Plant Cell, Tissue and Organ Culture (PCTOC) (2020) 143:573–581
https://doi.org/10.1007/s11240-020-01942-y

ORIGINAL ARTICLE

Establishment and triterpenoid production of *Ocimum basilicum* cambial meristematic cells

Alexander Mehring[1] · Janik Haffelder[1] · Jonas Chodorski[1] · Judith Stiefelmaier[1] · Dorina Strieth[1] · Roland Ulber[1]

Received: 10 June 2020 / Accepted: 1 October 2020 / Published online: 7 October 2020

Abstract

The application of plant suspension culture to produce valuable compounds, such as the triterpenoids oleanolic acid and ursolic acid, is a well-established alternative to the cultivation of whole plants. Cambial meristematic cells (CMCs) are a growing field of research, often showing superior cultivation properties compared to their dedifferentiated cell (DDC) counterparts. In this work, the first-time establishment of *O. basilicum* CMCs is demonstrated. DDCs and CMCs were cultivated in shake flasks and wave-mixed disposable bioreactors (wDBRs) and evaluated regarding triterpenoid productivity and biomass accumulation. CMCs showed characteristic small vacuoles and were found to be significantly smaller than DDCs. Productivities of oleanolic and ursolic acid of CMCs were determined at 3.02 ± 0.76 mg/(l*d) and 4.79 ± 0.48 mg/(l*d) after 19 days wDBR cultivation, respectively. These values were consistently higher than any productivities determined for DDCs over the observed cultivation period of 37 days. Elicitation with methyl jasmonate of DDCs and CMCs in shake flasks resulted in increased product contents up to 48 h after elicitor addition, with the highest increase found in CMCs at 232.30 ± 19.33% (oleanolic acid) and 192.44 ± 18.23% (ursolic acid) after 48 h.

Key message

For the first time, cambial meristematic cells of *Ocimum basilicum* were established and cultivated in a disposable bioreactor system. These cells outperform dedifferentiated cells of the same organism regarding productivity.

Keywords Plant cell culture · Cambial meristematic cells · Triterpenoids · Disposable bioreactor · Elicitation

Introduction

The perennial herb *Ocimum basilicum* (family *Lamiaceae*), commonly known as basil, is valued worldwide for its culinary uses, but its applications go well beyond that. Basil and its essential oil are often used in traditional medicine (Irondi et al. 2016; Ezeani et al. 2017; Bae et al. 2020), cosmetics (Vivas Castaño et al. 2016; Volpe et al. 2018; Yeşil et al. 2020), and pharmacology (Sestili et al. 2018; Zhan et al. 2020). Among the pharmacologically interesting compounds found in basil are linalool (Medeiros Venancio et al. 2016), rosmarinic (Kwon et al. 2019), oleanolic (OA; Qamar et al. 2020) and ursolic acid (UA; Arshad Qamar et al. 2010; Kümmritz et al. 2014). The latter are triterpenoids, a class of isoprenoid-based secondary metabolites. OA and UA were shown to possess antiinflammatory (Kashyap et al. 2016), antitumoral (Pięt and Paduch 2018), hepatoprotective (Gutiérrez-Rebolledo et al. 2016) and antioxidant (Srinivasan et al. 2020; Guo et al. 2020) properties among others. These properties make oleanolic and ursolic acid interesting candidates for clinical application, which could result in high future demand. The cultivation of whole plants for valuable substances is impractical from both economic and ecological stances because of long growth periods, huge crop areas and risk of crop loss. By utilizing plant cell culture, specific cell types can be used for production of valuables in a more controllable environment (Ramachandra Rao and Ravishankar 2002). Basil cell suspension culture

Communicated by Christophe Hano.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11240-020-01942-y) contains supplementary material, which is available to authorized users.

✉ Roland Ulber
ulber@mv.uni-kl.de

[1] Institute of Bioprocess Engineering, University of Kaiserslautern, Gottlieb-Daimler-Str. 49-502, 67663 Kaiserslautern, Germany

was established in the past for the production of rosmarinic acid (Kintzios et al. 2003) and more recently for phenolics and anthocyanins (Nazir et al. 2019) as well as oleanolic and ursolic acid (Pandey et al. 2019).

An interesting type of plant cell culture are cambial meristematic cells (CMC), which are derived from vascular cambium. These cells were described to grow at a faster rate, aggregate less and accumulate more product than dedifferentiated cells (DDCs) of the same plant (Ochoa-villarreal et al. 2015). CMCs were described for *Taxus cuspidata* (Lee et al. 2010), *Catharanthus roseus* (Moon et al. 2015; Zhu et al. 2018) and *Tripterygium wilfordii* (Song et al. 2019) among others, but not yet for *O. basilicum*.

The application of a suitable bioreactor system is crucial for plant cell cultivation in respect to biomass accumulation, cell viability and product formation (Eibl and Eibl 2008; Valdiani et al. 2019). While stirred-tank reactors are routinely used for plant cell culture (Arias et al. 2017; Pérez-Hernández et al. 2019), the mechanical stirring poses a source of hydrodynamic stress for the plant cells, decreasing cell viability during the process (Takeda et al. 1994). Alternatives without mechanical stirring are found in bubble column, airlift or some types of disposable bioreactors. Wave-mixed disposable bioreactors (wDBR) provide mass and energy transfer by a gentle rocking motion, reducing shear stress on the cells (Eibl and Eibl 2008). This reactor type was used in the past for cultivation of *Nicotiana tabacum* (Terrier et al. 2007; Raven et al. 2015), *Hordeum vulgare* (Ritala et al. 2008) and *Malus domesticus* (Schürch et al. 2008).

A popular means to enhance secondary metabolite formation in plant cell culture is known as elicitation (Giri and Zaheer 2016; Thakur et al. 2019). The application of an elicitor triggers stress or defence related responses in plant cells (Narayani and Srivastava 2017). Methyl jasmonate (MeJa) is a signal molecule involved in plant defence. When applied to plant cell culture, the production of secondary metabolites can be increased, for example rosmarinic acid in *O. basilicum* DDCs (Pandey et al. 2019) or flavonoids in *Pueraria candollei* (Udomsin et al. 2020).

In this study, CMCs of *O. basilicum* are described for the first time. Their productivity of the triterpenoids oleanolic and ursolic acid is described in shake flasks as well as wDBRs and compared to DDCs of the same organism. Additionally, eliciting effects of MeJa on triterpenoid production of CMCs are investigated in shake flasks.

Materials and methods

Establishment of CMC cell culture

Cell culture was established from commercially available *O. basilicum* plant. Young shoots were cut and surface sterilized by incubation in 0.56 mM ascorbic acid solution for 90 min, followed by 1 min incubation in 70% (v/v) ethanol and incubation in 1% (v/v) sodium hypochlorite for 20 min. Shoots were rinsed in sterile deionized water for 5 min after each sterilization step. Afterwards, shoots were quartered along the shoot axis and incubated on agar plates containing 30 g/l sucrose, 4.4 g/l Linsmaier-Skoog (LS) media, 4 g/l plant agar and 1 mg/l 2,4-dichlorophenoxyacetic acid. Subcultivation on the same medium was performed at a 2 week interval. Light microscopy was performed biweekly to check for the emergence of new cells in the cambial layer. When cells emerged, they were scraped off and transferred to agar plates for strain maintenance (see below). Cell morphology was routinely inspected with a light microscope (Nikon Eclipse NI, Düsseldorf, Germany).

Strain maintenance and pre-culture

CMCs were maintained on agar plates containing LS-medium (30 g/l sucrose, 4.4 g/l LS-media, 1 g/l MES and 1 mg/l 2,4-D, pH adjusted to 5.7 with sodium hydroxide) with an addition of 5.5 g/l plant agar. Original DDC culture was established from a commercial basil plant (unpublished results). LS-medium for DDCs additionally contained 1 mg/l 6-furfurylaminopurine. Subcultivation of agar plates was performed every 4 weeks. All agar plates were kept in an incubator (Binder, Tuttlingen, Germany) at 28 °C in darkness. To establish precultures, 1 g/l cell wet weight (CWW) of DDCs or CMCs were transferred into 500 ml shake flasks containing 50 ml LS-medium and incubated in an incubation shaker (Multitron, Infors GmbH, Einsbach, Germany) for 14 days at 28 °C, 120 rpm and 2.5 cm eccentricity in darkness. Additional homogenization of the cultured cells was not necessary, since all cultures were friable.

Cultivation in shake flasks

To assess growth and productivity in 500 ml shake flasks over time, 1 g/l CWW DDCs or CMCs from precultures were incubated in 50 ml LS-medium in an incubation shaker for up to 37 days at 28 °C and 120 rpm in darkness. Flasks were capped with cellulose plugs. Whole flasks were harvested by vacuum filtration starting after 4 days in a 3 days-rhythm and frozen at -20 °C until further use (n = 2).

Cultivation in disposable bioreactors

To assess productivity in wDBRs, a BIOSTAT RM 20 basic rocker (Sartorius AG, Göttingen, Germany) with 2 l Flexsafe RM basic SC bags was used. 1 g/l CWW DDCs or CMCs from precultures were incubated in their respective liquid media for 37 days at 7° tilt, 20 rpm, 50 ml/min aeration with ambient air and 28 °C in darkness. 1 ml samples containing

medium and biomass were drawn in a 3 days-rhythm starting after 4 days. Biomass was separated from media by centrifugation at 14,000 rpm for 5 min and frozen at −20 °C until further use.

Neutral red staining

50 mg cells from agar plates were incubated in 1 ml 0.005% (w/v) neutral red solution for 3 min. Suspensions were then centrifuged twice for 2 min at 14,000 rpm. Supernatant was discarded each time and cells were resuspended in 1 ml phosphate buffer (pH 7.2). All staining steps were performed in darkness. Suspended cells were pipetted on a microscopy slide and observed under a light microscope.

Determination of cell size

50 mg DDCs or CMCs from agar plates were suspended in 1 ml of the respective liquid media. Suspended cells were pipetted on a microscopy slide and observed under a light microscope. Six images were recorded from each of ten slides for both cell types (n(DDC) = 204, n(CMC) = 282).

Confocal laser scanning microscopy

Images were taken on a Leica SP5 II upright confocal laser scanning microscope (Leica Microsystems GmbH, Wetzlar, Germany) with a 63 × 0.9 water immersion objective. Pinhole size was 1 AU, Laser Power 15%, AOTF (Acusto-optical tunable filter) 20%. Excitation wavelength 488 nm, emission bandwidth 500–500 nm for chlorophyll a autofluorescence and 650–750 nm for neutral red, resolution was 1024px.

Elicitation

To investigate the effects of elicitation on triterpenoid production, 10 g/l DDCs or CMCs from precultures were incubated in 500 ml shake flasks containing 50 ml of their respective liquid media with an addition of 200 µM MeJa dissolved in 100% ethanol at 28 °C, 120 rpm and 2.5 cm eccentricity in darkness. Triplicates of whole flasks and one control flask were harvested after 24 h, 48 h, and 72 h.

Triterpenoid extraction and analysis

Frozen biomass was freeze-dried (Christ, Osterode, Germany) at −20 °C and 1.03 mbar for 24 h. Triterpenoids were extracted by grinding 50 mg dry biomass in a mortar with 250 mg sea sand (particle size 0.1 – 0.315 mm) and 750 µl ethanol for 2 min. Samples were collected in 15 ml reaction vessels by washing the mortar with another 750 µl of ethanol and stored at −20 °C until further use. Samples were prepared for analysis using a polytetrafluoroethylene filter (pore size 0.22 µm). Reversed-phase high performance liquid chromatography analysis was done on a Waters alliance 2695 with Waters PDA detector 2998 at isocratic flow (0.3 ml/min), 20 µl injection volume and 55 °C column temperature. The mobile phase consisted of methanol with 0.1% formic acid in a ratio of 92:8. A Supelco Discovery HS C18, 5 µm, 250 mm x 4.6 mm column was used. UV spectra were obtained using a detection wavelength of 250 nm. External standards of OA and UA were used for a seven-point calibration.

Statistical analysis

Values are presented as means including standard deviation unless otherwise noted. Shapiro–Wilk test was used to determine normality of data sets. Mann–Whitney U Test was used to determine significant differences between data sets; $p < 0.001$ is indicated by triple asterisk.

Results and discussion

Establishment of *O. basilicum* CMCs and their morphology

The emergence of new cells in the vascular cambium of *O. basilicum* explants could be observed with a light microscope as early as 7 days after preparation. Emergent cells were carefully scraped off and incubated on solid medium. CMCs were morphologically identified by their small size and abundance of small vacuoles (Fig. 1a). Abundance of small vacuoles and generally small cell size compared to DDCs from the same species are distinguishing features widely described for CMCs (Lee et al. 2010; Song et al. 2019; Zhou et al. 2015).To confirm the observed structures as small vacuoles in CMCs, they were stained with neutral red, which penetrates the cell wall and membranes, accumulating in vacuoles (Fig. 1c). *O. basilicum* DDCs were also stained this way, confirming the absence of smaller vacuoles (Fig. 1b). Neutral red has been used for decades as a reliable stain for plant vacuoles (Timmers et al. 1995; Dubrovsky et al. 2006; Kaur et al. 2018) due to the ion trap mechanism, during which neutral red is protonated in acidic pH and loses its ability to pass membranes. Since the pH of the plastid stroma is generally in neutral or alkaline ranges (Su and Lai 2017) and thylakoid lumen acidity is light-dependent, neutral red accumulation in plastids is unlikely. Additionally, cells were kept in the dark during cultivation and staining, making the generation of a proton gradient across the thylakoid membrane unlikely. To confirm this, neutral red stained CMCs were observed with confocal laser scanning microscopy, where no overlap of neutral red

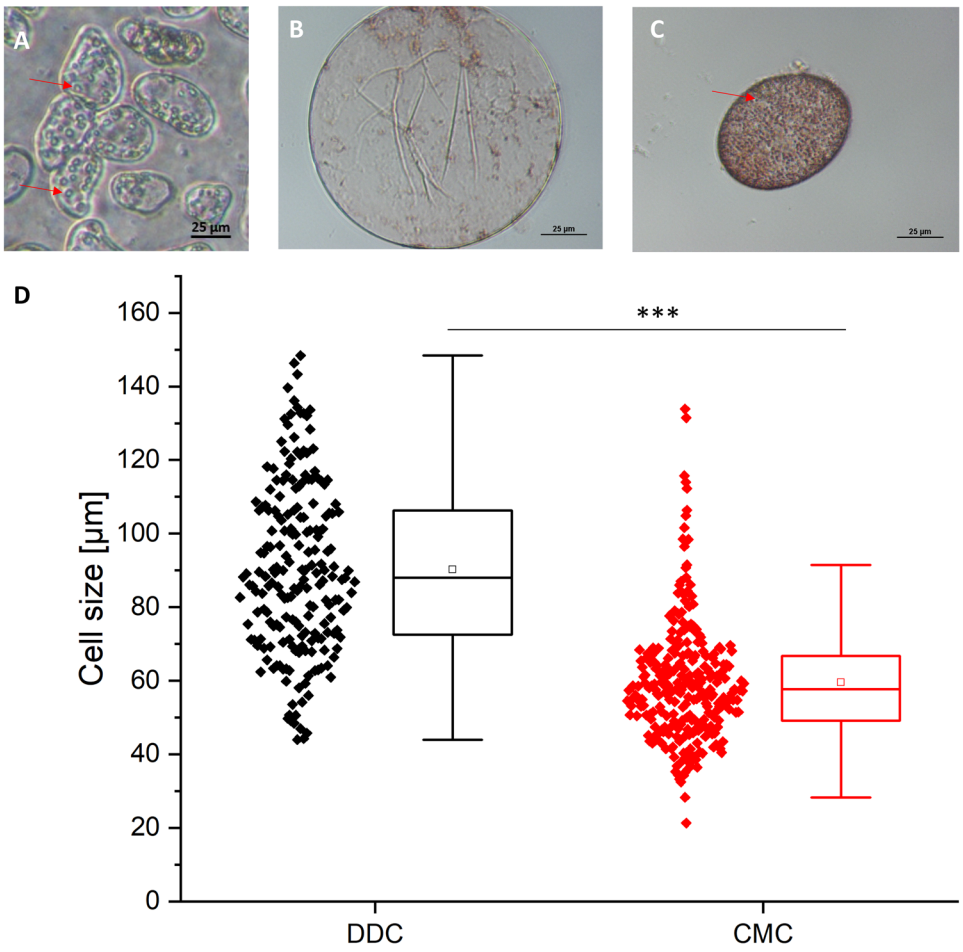

Fig. 1 Morphology of *O. basilicum* cambial meristematic cells (CMCs) compared to *O. basilicum* dedifferentiated cells (DDCs). **a** CMCs on agar plate. Arrows indicate small vacuoles within the cells. **b** DDC and **c** CMC stained with 0.005% neutral red. Scale bar: 25 µm. **d** Box plot depicting cell sizes for DDCs (left) and CMCs (right). Horizontal lines in boxes depict median values for the populations. Single data points beside corresponding boxes. n(DDC) = 204, n(CMC) = 282

fluorescence and chlorophyll a autofluorescence was found in vacuolar structures (Fig. S1, denoted by arrows). Cell size of DDCs were determined to spread around a median of 88.05 µm (Fig. 1d, black box, interquartile range 33.96 µm, min 43.96 µm, max 148.41 µm), which differed significantly from CMC size with a median of 57.66 µm (Fig. 1d, red box, interquartile range 17.60 µm, min 21.30 µm, max 133.893 µm). Although previous reports state that CMCs are smaller than DDCs (Lee et al. 2010; Song et al. 2019), their sizes were not comparatively quantified before.

CMC growth and productivity compared to DDCs

CMC growth was investigated in shake flasks over a period of 37 days (Fig. 2a). CMCs exhibited exponential growth between 16 and 25 days after inoculation, starting and ending 3 days earlier than DDCs (Fig. 2a, Fig. S2). Growth of CMCs continued until the last day of the cultivation period, reaching 203.02 ± 21.47 g_{CWW} /l after 37 days of cultivation. DDC CWW peaked at 261.55 ± 228.68 g/l after 28 days but declined to 55.97 ± 0.72 g_{CWW}/l after 37 days (Fig. S2), possibly due to cell death and lysis. While nutrient depletion cannot be ruled out as a reason for this decline, it was determined that sugars were not fully depleted at this time (data not shown). CWW concentrations are in the range of previously reported CWW values for DDCs of other plant species, for example *Panax ginseng* (Lian et al. 2002) and *Tribulus terrestris* (Khandy et al. 2017). Considering the extreme variation of DDC CWW between 22 and 31 days, CMCs appear to be superior to DDCs in biomass accumulation.

Productivity of oleanolic and ursolic acid of CMCs in shake flasks increased until 19 days after inoculation, where both peaked at 1.73 ± 0.49 mg/(l*d) and 2.73 ± 0.53 mg/(l*d), respectively (Fig. 2a). The productivity then declined until the end of the cultivation period, reaching 0.74 ± 0.46 mg/(l*d) (oleanolic acid) and 0.62 ± 0.25 mg/(l*d) (ursolic acid). While the variability of the productivities also increases for both triterpenoids towards the end of the cultivation, this is not considered problematic because productivities do not reach previously detected levels within their variability. The apparent relationship between cell growth and triterpenoid productivity is not yet described for *O. basilicum* cells, but a similar behaviour was reported in *Salvia officinalis*, where highest triterpenoid content preceded highest biomass and dropped off afterwards (Bolta

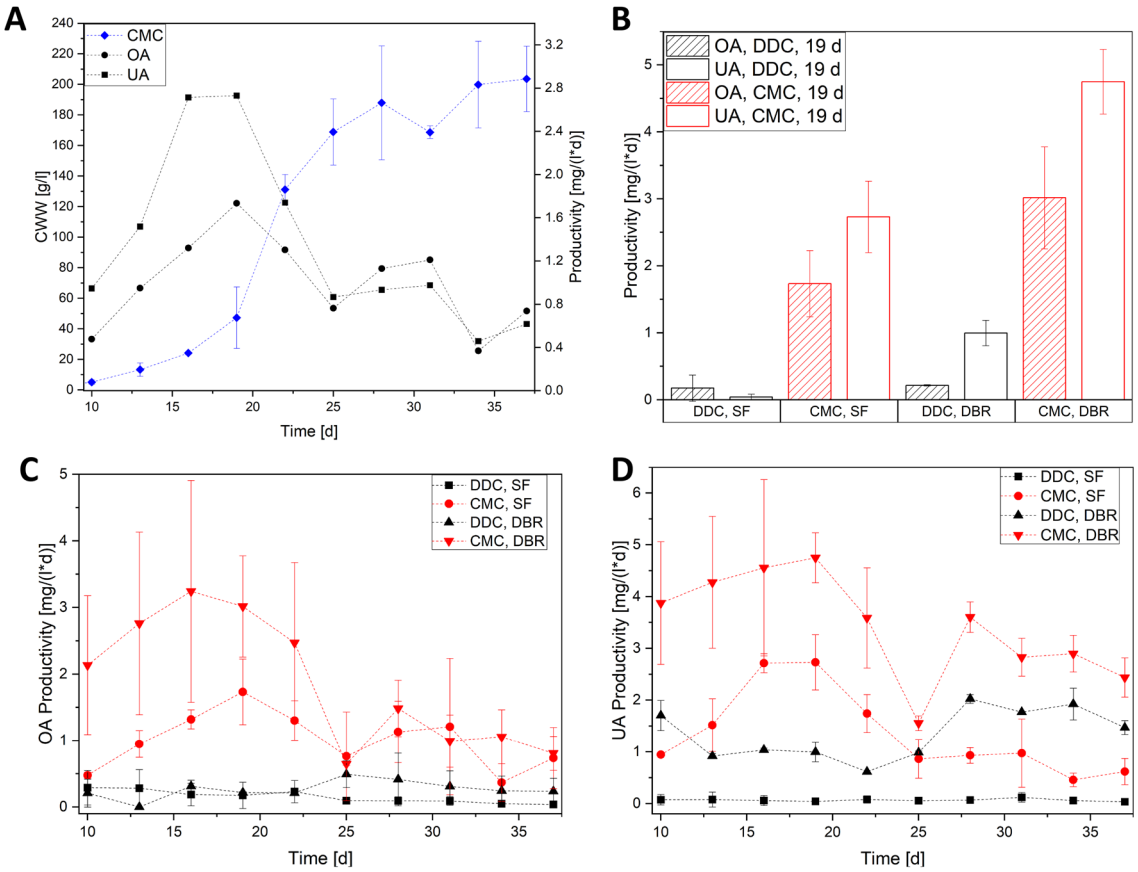

Fig. 2 Productivity of cambial meristematic cells (CMCs) compared to dedifferentiated cells (DDCs) in shake flasks (SF) and wave-mixed disposable bioreactors (wDBR). **a** CMC biomass accumulation and productivity of OA (circles) and UA (rectangles) in SF over the period of 37 days. **b** Productivity of OA and UA after 19 days cultivation in SF and wDBR. **c** OA productivity and **d** UA productivity in SF and wDBR over a 37 days cultivation period. n(DDC, SF) = 5; n(rest) = 2

et al. 2000; Haas et al. 2014). To visualize the differences between DDCs and CMCs at 19 days cultivation, productivities in both shake flasks and wDBR were observed in more detail (Fig. 2b). In shake flasks, oleanolic acid productivity of DDCs was found at 0.17 ± 0.20 mg/(l*d) and ursolic acid productivity at 0.04 ± 0.04 mg/(l*d) after 19 days. At that timepoint, CMC productivities of oleanolic and ursolic acid were 9.9-fold and 68.3-fold higher, respectively. Cultivation of DDCs in wDBR resulted in productivities of oleanolic and ursolic acid of 0.22 ± 0.01 mg/(l*d) and 1.00 ± 0.19 mg/(l*d) after 19 days, respectively. CMC productivities in shake flasks were still 7.8-fold (oleanolic acid) and 2.7-fold (ursolic acid) higher. At 19 days cultivation, the data indicates that CMCs are superior to DDCs in terms of productivity. Cultivation of CMCs in wDBR revealed even higher productivities for both triterpenoids, which were found at 3.02 ± 0.76 mg/(l*d) for oleanolic and 4.79 ± 0.48 mg/(l*d) for ursolic acid. Compared to CMC productivities in shake flasks, both oleanolic and ursolic acid productivities were increased 1.75-fold, indicating that cultivation of *O. basilicum* CMCs in wDBR is favourable. This is in line with a previous report, where cultivation of *O. basilicum* DDCs in a wDBR system lead to increased rosmarinic acid content (Kintzios et al. 2004). Conversely, oleanolic and ursolic acid content could not be increased when *O. basilicum* DDCs were cultivated in a stirred-tank reactor (Pandey et al. 2019). The indication for better productivities of CMCs in wDBR cultivation was confirmed by productivities obtained over the time courses of all cultivations (Fig. 2c, d). In a direct comparison, after 25 days, productivities of oleanolic acid determined in wDBR cultivation converged with those from shake flask cultivation within standard deviation margin. The productivities of ursolic acid determined in wDBR cultivation stayed above those from shake flasks over the rest of the cultivation period. Productivities of CMCs were higher than those of DDCs in both shake flasks and wDBR at most time points. Exceptions to this were found after 28 days of cultivation, where the highest productivity for ursolic acid in DDCs in wDBR was found at 2.03 ± 0.08 mg/(l*d). This value was 2.18-fold higher than ursolic acid productivity of CMCs in shake flasks at 28 days. In wDBR at 28 days, ursolic acid productivity of CMCs was still 1.77-fold higher.

This observed pattern continued until the end of the cultivation period. Regarding oleanolic acid, no such exception was found. These findings are consistent with previous reports on CMCs. In *Taxus cuspidata* CMCs, paclitaxel yield was found to be eightfold higher in CMCs than in DDCs (Lee et al. 2010). *Tripterygium wilfordii* CMCs also yielded at least twofold higher terpenoid contents than DDCs (Song et al. 2019). Across most cultivations in this work, productivities of oleanolic acid were found to be lower than of ursolic acid, which is consistent with previous reports on *O. basilicum* (Pandey et al. 2019). An exception was found in DDCs in SF, where both productivities were around the same level over the cultivation period, but generally very low. There was no indication for a possible cultivar influence on the triterpenoid levels of CMCs and DDCs, as was reported for other metabolites in whole basil plants before (Kwee and Niemeyer 2011; Flanigan and Niemeyer 2014), when comparing the triterpenoid levels of six *Ocimum* lines established in our lab to those of their parent plants (data not shown).

For the first time, *O. basilicum* cells were cultivated in a wDBR. In a previous study, the rosmarinic acid production of *O. basilicum* DDCs was shown to be increased when cultivated in a disposable airlift reactor (Kintzios et al. 2004). In general, wDBR systems were used in the past for plant cell suspensions of various uses, often yielding satisfactory results (Eibl et al. 2009). Oleanolic and ursolic acid production were not investigated before in wDBRs. Considering the negative influence of hydrodynamic stress on plant cells, most pronounced by stirring (Kieran et al. 1997), it appears reasonable to favour non-stirred cultivation systems like wave wDBRs. However, it was recently postulated that careful adjustment of stirring speed may enhance limonoid production in *Azadirachta indica* cell culture (Villegas-Velásquez et al. 2017). During cultivation of CMCs in a stirred tank reactor at 120 rpm stirring speed, the highest productivities of oleanolic and ursolic acid were determined at 2.26 mg/ml*d and 2.10 mg/ml*d after 13 days cultivation, respectively (Fig. S3). These values were 1.33-fold (oleanolic acid) and 2.36-fold (ursolic acid) lower than the highest productivities obtained in wDBR cultivation. In summary, productivities were shown to improve when cultivating CMCs over DDCs. Among the investigated cultivation strategies, wDBR currently appears to be the best strategy to obtain high productivities in CMCs.

Elicitation

To investigate a potential increase in product contents, elicitation with 200 µM MeJa was performed in DDCs and CMCs in SF (Fig. 3). Highest increase from control in oleanolic and ursolic acid concentration in DDCs was found after 48 h incubation at 169.16 ± 19.91% and 184 ± 38.27%, respectively. In CMCs, highest increases from control were found after 48 h incubation as well at 232.30 ± 19.33% for oleanolic acid and 192.44 ± 18.23% for ursolic acid, both of which were higher than increases found in DDCs. A strong positive effect of MeJa elicitation on product contents of both metabolites is thus indicated. In contrast to these findings, a recent study reported only a slight increase in oleanolic acid concentration and a decrease in ursolic acid concentration in *O. basilicum* under similar conditions (Pandey et al. 2019). In whole plants, however, MeJa elicitation led to increases in both triterpenoids (Misra et al. 2014). Other studies reported increased rosmarinic acid contents following MeJa elicitation in *O. basilicum* cultured cells (Pandey et al. 2015) and whole plants (Kim et al. 2006). After 72 h incubation with MeJa, a decrease of both triterpenoid concentrations was determined in CMCs at 91.13 ± 28.92% for oleanolic acid and 89.05 ± 24.76% for ursolic acid. This decrease indicates a detrimental effect of prolonged incubation with MeJa. While high elicitor concentration was reported to decrease product concentration in *Linum album* (Baldi et al. 2010), prolonged exposition alone did not decrease metabolite content below control levels in *Withania somnifera* (Sivanandhan et al. 2013) and *Gingko biloba* (Kang et al. 2009). Secretion of triterpenoids into the surrounding media could not be detected. Despite this, MeJa elicitation appears to be an efficient tool to increase product content in *O. basilicum* DDCs and CMCs.

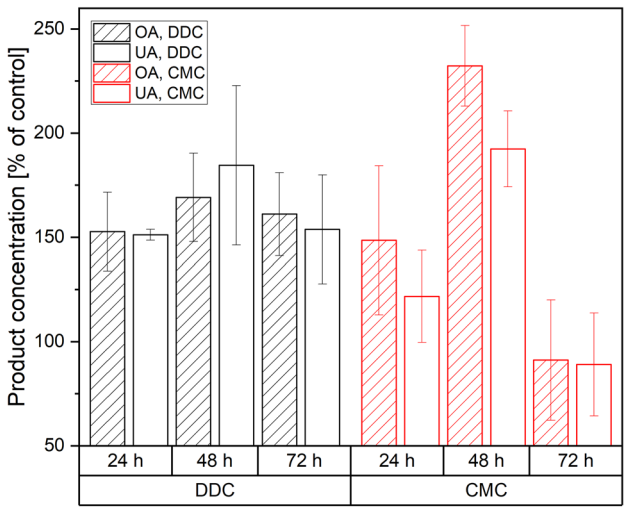

Fig. 3 Elicitation of DDCs and CMCs with 200 µm methyl jasmonate in shake flasks. Product concentrations of triterpenoids are shown 24 h, 48 h and 72 h after methyl jasmonate addition. Data expressed as % of unelicited control. n = 3

Conclusion and outlook

The establishment and cultivation of a CMC line from *O. basilicum* could be successfully demonstrated for the first time. Characteristic morphological features of CMCs, their relatively small size (median: 57.66 µm) and abundance of small vacuoles, were found to be consistent with previous works. Productivities of DDCs and CMCs were elucidated in shake flasks and wDBR. The highest productivities of the valuable triterpenoids oleanolic and ursolic acid were achieved in CMC cultivation in a wDBR after 19 days at 3.02 ± 0.76 mg/(l*d) for oleanolic and 4.79 ± 0.48 mg/(l*d) for ursolic acid, outperforming DDC productivities at least 1.77-fold. The findings provide a novel basis upon which the cultivation strategy for CMCs can be expanded and optimized. One enhancement is elicitation, which was shown to nearly double oleanolic and ursolic acid contents in shake flask experiments. Long-term suspension cultures of *Ocimum* cells are currently under investigation. The presence of triterpenoid glycosides was not shown in cultured *Ocimum* cells before and would also be interesting to elucidate. Future experiments should also compare process engineering parameters between stirred and wave-mixed cultivation systems. *O. basilicum* CMCs thus possess a great perspective for future applications as productive systems for valuable triterpenoids.

Acknowledgements Parts of this work (establishment of CMC cell line) has been funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)–Project-ID 172116086 – SFB 926.

Author contributions AM: Conceptualization, Methodology, Formal analysis and investigation, Writing—original draft preparation; JH: Formal analysis and investigation; JC: Formal analysis and investigation, JS: Writing—review and editing; DS: Writing—review and editing; RU: Conceptualization, Supervision, Funding acquisition, Writing—review and editing.

Funding Open Access funding enabled and organized by Projekt DEAL. Parts of this work (establishment of CMC cell line) has been funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)–Project-ID 172,116,086 – SFB 926.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

Arias JP, Zapata K, BenjamЄRojano, et al (2017) Plant cell suspension culture of Thevetia peruviana (Pers.) K. Schum. in shake flask and stirred tank reactor scale: a comparative study. African J Biotechnol 16:2355–2363. https://doi.org/10.5897/ajb2017.16281

Arshad Qamar K, Dar A, S. Siddiqui B, et al (2010) Anticancer activity of *Ocimum basilicum* and the effect of Ursolic acid on the Cytoskeleton of MCF-7 Human breast cancer cells. Lett Drug Des Discov 7:726–736. https://doi.org/10.2174/157018011007010726

Bae AH, Kim G, Seol GH et al (2020) Delta- and mu-opioid pathways are involved in the analgesic effect of *Ocimum basilicum* L in mice. J Ethnopharmacol 250:112471. https://doi.org/10.1016/j.jep.2019.112471

Baldi A, Farkya S, Jain A et al (2010) Enhanced production of podophyllotoxins by co-culture of transformed Linum album cells with plant growth-promoting fungi. Pure Appl Chem 82:227–241. https://doi.org/10.1351/PAC-CON-09-02-09

Bolta I, Bari evi D, Bohanec B, Andren ek S, (2000) A preliminary investigation of ursolic acid in cell suspension culture of Salvia officinalis. Plant Cell Tissue Organ Cult 62:57–63. https://doi.org/10.1023/A:1006498431099

Dubrovsky JG, Guttenberger M, Saralegui A et al (2006) Neutral red as a probe for confocal laser scanning microscopy studies of plant roots. Handb Environ Chem Vol 5 Water Pollut 97:1127–1138. https://doi.org/10.1093/aob/mcl045

Eibl R, Eibl D (2008) Design of bioreactors suitable for plant cell and tissue cultures. Phytochem Rev 7:593–598. https://doi.org/10.1007/s11101-007-9083-z

Eibl R, Werner S, Eibl D (2009) Disposable bioreactors for plant liquid cultures at litre-scale. Eng Life Sci 9:156–164. https://doi.org/10.1002/elsc.200800102

Ezeani C, Ezenyi I, Okoye T, Okoli C (2017) *Ocimum basilicum* extract exhibits antidiabetic effects via inhibition of hepatic glucose mobilization and carbohydrate metabolizing enzymes. J Intercult Ethnopharmacol 6:22. https://doi.org/10.5455/jice.20161229054825

Flanigan PM, Niemeyer ED (2014) Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (Ocimum basilicum L.). Food Chem 164:518–526. https://doi.org/10.1016/j.foodchem.2014.05.061

Giri CC, Zaheer M (2016) Chemical elicitors versus secondary metabolite production in vitro using plant cell, tissue and organ cultures: recent trends and a sky eye view appraisal. Plant Cell Tissue Organ Cult 126:1–18. https://doi.org/10.1007/s11240-016-0985-6

Guo Q, He J, Zhang H et al (2020) Oleanolic acid alleviates oxidative stress in Alzheimer's disease by regulating stanniocalcin-1 and uncoupling protein-2 signalling. Clin Exp Pharmacol Physiol 1440–1681:13292. https://doi.org/10.1111/1440-1681.13292

Gutiérrez-Rebolledo GA, Siordia-Reyes AG, Meckes-Fischer M, Jiménez-Arellanes A (2016) Hepatoprotective properties of oleanolic and ursolic acids in antitubercular drug-induced liver damage. Asian Pac J Trop Med 9:644–651. https://doi.org/10.1016/j.apjtm.2016.05.015

Haas C, Hengelhaupt K-C, Kümmritz S et al (2014) Salvia suspension cultures as production systems for oleanolic and ursolic acid. Acta Physiol Plant 36:2137–2147. https://doi.org/10.1007/s11738-014-1590-0

Irondi E, Agboola S, Oboh G, Boligon A (2016) Inhibitory effect of leaves extracts of *Ocimum basilicum* and *Ocimum gratissimum* on two key enzymes involved in obesity and hypertension in vitro. J Intercult Ethnopharmacol 5:396. https://doi.org/10.5455/jice.20160814112756

Kang S-M, Min J-Y, Kim Y-D et al (2009) Effect of biotic elicitors on the accumulation of bilobalide and ginkgolides in Ginkgo biloba cell cultures. J Biotechnol 139:84–88. https://doi.org/10.1016/j.jbiotec.2008.09.007

Kashyap D, Sharma A, Tuli HS et al (2016) Ursolic Acid and Oleanolic Acid: Pentacyclic Terpenoids with promising anti-inflammatory activities. Recent Pat Inflamm Allergy Drug Discov 10:21–33. https://doi.org/10.2174/1872213x10666160711143904

Kaur P, Gonzalez P, Dutt M, Etxeberria E (2018) Identification of sieve elements and companion cell protoplasts by a combination of brightfield and fluorescence microscopy. Appl Plant Sci 6:1–8. https://doi.org/10.1002/aps3.1179

Khandy MT, Kochkin DV, Tomilova SV et al (2017) Obtaining and study of callus and suspension plant cell cultures of *Tribulus terrestris* L., a producer of steroidal glycosides. Appl Biochem Microbiol 53:800–806. https://doi.org/10.1134/S000368381708003 8

Kieran P, MacLoughlin P, Malone D (1997) Plant cell suspension cultures: some engineering considerations. J Biotechnol 59:39–52. https://doi.org/10.1016/S0168-1656(97)00163-6

Kim H-J, Chen F, Wang X, Rajapakse NC (2006) Effect of methyl jasmonate on secondary metabolites of sweet basil (Ocimum basilicum L.). J Agric Food Chem 54:2327–2332. https://doi.org/10.1021/jf051979g

Kintzios S, Kollias H, Straitouris E, Makri O (2004) Scale-up micropropagation of sweet basil (Ocimum basilicum L.) in an airlift bioreactor and accumulation of rosmarinic acid. Biotechnol Lett 26:521–523. https://doi.org/10.1023/B:BILE.0000019561.89044.30

Kintzios S, Makri O, Panagiotopoulos E, Scapeti M (2003) In vitro rosmarinic acid accumulation in sweet basil (*Ocimum basilicum* L.). Biotechnol Lett 25:405–408. https://doi.org/10.1023/A:1022402515263

Kümmritz S, Haas C, Pavlov AI et al (2014) Determination of triterpenic acids and screening for valuable secondary metabolites in salvia suspension cultures. Nat Prod Commun. https://doi.org/10.1177/1934578X1400900107

Kwee EM, Niemeyer ED (2011) Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. Food Chem 128:1044–1050. https://doi.org/10.1016/j.foodchem.2011.04.011

Kwon DY, Li X, Kim JK, Park SU (2019) Molecular cloning and characterization of rosmarinic acid biosynthetic genes and rosmarinic acid accumulation in *Ocimum basilicum* L. Saudi J Biol Sci 26:469–472. https://doi.org/10.1016/j.sjbs.2017.03.010

Lee EK, Jin YW, Park JH et al (2010) Cultured cambial meristematic cells as a source of plant natural products. Nat Biotechnol 28:1213–1217. https://doi.org/10.1038/nbt.1693

Lian M-L, Chakrabarty D, Paek K-Y (2002) Effect of plant growth regulators and medium composition on cell growth and saponin production during cell-suspension culture of mountain ginseng (Panax ginseng C. A. mayer). J Plant Biol 45:201–206. https://doi.org/10.1007/BF03030360

Medeiros Venancio A, Ferreira-da-Silva FW, da Silva-Alves KS et al (2016) Essential oil of *Ocimum basilicum* L. and (−)-Linalool blocks the excitability of rat sciatic nerve. Evidence-Based Complement Altern Med 2016:1–7. https://doi.org/10.1155/2016/9012605

Misra RC, Maiti P, Chanotiya CS et al (2014) Methyl jasmonate-elicited transcriptional responses and pentacyclic triterpene biosynthesis in sweet basil. Plant Physiol 164:1028–1044. https://doi.org/10.1104/pp.113.232884

Moon SH, Venkatesh J, Yu J, Park SW (2015) Comptes rendus biologies differential induction of meristematic stem cells of *Catharanthus roseus* and their characterization. Comptes rendus - Biol 338:745–756. https://doi.org/10.1016/j.crvi.2015.05.005

Narayani M, Srivastava S (2017) Elicitation: a stimulation of stress in in vitro plant cell/tissue cultures for enhancement of secondary metabolite production. Phytochem Rev 16:1227–1252. https://doi.org/10.1007/s11101-017-9534-0

Nazir M, Tungmunnithum D, Bose S et al (2019) Differential production of phenylpropanoid metabolites in callus cultures of *Ocimum basilicum* L. with distinct in vitro antioxidant activities and in vivo protective effects against UV stress. J Agric Food Chem 67:1847–1859. https://doi.org/10.1021/acs.jafc.8b05647

Ochoa-villarreal M, Howat S, Jang MO et al (2015) Cambial meristematic cells : a platform for the production of plant natural products. N Biotechnol 32:581–587. https://doi.org/10.1016/j.nbt.2015.02.003

Pandey H, Pandey P, Singh S et al (2015) Production of anti-cancer triterpene (betulinic acid) from callus cultures of different Ocimum species and its elicitation. Protoplasma 252:647–655. https://doi.org/10.1007/s00709-014-0711-3

Pandey P, Singh S, Banerjee S (2019) Ocimum basilicum suspension culture as resource for bioactive triterpenoids: yield enrichment by elicitation and bioreactor cultivation. Plant Cell Tissue Organ Cult 137:65–75. https://doi.org/10.1007/s11240-018-01552-9

Pérez-Hernández J, del Nicasio-Torres M, P, Sarmiento-López LG, Rodríguez-Monroy M, (2019) Production of anti-inflammatory compounds in Sphaeralcea angustifolia cell suspension cultivated in stirred tank bioreactor. Eng Life Sci 19:196–205. https://doi.org/10.1002/elsc.201800134

Pięt M, Paduch R (2018) Ursolic and Oleanolic acids as potential anticancer agents acting in the gastrointestinal tract. Mini Rev Org Chem 16:78–91. https://doi.org/10.2174/1570193X15666180612090816

Qamar KA, Farooq AD, Siddiqui BS et al (2020) Antiproliferative effects of Ocimum basilicum methanolic extract and fractions, Oleanolic acid and 3-epi-Ursolic acid. Curr Tradit Med 6:134–146. https://doi.org/10.2174/2215083805666191010152439

Ramachandra Rao S, Ravishankar G (2002) Plant cell cultures: chemical factories of secondary metabolites. Biotechnol Adv 20:101–153. https://doi.org/10.1016/S0734-9750(02)00007-1

Raven N, Rasche S, Kuehn C et al (2015) Scaled-up manufacturing of recombinant antibodies produced by plant cells in a 200-L orbitally-shaken disposable bioreactor. Biotechnol Bioeng 112:308–321. https://doi.org/10.1002/bit.25352

Ritala A, Wahlström EH, Holkeri H et al (2008) Production of a recombinant industrial protein using barley cell cultures. Protein Expr Purif 59:274–281. https://doi.org/10.1016/j.pep.2008.02.013

Schürch C, Blum P, Zülli F (2008) Potential of plant cells in culture for cosmetic application. Phytochem Rev 7:599–605. https://doi.org/10.1007/s11101-007-9082-0

Sestili P, Ismail T, Calcabrini C et al (2018) The potential effects of *Ocimum basilicum* on health: a review of pharmacological and toxicological studies. Expert Opin Drug Metab Toxicol 14:679–692. https://doi.org/10.1080/17425255.2018.1484450

Sivanandhan G, Kapil Dev G, Jeyaraj M et al (2013) Increased production of withanolide A, withanone, and withaferin a in hairy root cultures of *Withania somnifera* (L.) Dunal elicited with methyl jasmonate and salicylic acid. Plant Cell, Tissue Organ Cult 114:121–129. https://doi.org/10.1007/s11240-013-0297-z

Song Y, Chen S, Wang X et al (2019) A novel strategy to enhance terpenoids production using cambial meristematic cells of Tripterygium wilfordii Hook. f. Plant Methods 15:1–13. https://doi.org/10.1186/s13007-019-0513-x

Srinivasan R, Aruna A, Lee JS et al (2020) Antioxidant and Antiproliferative potential of bioactive molecules Ursolic acid and Thujone isolated from *Memecylon edule* and *Elaeagnus indica*

and their inhibitory effect on topoisomerase II by molecular docking approach. Biomed Res Int 2020:1–12. https://doi.org/10.1155/2020/8716927

Su PH, Lai YH (2017) A reliable and non-destructive method for monitoring the stromal pH in isolated chloroplasts using a fluorescent pH probe. Front Plant Sci 8:1–10. https://doi.org/10.3389/fpls.2017.02079

Takeda T, Seki M, Furusaki S (1994) Hydrodynamic damage of cultured cells of *Carthamus tinctorius* in a stirred tank reactor. J Chem Eng JAPAN 27:466–471. https://doi.org/10.1252/jcej.27.466

Terrier B, Courtois D, Hénault N et al (2007) Two new disposable bioreactors for plant cell culture: the wave and undertow bioreactor and the slug bubble bioreactor. Biotechnol Bioeng 96:914–923. https://doi.org/10.1002/bit.21187

Thakur M, Bhattacharya S, Khosla PK, Puri S (2019) Improving production of plant secondary metabolites through biotic and abiotic elicitation. J Appl Res Med Aromat Plants 12:1–12. https://doi.org/10.1016/j.jarmap.2018.11.004

Timmers ACJ, Tirlapur UK, Schel JHN (1995) Vacuolar accumulation of acridine orange and neutral red in zygotic and somatic embryos of carrot (Daucus carota L.). Protoplasma 188:236–244. https://doi.org/10.1007/BF01280375

Udomsin O, Yusakul G, Kitisripanya T et al (2020) The Deoxymiroestrol and Isoflavonoid production and their elicitation of cell suspension cultures of *Pueraria candollei* var. mirifica: from Shake Flask to Bioreactor. Appl Biochem Biotechnol 190:57–72. https://doi.org/10.1007/s12010-019-03094-y

Valdiani A, Hansen OK, Nielsen UB et al (2019) Bioreactor-based advances in plant tissue and cell culture: challenges and prospects. Crit Rev Biotechnol 39:20–34. https://doi.org/10.1080/07388551.2018.1489778

Villegas-Velásquez S, Martínez-Mira AD, Hoyos R et al (2017) Hydrodynamic stress and limonoid production in *Azadirachta indica* cell culture. Biochem Eng J 122:75–84. https://doi.org/10.1016/j.bej.2017.03.004

Vivas Castaño AM, Beltrán Cifuentes MC, Cañón Rincón DJ (2016) Antioxidant activity of two varieties of *Ocimum basilicum* L. for potential use in phytocosmetics. Rev Fac Nac Agron Medellín 69:7965–7973. https://doi.org/10.15446/rfna.v69n2.59141

Volpe V, Nascimento DS, Insausti M, Grünhut M (2018) Octyl p-methoxycinnamate loaded microemulsion based on *Ocimum basilicum* essential oil. Characterization and analytical studies for potential cosmetic applications. Colloids Surfaces A Physicochem Eng Asp 546:285–292. https://doi.org/10.1016/j.colsurfa.2018.02.070

Yeşil M, Öztürk I, Yeşil Duymuş Z, Özcan MM (2020) Evaluating the effect of some medicinal plants (*Mentha piperita*, *Ocimum basilicum*, *Rosmarinus officinalis*, *Salvia officinalis*) on whitening of the permanent teeth. Turkish J Agric - Food Sci Technol 8:1. https://doi.org/10.24925/turjaf.v8i1.1-6.2508

Zhan Y, An X, Wang S et al (2020) Basil polysaccharides: a review on extraction, bioactivities and pharmacological applications. Bioorg Med Chem 28:115179. https://doi.org/10.1016/j.bmc.2019.115179

Zhou P, Yang J, Zhu J et al (2015) Effects of β-cyclodextrin and methyl jasmonate on the production of vindoline, catharanthine, and ajmalicine in *Catharanthus roseus* cambial meristematic cell cultures. Appl Microbiol Biotechnol 99:7035–7045. https://doi.org/10.1007/s00253-015-6651-9

Zhu J, He S, Zhou P et al (2018) Eliciting effect of catharanthine on the biosynthesis of vallesiachotamine and isovallesiachotamine in *Catharanthus roseus* cambial meristematic cells. Nat Prod Commun. https://doi.org/10.1177/1934578X1801300508