 1998).

For standards preparation, compounds **1a**, **2a**, **3a** and **6a** were treated with $NaBH_4$, in absence or presence of $CeCl_3$ in methanol to obtain the racemic mixtures of the corresponding saturated and allylic alcohols, respectively. Racemic **2b**, **3b** and **6b** ketones were prepared from by Jones oxidation ($CrO_3/H^+/H_2O$) of the totally reduced derivatives (Bodwen *et al.*, 1946).

(2-nitroethyl)benzene (**5b**) was prepared by reduction of **5a** with 2-phenylbenzimidazoline (Chikashita *et al.*, 1985).

All products were identified by NMR and GC–MS.

2.2. Biocatalysts

2.2.1. Undifferentiated cells (calli)

Plant calli were initiated from *Baccharis crispa*, *Daucus carota*, *Gardenia jasminoides*, *Grindelia pulchella*, *Capsicum annuum* and *Tessaria absinthioides* and maintained on Murashige Skoog (MS) agar (Murashige and Skoog, 1962) supplemented with sucrose (30 g L^{-1}), auxins and cytokinins at different ratios as previously reported (Orden *et al.*, 2008).

Friable calli from *Medicago sativa* and *Ocimun basilicum* were initiated from leaves and stems of *in vitro* germinated sprouts. Calli were maintained on MS solid medium supplemented with sucrose (30 g L^{-1}) and 20 µM indol-3-butyric acid and 0.18 µM 6-benzylaminepurine, under a 16-h light/8-h dark cycle by fluorescent lamps at an irradiance of approximately 1.8 W m^2.

2.2.2. Hairy root (HR) cultures

HR cultures were obtained from leaf and stem explants of *in vitro* germinated sprouts of *Brassica napus*, *Solanum lycopersicum*, *C. annuum*, *Cucumis sativus*, *N. tabacum* and *Raphanus sativus* by infection with *Agrobacterium rhizogenes* LBA 9402 as previously described (Orden *et al*., 2009). The HR cultures were maintained at 22 ± 2 ºC, in the dark on an orbital shaker at 120 rpm, and subcultured once a month to fresh liquid hormone free MS enriched with vitamins (MSRT) (Agostini *et al*., 1997) using an inoculum of 100-200 mg of roots.

2.3. *Biotransformation procedures*

The 20-day-old calli were mechanically disrupted by gently pressing them with a spatula under sterile conditions. The mashed biomass was transferred to flasks containing 20 mL of MS medium pH 5.7 (2.5 g of fresh weight per flask). Each substrate (50 µmol) dissolved in DMSO was added and incubation was carried out for 4 days on an orbital shaker at 120 rpm and 22 ± 2 ºC under a 16-h light/8-h dark cycle.

HR (4 tips) were transferred to 20 mL fresh MSRT medium and incubated at 120 rpm and 22 ± 2 ºC in the dark. After 30 days, the biomass was transferred to flasks containing 20 mL of MSRT medium pH 5.7 (4.5 g of fresh weight per flask) and substrates (50 µmol) dissolved in DMSO were added to the cultures and incubated for 4 more days.

Afterwards, in every case, the culture media were filtered and extracted with EtOAc (x3), the solvent dried over Na_2SO_4, filtered and removed *in vacuo*.

Blank assays without substrates and without plant cultures were carried out. For each experiment, three technical replicates were performed.

148

149 *2.4. Conversion rates analysis*

150 Biotransformation progress was monitored by GC-FID and the conversions rates were
151 calculated based on peak areas from the recovered material. The secondary alcohols were
152 derivatized by the addition of 200 µL of acetic anhydride and a catalytic amount of DMAP (4-
153 dimethylaminopyridine, 2 mg) to the organic phase. The reaction mixture was shaken in an
154 Eppendorf tube for 90 minutes and 130 rpm at room temperature. Afterwards, 400 µL of
155 water was added and the mixture was centrifuged (4 min). The organic phase was dried over
156 Na_2SO_4 anhydrous and analyzed by GC-FID on a Perkin-Elmer Clarus 500 instrument, equipped
157 with a chiral β-DEX-column Restek (30 m, 0.25 mm ID and 0.25 µm df), injector 200 ºC, FID 300
158 ºC and N_2 carrier gas (28 cm s^{-1}) for the cyclic enones **1a**, **2a** and **3a** and their bioreduction
159 products. For substrates **4a**, **5a** and **6a**, conversions were determined by GC-FID equipped with
160 a 007 methyl 5% phenyl silicone column (30 m, 0.25 mm ID and 0.25 µm df), injector 200 ºC,
161 FID 300 ºC and N_2 carrier gas (25 cm s^{-1}). The bioreduction products were identified by
162 comparison with the standards prepared as described above.

163

164 *2.5. GC-MS analysis*

165 EI-LRMS analysis of chalcones were performed at 70 eV using an ion trap (GCQ Plus)
166 with MSn (Finnigan, Thermo-Quest, Austin, TX, USA), operated at a fundamental rf-drive of
167 1.03 MHz. Helium was used as the damping gas at an uncorrected gauge reading of 6×10^{-5}
168 Torr. For the analysis of tandem mass spectrometric (MS/MS) product ions, the precursor ion
169 was selected using a MS/MS standard function, with a peak width of 0.5–1.0 m/z units, and
170 dynamically programmed scans. The supplementary voltage was in the range 0.5–1.0 V, as
171 previously described (Ardanaz *et al*., 1991). The alcohols obtained from chalcones were
172 analyzed as acetyl derivatives.

173 In order to validate the GC-MS analysis, the bioreduction of deuterated chalcone (**7a**)
174 by *M. sativa* calli was carried out.

175

176 **3. Results and discussion**

177 *3.1. Screening for ER activity*

178 Thirteen different plant tissue cultures from relevant agronomic and endemic species
179 of Argentina were tested for their ability to hydrogenate the C=C double bond of 2-cyclohexen-
180 1-one (**1a**) (Table 1) used as model compound to screen OYE activity (Lonsdale and Reetz,
181 2015). Due to the presence of both C=C and C=O bonds, **1a** was a suitable substrate to also

assess the chemoselectivity of the reaction, which is often poor in whole cells as a result of competitive endogenous KR activity. Four plant cultures, *C*. *annuum*, *M*. *sativa*, *G. jasminoides* and *T*. *absinthioides*, showed ER activity yielding **1b**. Small amounts of the alcohol **1d** were detected in the cases of *C. annuum* and *T. absinthioides* whereas *M. sativa* and *G. jasminoides* cells afforded **1d** in 8 and 12% conversion, respectively. The results observed with *M. sativa* are in agreement with the ER and KR activities toward (-)-carvone reported by Kergomard *et al*., (1988). The analysis of the blank assays confirmed that neither unspecific oxidation nor reduction of the substrate occurred.

Strassner *et al*. (1999) reported *Le*OPR activity from tomato. In our experiment, **1a** was not accepted by *S. lycopersicum* HR. This different behavior might be explained by changes in the gene expression levels in HR clones and untransformed plant tissues. It should also be considered that the alluded seminal work was performed with different tissues coming from *S. lycopersicum* (formerly known as *Lycopersicon esculentum*) cv. Castlemart II and the tomato HR clone used here was generated from *S. lycopersicum* cv. Pera.

The bioreduction of **1a** by *G. jasminoides* undifferentiated cells yielded **1b** (*c*: 8%) and **1d** alcohols (*c*: 12%), showing no chemoselectivity, although this is the first report of ER activity for this species.

3.2. Substrate scope for selected biocatalysts

In order to study both the substrate scope and the stereoselectivity of *C. annuum* HR, *T. absinthioides* and *M. sativa* calli as biocatalysts, we selected two isomeric cyclopentenones (**2a** and **3a**) to assess OYE activity. Also, the nitroolefin (**5a**) was selected to test non-flavin ER activity. Besides, *N*-phenyl-maleimide (**4a**) was chosen since it has been proposed as a good substrate for the two types of the ER enzymes (Gatti *et al*., 2014) (Figure 1). The results are summarized in Table 2. As a positive control, we performed the bioreduction of **2a** and **3a** with *Aspergillus fumigatus*, since OYE ER activity has been reported for this fungal species (Sortino *et al*., 2009) (experiments are described in the Supplementary Material).

The C=C double bonds of cyclopentenones **2a** and **3a** were poorly reduced by our plant systems. Low conversions of **3a** have been observed also with the ER enzymes *Le*OPR-1, YqjM from *Bacillus subtilis* (Hall *et al*., 2008) and TOYE from *Thermoanaerobacter pseudethanolicus* (Adalbjörnsson *et al*., 2010). On the other hand, neither allylic nor saturated alcohols were detected in these experiments, leading to assume that (competitive) KR activities did not occur. Table 2 shows that **2a** was better substrate than **3a**. Although *C. annuum* HR were the best biocatalysts, only 12% of the 2-alkylated derivative was bioreduced to **2b.** Additionally,

only a small amount of the ketone **2b** (6% conversion) was detected with *M. sativa* calli. On the other hand, *T. absinthioides* was not capable to transform these substrates.

The biohydrogenation of the C=C double bond of the maleimide **4a** by *C. annuum* HR and *T. absinthiodes* calli was very good (85 and 81%, respectively) after 4 days of reaction. Moreover, *M. sativa* calli yielded **4b** in 99% conversion. In this regard, other researchers have also reported high conversions when using *N. tabacum* and *C. roseus* calli as biocatalysts (Hirata *et al*., 2004). Interestingly, the ER activities toward **4a** were much higher than those observed for **1a** (5-folds), which indicates the preference of our biocatalysts toward activated substrates as well as their ability to accept bulky alkenes.

(*E*)-β-nitrostyrene (**5a**) was also well-accepted by *C. annuum* HR and *M. sativa* calli. After 2 days of biotransformation, conversions reached values higher than 70%. No secondary reduction products such as 2-phenylethan-1-amine or 2-phenylethanal, which might be obtained by some OYEs, were detected throughout the 4 days of bioreduction. These results led us to think that *C. annuum* HR and *M. sativa* calli present substrate profiles corresponding to the non-flavin ERs, since the best activities were obtained on nitroalkenes rather than enones (Durchschein *et al*., 2012). By performing preliminary homology searches, we found both types of ERs –OYE and non-flavin- in the genomes of *C. annuum* and *M. truncatula*, closely related species to *M. sativa* (data not shown). Therefore, we were unable to confirm which kind of ER would be responsible of the observed activity in both species.

3.3. Application of selected plant biocatalysts in the bioreduction of chalcones

Due to the well-known bioactivities of chalcones, many authors have reported their biotransformations with microorganisms. A huge number of metabolites have been reported because of their diverse enzymatic repertoire, catalyzing cyclizations, *O*-demethylations, reductions, oxidations and hydroxylations (Corrêa *et al.*, 2011). Conversely, there are only a few examples concerning C=C double bond biohydrogenations to obtain dihydrochalcones and the C=O reduction to yield *sec*-alcohols (Janezco *et al.*, 2013; Salokhe *et al.*, 2010, Gall *et al*., 2014).

Considering this background, we decided to test the ability of *C. annuum* HR and *T. absinthioides* and *M. sativa* calli to perform the reduction of chalcone **6a**. After 4 days of biotransformation, these cultures were able to reduce the C=C double bond as well as the carbonyl group, furnishing the ketone **6b** and the saturated alcohol **6d** as major products (Table 3). These results suggest that these plant cell cultures exhibit ER and KR activities in a sequential fashion. Initially, an ER would catalyze the reduction of the activated C=C double

bond of **6a** to give **6b** which would be subsequently reduced to **6d** by a KR. In order to test this hypothesis, we decided to carry out a time course analysis of the biotransformation of **6a** by these cultures (Figure 2).

The biohydrogenation of **6a** was chemoselective with *T. absinthioides* calli, yielding the dihydrochalcone **6b** (c: 38%) and a small amount of the alcohol **6d** after 4 days. Due to the negligible KR activity observed in this study, this biocatalyst is promising to chemoselectively reduce the C=C double bond of chalcones so as to prepare dihydrochalcones.

Besides, in the experiments carried out with *C. annuum* HR, **6b** was the first metabolite detected due to the early ER activity. Then **6b** was reduced to **6d** by KR activity. A similar behavior was observed with *M. sativa* calli, although it was also detected KR activity toward **6a**. In fact, a low percentage of the allylic alcohol **6c** (8%) was detected at the second day of biotransformation. However, this metabolite decreased rapidly and disappeared after 96 h of reaction. In order to understand this, **6c** was chemically prepared and added as substrate to *M. sativa* calli. After 4 days, only 44% of **6c** was recovered untransformed and neither **6b** nor **6d** were detected. This could be explained considering that these cells might redirect the allylic alcohol **6c** to others metabolic pathways different to the ER-mediated-reductions.

Even though *T. absinthioides* calli was the only chemoselective biocatalyst as regard ER activity, a new KR activity was detected in both *C. annuum* HR and *M. sativa* calli. This is an interesting result, as only few bioreductions on bulky-bulky ketones catalyzed by wild type cells have been reported, being bacteria and yeasts the main organisms (Lavandera *et al*., 2008). In particular, *M. sativa* could accept the ketone **6a** which is flanked by two bulky phenyl substituents, and thus producing steric hindrance in most of the KR enzymes (Kroutil *et al*., 2004). When ketone **6b** is desired, a further chemical oxidation step of alcohol **6d** could be added to the process (Matsumoto *et al.*, 1998).

3.4. Bioreduction of a series of substituted chalcones by M. sativa calli

Based on the results of the chalcone **6a** biotransformation, we selected *M. sativa* calli to explore the potential of KR activity on a series of substituted chalcones whose derivatives are particularly interesting as building blocks in the synthesis of pharmaceuticals (Salokhe *et al.*, 2010). A work on the biohydrogenation of these compounds using *Saccharomyces cerevisiae* in a biphasic system has been reported (Silva *et al.*, 2010).

M. sativa calli were able to hydrogenate the C=C double bonds as well as the carbonyl groups producing the 1,3-diarylpropanols from all the tested chalcone derivatives. The conversion values were good to excellent and it seems not to depend on the substituent nature (Table 4).

No significant differences were observed among the derivatives substituted with EWG and electron-donating-groups (EDG), although 100% was reached with only the methylated derivative.

The conversions of the fluorinated and chlorinated derivatives (**11a** and **12a**) were higher than 90% after 4 days, while the bioreduction of brominated **13a** just achieved 51% conversion. This result is in agreement with the observations reported by Otha *et al*. (1985) in a study of the chemoselective reduction of substituted chalcones by *Corynebaterium equi* IFO 3730. These authors suggest the reaction could be influenced by steric rather than electronic factors.

3.5. Prospects and scope

The study of *in vitro* axenic plant cell and tissue cultures is a valuable strategy to detect biocatalytic activities which could contribute to expand the enzyme toolbox to perform specific chemical reactions, such as the C=C and C=O double bonds reductions. It also offers an alternative to screen natural diversity and develop enzymatic platforms. Furthermore, these wild-type whole cell systems are applicable to prepare building blocks and selectively transform interesting compounds (e.g. the chalcone derivatives here reported) by eco-friendly methodologies.

4. Conclusions

Ene-reductase activity was detected in axenic plant cell tissues. According to the substrate scope, this activity may be attributed to NAD(P)H-dependent flavin-independent ERs rather than Old Yellow Enzymes. *T. absinthioides* calli are capable of reducing the activated C=C double bonds of bulky substrates in a chemoselective fashion, meanwhile *M. sativa* calli and *C. annuum* hairy roots show both ER and ketoreductase activity toward different α,β-unsaturated carbonyl compounds. The new biocatalytic systems were successfully applied in the selective transformation of chalcones into potentially bioactive derivatives.

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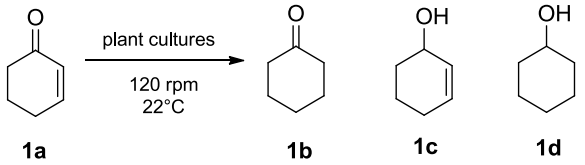
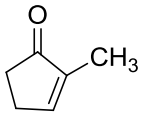
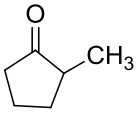
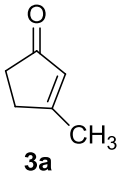
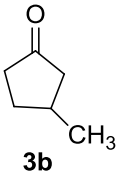
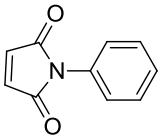
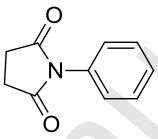
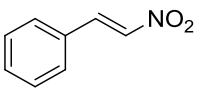
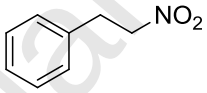
Table 1. Bioreduction of **1a** (50 µmol) by *in vitro* plant cell and tissue cultures after 4 days



Entry	Biocatalyst	*c* (%)[a]			Total ER activity (%)	Specific conversion (%)[b]
		1b	**1c**	**1d**		
Hairy roots						
1	*B. napus*	2	-	1	3	0.49
2	*C. annuum*	12	-	2	14	3.12
3	*C. sativus*	1	-	1	2	0.27
4	*N. tabacum*	1	-	1	2	0.54
5	*R. sativus*	2	-	1	3	0.62
6	*S. lycopersicum*	-	-	-	-	-
Undifferentiated cells						
7	*B. crispa*	1	-	1	2	0.41
8	*B. crispa*[c]	1	2	3	4	1.86
9	*D. carota*	2	3	1	3	1.16
10	*G. jasminoides*	8	-	12	20	8.06
11	*G. pulchella*	2	-	1	3	1.21
12	*M. sativa*	20	-	8	28	11.12
13	*T. absinthioides*	4	-	1	5	2.02
14	*T. absinthioides*[c]	7	-	1	8	3.06
15	*O. basilicum*	1	-	-	1	0.22

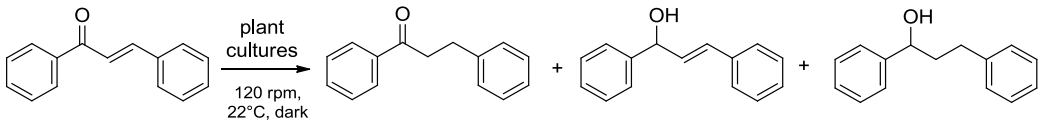
[a]Conversion determined by GC-FID. [b][total ER activity/g of fresh weight] [c]Plant cells grown in the dark.

Table 2. Bioreduction of activated alkenes by plant cultures

Entry	Biocatalyst	Substrate	Product	*c* (%)[a]	Specific conversion (%)[b]
1	*C. annuum*			12	2.76
2	*T. absinthioides*			-	-
3	*M. sativa*			6	2.53
4	*A. fumigatus*	**2a**	**2b**	86	43.2
5	*C. annuum*			4	0.97
6	*T. absinthioides*			-	-
7	*M. sativa*			-	-
8	*A. fumigatus*	**3a**	**3b**	1	0.66
9	*C. annuum*			85	18.91
10	*T. absinthioides*			81	32.46
11	*M. sativa*	**4a**	**4b**	99	39.56
12	*C. annuum*			75	16.75
13	*T. absinthioides*			2	0.94
14	*M. sativa*	**5a**	**5b**	77	30.75

[a] Conversion determined by GC-FID. [b][total ER activity/g of fresh weight]. Substrate conc.: 50 µmol. Bioreaction time: 4 d (in the dark).

Table 3. Bioreduction of **6a** by *C. annuum* hairy roots and *T. absinthioides* and *M. sativa* calli after 4 days

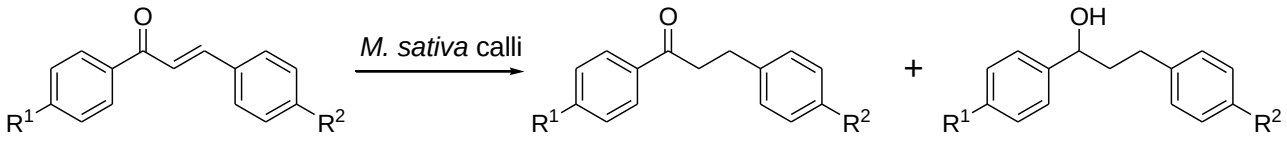


6a **6b** **6c** **6d**

Entry	Biocatalyst	**6b** *c* (%)[a]	**6c** *c* (%)[a]	**6d** *c* (%)[a]	Total ER activity (%)	Specific conversion (%)[b]
1	*C. annuum*	19	1	30	49	11.19
2	*M. sativa*	4	-	83	87	34.39
3	*T. absinthioides*	38	-	-	38	15.33

[a]Conversion determined by GC-FID and GC-MS. [b][total ER activity/g of fresh weight].

Table 4. Bioreduction of a series of substituted chalcones by *M. sativa* calli after 4 days

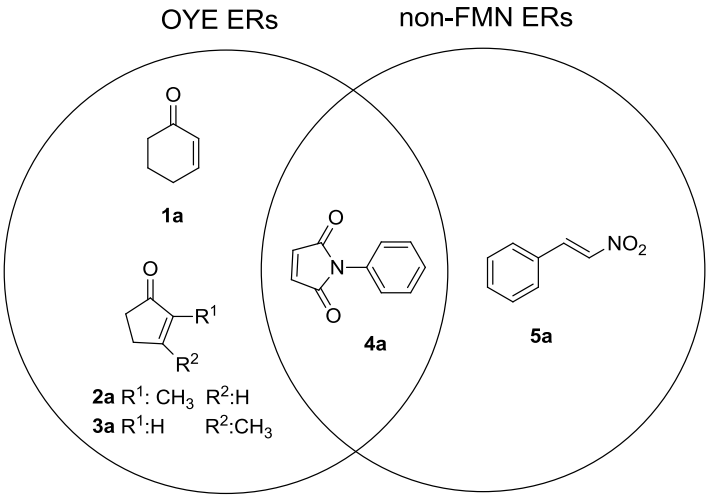


8b-13b **8d-13d**

8a: R^1 = H R^2 = OCH_3
9a: R^1 = CH_3 R^2 = H
10a: R^1 = OCH_3 R^2 = H
11a: R^1 = F R^2 = H
12a: R^1 = Cl R^2 = H
13a: R^1 = Br R^2 = H

Entry	Substrate	**b** *c* (%)[a]	**d** *c* (%)[a]	Total ER activity (%)
1	**8a**	18	65	83
2	**9a**	28	72	100
3	**10a**	15	67	82
4	**11a**	11	82	93
5	**12a**	21	71	92
6	**13a**	9	42	51

[a]Conversion determined by GC-FID and GC-MS.



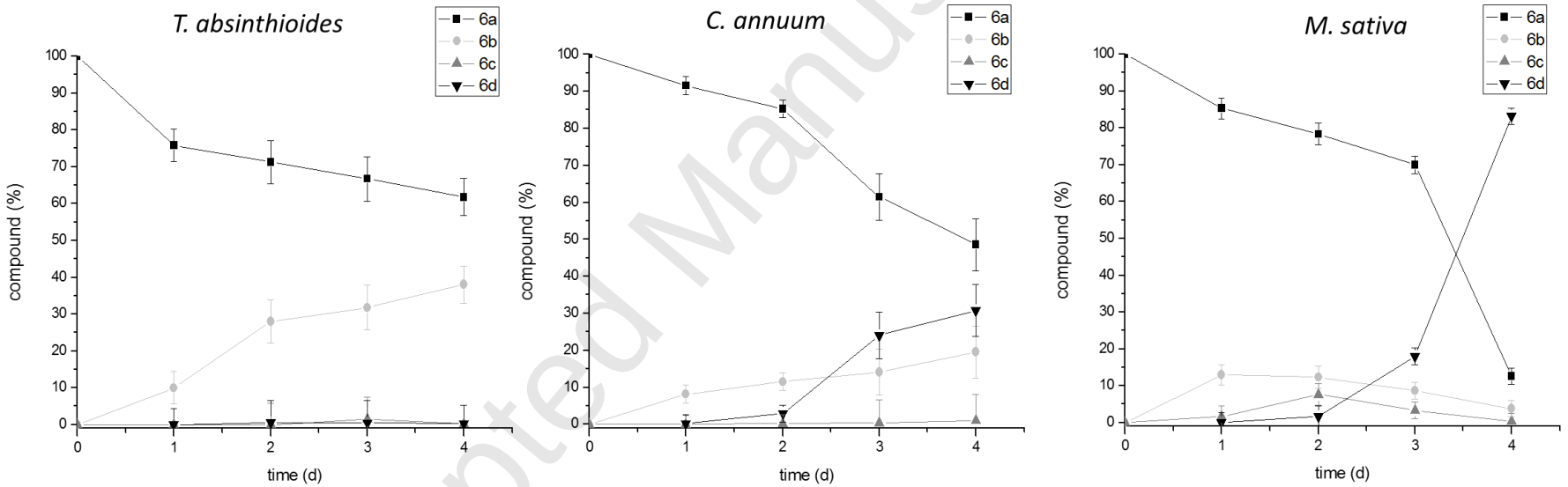
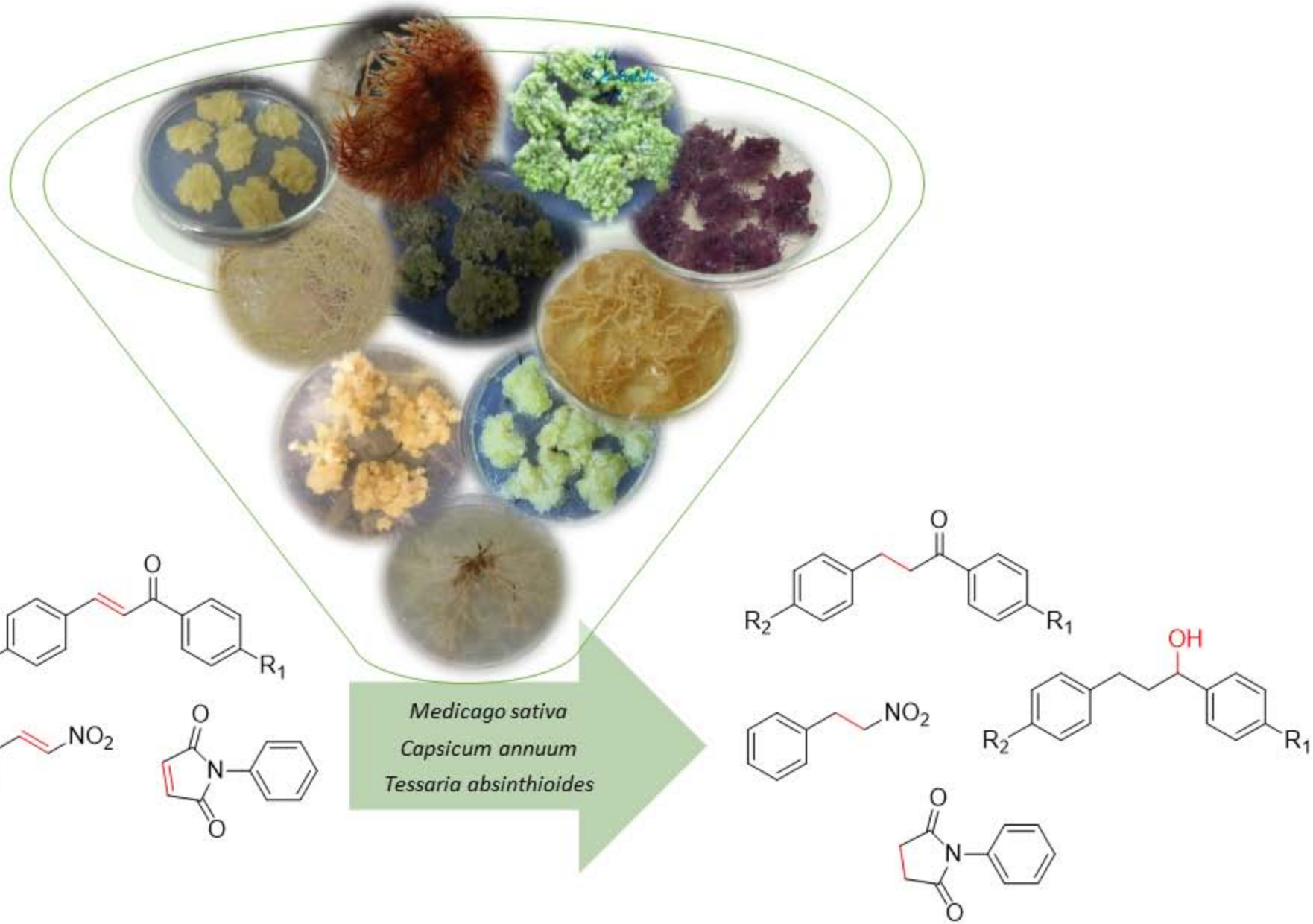


Figure 1. Substrates tested to assess the ER preference to reduce C=C double bonds by plant cell cultures

Figure 2. Time course profiles of the biotransformation of chalcone **6a** by *T. absinthioides* calli, *C. annuum* HR and *M. sativa* calli


O
R2
R1
NO2
O
N
O
Medicago sativa
Capsicum annuum
Tessaria absinthioides
O
R2
R1
NO2
OH
R2
R1
O
N
O