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ORIGINAL ARTICLE

Yeast-extract improved biosynthesis of lignans and neolignans in cell suspension cultures of *Linum usitatissimum* L.

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Abstract

Lignans and neolignans are important biologically active ingredients (BAIs) biosynthesized by *Linum usitatissimum*. These BAIs have multi-dimensional effects against cancer, diabetes and cardio vascular diseases. In this study, yeast extract (YE) was employed as an elicitor to evaluate its effects on dynamics of biomass, BAIs and antioxidant activities in *L. usitatissimum* cell cultures. During preliminary experiments, flax cultures were grown on different concentrations of YE (0–1000 mg/L), and 200 mg/L YE was found to be optimum to enhance several biochemical parameters in these cell cultures. A two-fold increase in fresh (FW) and dry weight (DW) over the control was observed in cultures grown on MS medium supplemented with 200 mg/L YE. Similarly, total phenolic (TPC; 16 mg/g DW) and flavonoids content (TFC; 5.1 mg/g DW) were also positively affected by YE (200 mg/L). Stimulatory effects of YE on biosynthesis of lignans and neolignans was also noted. Thus, 200 mg/L of YE enhanced biosynthesis of secoisolariciresinol diglucoside (SDG; 3.36-fold or 10.1 mg/g DW), lariciresinol diglucoside (LDG; 1.3-fold or 11.0 mg/g DW) and dehydrodiconiferyl alcohol glucoside (DCG; 4.26-fold or 21.3 mg/g DW) in *L. usitatissimum* cell cultures with respect to controls. This elicitation strategy could be scaled up for production of commercially feasible levels of these precious metabolites by cell cultures of *Linum*.

Keywords *Linum usitatissimum* · Cell cultures · Lignans · Neolignans · Yeast extract · Elicitation

Abbreviations

YE	Yeast extract
NAA	α-Naphthalene acetic acid
TPC	Total phenolic content
TFC	Total flavonoid content
RSA	Radical scavenging activity
SDG	Secoisolariciresinol diglucoside
LDG	Lariciresinol diglucoside
DCG	Dehydrodiconiferyl alcohol glucoside
GGCG	Guaiacylglycerol-β-coniferyl alcohol ether glucoside (GGCG)
PLRs	Pinoresinol–lariciresinol reductases

Introduction

Flaxseed, *Linum usitatissimum*, in the family Linaceae (Singh et al. 2011) is one of the oldest crops and has been used for various medicinal and industrial applications. Flax is also consumed as a functional food, used in making salad, beverages and dairy products (Shim et al. 2014). Flax has also been used in folklore medicinal therapies to cure lethargy, cough and anti-aging agent. Besides, flax anti-inflammatory, antioxidant, anti-malignant, and anti-diabetic potential have also been documented (Oomah 2001). These medicinal properties are attributed to the presence of phenolic compounds, minerals, vitamins, fatty acids, and cyclic peptides (Shim et al. 2014). The most notable class of compounds found in flax is lignans and neolignans, having

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various pharmacological potentials with both in vivo and in vitro experiments (Adlercreutz 2007). In human gut, the stereo chemistry of lignans is changed by mammalian bacteria to alternative class of lignans called enterolactones and enterolignans, also known as phyto-estrogens with improved anti-cancer potential against breast, prostate and intestinal tumors (Shim et al. 2014; Hu et al. 2007). With such important nutritional, physiological and medicinal properties lignans and neolignans are presently well recognized (Touré and Xueming 2010). Many reports are elucidating their biosynthetic pathway, regulation and accumulation in plants (Hano et al. 2006; Renouard et al. 2014).

Plants are source of biologically active ingredients (BAIs) but their production is either inadequate or compromised by various environmental factors (Nowak 1998), and plant cell culture systems have been established with numerous applications and advantages (Bhojwani and Razdan 1986). Various plant tissue culture methods have been successfully adopted for the in vitro multiplication, and to meet the agronomical demand for production of quality controlled flax cultivars via organogenesis, anther culture, somatic embryogenesis and adventitious root cultures (McHughen 1990; Beejmohun et al. 2007). Conversely, very few studies are available on in vitro production of pharmacologically important lignans and neolignans with this species (Hano et al. 2006). Thus, no report is available to date on elicitation of lignans and neolignans in flax by using YE. Therefore, in current study, the potential effects of YE on the dynamics of lignans and neolignans biosynthesis in cell cultures of flax have been investigated. Moreover, dynamics of total phenolic content, flavonoid content and antioxidant potential were also evaluated.

Materials and methods

Plant materials

Seeds of *Linum usitatissimum* (brown variety) were collected from Swat KPK, Pakistan (35.2227°N, 72.4258°E). Plants were identified by plant taxonomist and voucher specimen was deposited in university herbarium. Seeds were surface sterilized as in Anjum and Abbasi (2016), and geminated on MS basal medium (Murashige and Skoog 1962). For callus culture establishment, five pieces of stem explants (0.2–0.3 cm) from 28 days old seedling were inoculated on MS basal medium fortified with sucrose (30 g/L), agar (8 g/L) and α-naphthalene acetic acid (NAA; 1 mg/L; Anjum et al. 2017b). Erlenmeyer flasks (100 mL) were used and cultures were maintained at 25 ± 1 °C at 16/8 h photoperiod under light intensity of ~ 45 to 50 µmol/m^2/s, supplied by fluorescent bulbs (Philips Tornado Spiral). Sub culturing was performed every 28 days on the same medium.

Establishment of suspension culture

For establishment of suspension cultures, ~ 1 g of fresh callus cells was transferred to 250 mL Erlenmeyer flasks containing 50 mL of liquid medium as above, kept at the same culture conditions above, on a rotary shaker (speed) for 15 days, and subcultured every 2 weeks thereafter.

Yeast extract treatments and elicitor preparation

Yeast extract stock solution was prepared by the method of Hahn and Albersheim (1978). Subsequently, the supernatant was discarded and its precipitate used for experiments. Different concentrations of YE (0, 10, 50, 100, 200, 500 and 1000 mg/L) were added to MS medium supplemented with 1 mg/L of NAA and sucrose (30 g/L). About 10 mL of inoculum from 15 days old suspension culture was added to each flask and then placed in growth room on rotary shaker.

Sample extraction

For fresh weight determination, cultures were harvested and filtered through Whatman filter paper to remove extra water content. After removal of excess water callus residue was weighed. To determine dry weights, fresh calli were dried in oven at 50 °C for 24 h and weighed. Extraction of cell cultures was performed by using a modified method of Abbasi et al. (2011). Briefly, about 0.1 g of each dried sample was dissolved in 0.5 mL methanol, left overnight on rotatory shaker (80 rpm) for 24 h at room temperature. The mixture was sonicated for 30 min and then vortexed. Extract was centrifuged for 15 min at 10,000 rpm and supernatant was preserved at 4 °C.

Total phenolic content (TPC)

Total phenolic content (TPC) was determined via Folin–Ciocalteu's reagent (FC) according to Singleton and Rossi (1965). Briefly, 20 µL of each sample was diluted with 90 µL of FC reagent then 90 µL of sodium carbonate was added. Gallic acid and DMSO was used as positive and negative control, respectively. The 96 micro well plate reader was used for measuring absorbance at 630 nm.

Total flavonoid content (TFC)

The method of Ul-Haq et al. (2012), was followed for determination of total flavonoid content. Briefly, 20 µL of each sample was mixed with 10 µL of potassium acetate, 10 µL of aluminum chloride and 160 µL of distilled water was added and incubated for 30 min. Quercetin and methanol

was used as positive and negative control, respectively. The 96 micro well plate-reader was used for measuring absorbance at 415 nm.

Determination of antioxidant activity

For antioxidant activity determination, 2,2-diphenyl-1-picrylhydrazy (DPPH) free radical scavenging assay, ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity power (ORAC) were used. Free radical scavenging potential was measured as in Ahmad et al. (2010). Briefly, 20 µL of each sample was diluted with 180 µL of DPPH solution. The dilutions were kept in dark for 1 h at room temperature. The absorbance was measured at 517 nm. To calculate FRSA following formula was used:

$$\%\ \text{scavenging DPPH free radical} = 100 \times (1 - AE/AD) \quad (1)$$

AE denotes absorbance of the mixture at 517 nm and AD denotes the absorbance of the DPPH solution without adding anything.

Ferric reducing antioxidant power (FRAP) was measured as described by Benzie and Strain (1996) with little modifications. Briefly, 10 µL of the extracted sample with concentrations used in the range of 8 µM to 1 mM as described by Mishra et al. (2013) was mixed with 190 µL of FRAP [10 mM TPTZ; 20 mM $FeCl_3$ $6H_2O$ and 300 mM acetate buffer pH 3.6; ratio 1:1:10 (v/v/v)]. Incubation lasted 15 min at room temperature. Absorbance of the reaction mixture was measured at 630 nm with a BioTek ELX800 Absorbance Microplate Reader. Oxygen radical absorbance capacity (ORAC) assay was performed as described by Prior et al. (2003). Briefly, 10 µL of the extracted sample with concentrations used in the range of 8 µM to 1 mM (Mishra et al. 2013) was mixed 190 µL of fluorescein (0.96 µM) in 75 mM phosphate buffer pH7.4 and incubated for at least 20 min at 37 °C with intermittent shaking. Then 20 µL of 119.4 mM 2,2′-azobis-amidinopropane (ABAP, Sigma Aldrich) was added and the fluorescence intensity was measured every 5 min during 2.5 h at 37 °C using a fluorescence spectrophotometer (BioRad) set with an excitation at 485 nm and emission at 535 nm. Assays were realized in triplicate and antioxidant capacity was expressed as Trolox C equivalent antioxidant capacity (TAEC).

HPLC Analysis

Lignans and neolignans were extracted by the method of Corbin et al. (2013) and Renouard et al. (2010), respectively. Approximately, 250 mg of cells were placed in 80% methanol (v/v) and was sonicated for 1 h at 25 °C. Later sonication sample was centrifuged and supernatant taken and evaporated at 40 °C to remove the methanolic fraction and then placed in 1 mL of 0.1 M citrate–phosphate pH 4.8 buffer comprising 5 unit/mL of β-glucosidase (Sigma) to release aglycone residues. The extract was then homogenized and supernatant was passed through (0.45 µm) filter paper prior injection. The lignans and neolignans profile of flax cell cultures were quantified by RP-HPLC using a Varian liquid chromatographic system comprising Varian Prostar 230 pump, Metachem Degasit, Varian Prostar 410 auto sampler and Varian Prostar 335 Photodiode Array Detector (PAD) and equipped with Galaxie version 1.9.3.2 software. The separation was achieved by using Purospher (Merck) RP-18 column (250 × 4.0 mm i.d.; 5 µm) at 40 °C by using the method of Renouard et al. (2010). The same extract was also investigated without enzymatic treatment which confirms the relative proportion of aglycones vs glycosides. Lignans and neolignans were quantified by using the calibration curves and the results are expressed as mg of glucoside (Secoisolariciresinol diglucoside, lariciresinol diglucoside, dehydrodiconiferyl alcohol glucoside, guaiacylglycerol-β-coniferyl alcohol) equal to per gram dry weight. Results were then compared with published data and molecular weight of the specific compounds was used for each compound.

Statistical analysis

All the experiments were performed in triplicates. For statistical analysis Graph Pad Prism Windows, v5.0 was exploited. Origin v8.5 was used to draw graphs. One-way ANOVA (Tukey's multiple comparisons test) was used to determine significance at $p < 0.05$.

Results and discussion

Various strategies have been devised to improve the production of BAIs in in vitro cultures of medicinal plants. Among these approaches, elicitation has been the most renowned (Ali and Abbasi 2013; Yue et al. 2016). Yeast extract (YE) is a biotic elicitor reported to enhance valuable BAIs in cell cultures of several plant species (Sánchez-Sampedro et al. 2005; Yan et al. 2006; Zhao et al. 2014). Though the exact mechanism of elicitation is unknown it could be due to the presence of various organic and inorganic derivatives, which stimulated biosynthesis of BAIs (Boller 1995). In the present study, different concentrations of YE (10–1000 mg/L) were tested on cell biomass, TPC and TFC in cell cultures of flax (Table 1). As a whole, results showed that 200 mg/L YE resulted in optimum biomass, TPC and TFC as compared to control, and was selected for further investigations (growth kinetics). Higher doses (1000 and 500 mg/L) of YE did not significantly enhance growth and secondary metabolites as compared to control. In agreement with our results, Cai et al. (2014) and Ali et al. (2018)

Table 1 Effects of different concentrations of Yeast extract on biomass accumulation (FW, DW) and biosynthesis of total phenolic and flavonoid content in flax cell cultures

YE Conc mg/L	FW (g/L)	DW (g/L)	TPC (mg/g DW)	TFC (mg/DW)
0	62.1 ± 4.3^{a}	5.4 ± 0.250^{a}	8.2 ± 0.02^{a}	3.5 ± 0.063^{a}
10	72.23 ± 3.1^{b}	7.1 ± 0.321^{b}	9.1 ± 0.04^{b}	3.67 ± 0.065^{b}
50	76.67 ± 5.4^{c}	7.34 ± 0.553^{b}	11.6 ± 0.020^{c}	3.89 ± 0.023^{c}
100	79.32 ± 6.18^{d}	7.68 ± 0.569^{b}	14.23 ± 0.021^{d}	4.2 ± 0.065^{d}
200	84.36 ± 5.6^{e}	8.27 ± 0.579^{c}	16 ± 0.032^{e}	4.9 ± 0.045^{e}
500	64.64 ± 6.14^{a}	6.12 ± 0.400^{d}	12.25 ± 0.09^{b}	3.91 ± 0.025^{c}
1000	60.7 ± 5.12^{a}	5.6 ± 0.398^{a}	8.23 ± 0.10^{a}	3.4 ± 0.032^{a}

Values designates mean ± SE. Columns with same words are not significantly different ($p < 0.05$)

reported that a moderate concentration of YE showed a positive impact on cell cultures of apple and ginger. Our study is also in harmony with a previous report by El-Nabarawy et al. (2015), where higher concentrations of YE inhibited cellular growth in *Zingiber officinale* callus cultures. No significant morphological changes were observed at lower and moderate concentrations. However, higher concentrations induced browning in cultures. Such observations were also made for *Silybum marianum* cultures grown at higher concentration of YE (Sánchez-Sampedro et al. 2005).

Biomass accumulation

Growth dynamics of flax cell cultures grown on YE was evaluated with a sampling time of 5 days for a period of 45 days. As shown in Figs. 1a and 2b, flax cells grown on 200 mg/L YE showed two fold enhancement in FW and DW than their respective controls on 35th day of culture. The biomass accumulation may be attributed to the high amount of minerals, amino acids and vitamins present in YE (Vasil and Hildebrandt 1966). Similar stimulatory effects have also been reported by Bayraktar et al. (2016), where YE improved biomass accumulation in *Stevia rebaudiana* plantlets. Enhanced biomass accumulation of elicited cultures compared to that of control has been noted in shoot cultures of *Eryngium planum* L (Kikowska et al. (2015), *Salvia castanea* (Li et al. 2016) and hairy root cultures of *Tropaeolum majus* (Wielanek and Urbanek 2006) treated with YE.

Total phenolic and flavonoid contents

In this study, 200 mg/L YE induced optimum biosynthesis of total phenolic and flavonoid contents in cell cultures of flax. However, optimum TPC was recorded on 35th day of culture (Fig. 2a). The enhanced production of phenolic compounds may be linked to an activation of defense responses happened due to environment-induced stress (Pauw et al. 2004). These phenolic compounds are synthesized through phenylpropanoid metabolic pathway (Yan et al. 2006; Fazal et al. 2016). Similar observations were made for cultures of

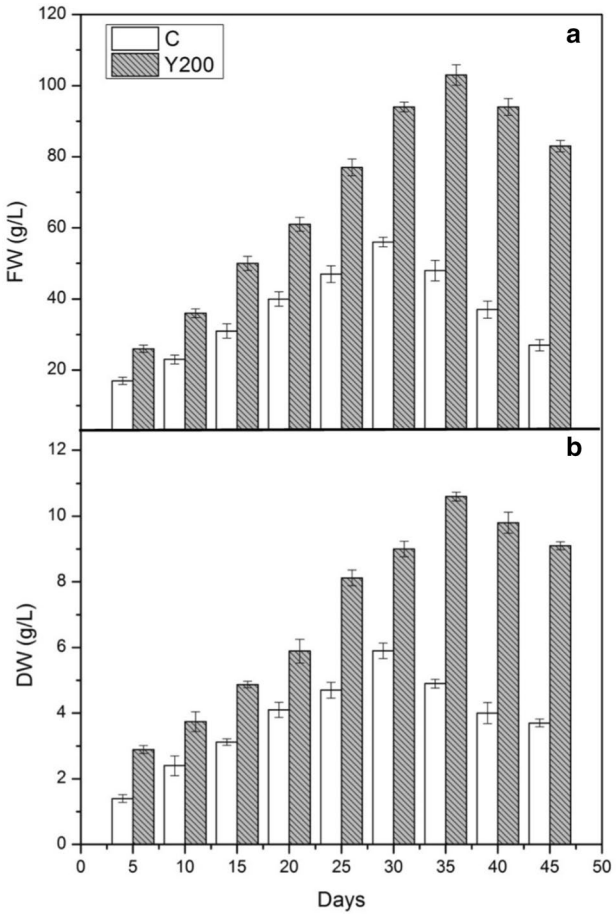

Fig. 1 Biomass accumulation in cell cultures of flax grown on 200 mg/L Yeast extract. **a** FW, **b** DW. Values are mean ± SE of three replicates

Astragalus chrysochlorus (Cakir and Ari 2009) and *Curcuma manga* (Abraham et al. 2011).

Likewise, 1.5-fold higher accumulation in flavonoids (TFC) was also noted on 35th day of culture (Fig. 2b). Similar observations have also been reported showing the positive impact of YE on accumulation of flavonoids in sprout cultures of *Fagopyrum tataricum* (Zhao et al. 2014) and in suspension cultures of *Andrographis paniculata* and

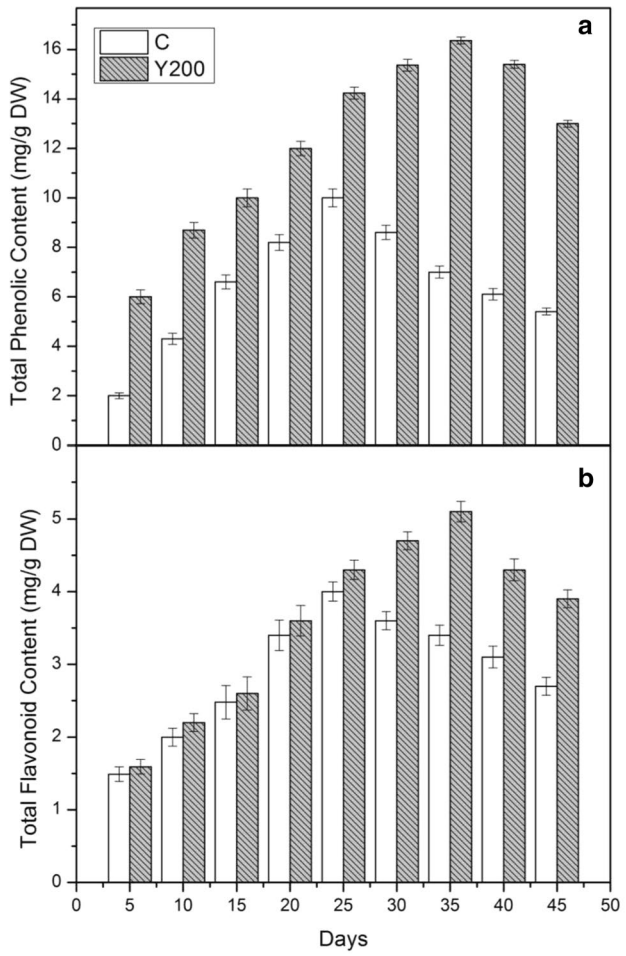

Fig. 2 Accumulation of total phenolic content in cell cultures of flax grown on 200 mg/L yeast extract (**a**), and total flavonoid content (**b**), in cell cultures of flax. Values are mean ± SE of three replicates

Hypericum perforatum (Mendhulkar et al. 2013; Simic et al. 2015). The enhanced biosynthesis of TFC might be attributed to the triggering of jasmonate thus yielding high levels of flavonoids (Sánchez-Sampedro et al. 2005). Moreover, a positive correlation was found between the total flavonoid content and total phenolic content which is in agreement with previous studies of Fazal et al. (2016).

Antioxidant activities

DPPH, FRAP and ORAC antioxidant assays were performed in order to evaluate the antioxidant potential of flax in vitro cultures (Table 2). According to Prior and Cao (2001), all these assays have different mechanism of reaction and do not predict parallel results. Elicited flax cell cultures showed high antioxidant activities as shown in Table 2. Maximum DPPH (92.2%), FRAP (20.41 µmol TAEC mg /DW) and ORAC (24.63 µmol TAEC mg /DW) activity were noted at 35th day, which were 1, 1. 5 and 2.81 times higher than their respective controls. Many researchers have stated similar observations for other cell cultures grown on YE (Ali et al. 2018; Złotek and Świeca 2016). YE treated apple cell cultures resulted in enhanced FRAP activity which also endorses our results. A strong positive correlation was established among TPC, TFC and antioxidants, in agreement with previous reports (Luximon-Ramma et al. 2002; Liaudanskas et al. 2014). High antioxidant potential of flax elicited cultures has been correlated with high concentration of phenolic acids and other metabolites. Furthermore, a positive correlation also existed among DPPH, FRAP and ORAC, in line with a previous report of Dudonné et al. (2009). Similarly, a positive correlation was found between polyphenols and FRSA, in agreement with previous studies of Anjum et al. (2017a, b), which suggests that the increase in FRSA might be linked with enhanced biosynthesis of free-radicle

Table 2 Antioxidant potential (DPPH, FRAP and ORAC) of cell cultures of flax grown on 200 mg/L yeast extract

Day	ORAC (TE*)		FRAP (TE*)		DPPH (%)	
	Control	Y200	Control	Y200	Control	Y200
5	2.1 ± 0.76^{a}	2.89 ± 0.77^{a}	4.4 ± 0.72^{a}	4.8 ± 0.69^{a}	58.12 ± 1.16^{a}	62.3 ± 1.27^{a}
10	2.7 ± 0.12^{a}	3.78 ± 0.14^{b}	4.89 ± 0.63^{a}	6.12 ± 0.78^{b}	61.46 ± 1.23^{a}	68.2 ± 1.34^{b}
15	3.12 ± 0.66^{b}	4.40 ± 0.39^{c}	5.21 ± 0.44^{a}	7.73 ± 0.60^{b}	70.23 ± 1.34^{b}	74.63 ± 1.13^{c}
20	4.64 ± 0.71^{c}	7.47 ± 0.97^{d}	7.69 ± 0.36^{b}	13.28 ± 0.91^{c}	80.74 ± 1.11^{c}	84.22 ± 1.36^{d}
25	6.34 ± 0.22^{d}	12.16 ± 0.72^{e}	9.67 ± 0.47^{c}	16.56 ± 2.15^{d}	85.36 ± 1.41^{d}	87.44 ± 1.46^{e}
30	10.63 ± 0.29^{e}	21.33 ± 1.17^{f}	15.3 ± 0.73^{d}	29.46 ± 0.11^{e}	81.12 ± 1.46^{c}	89.06 ± 1.64^{e}
35	8.64 ± 0.77^{f}	24.63 ± 0.71^{g}	12.36 ± 0.4^{e}	31.19 ± 1.58^{e}	79.39 ± 1.64^{c}	92.2 ± 1.34^{f}
40	7.34 ± 0.64^{d}	16.34 ± 0.81^{h}	8.63 ± 0.22^{c}	20.41 ± 0.34^{f}	74.4 ± 1.33^{e}	87.6 ± 1.42^{e}
45	4.6 ± 0.33^{b}	7.4 ± 0.74^{d}	6.23 ± 0.11^{b}	10.36 ± 0.76^{c}	71.74 ± 1.11^{b}	84.3 ± 1.21^{d}

Values are mean ± SE of three replicates. Columns with same words are not significantly different ($p < 0.05$)

**TE* expressed in in µmol of Trolox C equivalent per mg DW of extract

scavengers; lignans, neolignans and other metabolites (Hu et al. 2007).

Lignans and neo lignans

Contrary to seed that naturally only accumulates lignans [i.e. mainly (+)-secoisolariciresinol (Ford et al. 2001; Hano et al. 2006)], in vitro cultures of flax accumulated both lignans SDG (Hano et al. 2006) and LDG (Beejmohun et al. 2007; Anjum et al. 2017) but also substantial amounts of neolignans DCG (Attoumbré et al. 2006) and GGCG (Beejmohun et al. 2007; Anjum et al. 2017a, b). Here, accumulation of lignans and neolignans was investigated using authentic standards and a validated HPLC-DAD method as described in our previous work (Anjum et al. 2017a, b). Results showed that the highest accumulation of secoisolariciresinol diglucoside (SDG) and lariciresinol diglucoside (LDG) at Y200 was observed at day 35, with 3.36 and 1.3 fold increase over control, respectively (Fig. 3a, b). The highest concentration of dehydrodiconiferyl alcohol glucoside (DCG) was also noted in medium supplemented with Y200 after 35 days and it was almost 4.26 fold that of control (Fig. 3c). While no significant increase was noted in GCGG accumulation as shown in Fig. 3d. Anjum et al. (2017a), reported the polyphenol SDG in UV elicited cultures of flax which was almost 0.73 fold lower than observed here. Corbin et al. (2015), also documented the accumulation of SDG in ABA induced cell cultures of flax, which was almost two-fold lesser than YE elicited cultures. Moreover the amount of DCG was also found to be 1.7 times higher than previous reports of Hano et al. (2006), when flax cells were elicited with various fungal elicitors, while minute amount of SDG and LDG was accumulated.

Pinoresinol–lariciresinol reductases (PLRs) have been isolated and characterized in several species including flax, and have been reported to be involved in one or two steps

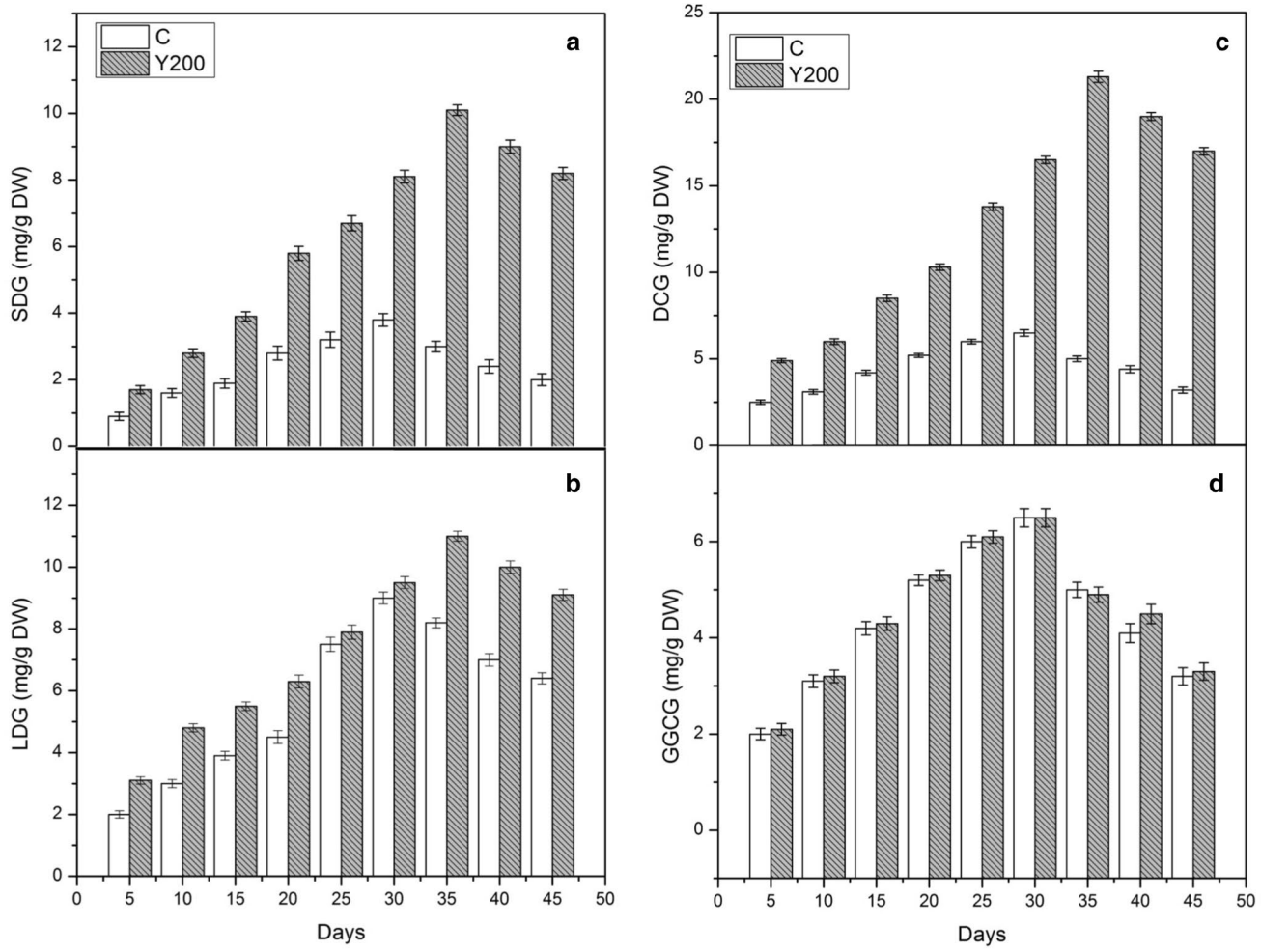

Fig. 3 Biosynthesis of lignans; **a** Secoisolariciresinol diglucoside (SDG), **b** Lariciresinol diglucoside (LDG) and neolignans; **c** dehydrodiconiferyl alcohol glucoside (DCG) and **d** Guaiacylglycerol-β-coniferyl alcohol ether glucoside (GGCG) in cell cultures of flax grown on 200 mg/L yeast extract. Values are mean ± SE of three replicates

reduction of pinoresinol leading to lariciresinol or secoisolariciresinol, respectively. In flax, two PLRs with opposite stereo specificity and differing gene expression patterns have been functionally characterized (von Heimendahl et al. 2005; Renouard et al. 2014; Corbin et al. 2017). However, both were reported to catalyze two step reduction of opposite pinoresinol isomers leading to opposite secoisolariciresinol isomers. Here, detection of lariciresinol is not necessarily contrary to these observations since both aglycone and glycosylated forms of lariciresinol have been detected in flax in stem (Huis et al. 2012), and isolariciresinol in seed (Ramsay et al. 2017). The action of pinoresinol reductases, catalyzing the sole first step reduction of pinoresinol, could partially explain these accumulations of lariciresinol forms. In *Arabidopsis thaliana* and *Isatis indigotica*, pinoresinol reductases have been already characterized (Nakatsubo et al. 2008; Xiao et al. 2015).

PLRs belong to the family of short-chain dehydrogenase/reductases (SDRs), named PIP family grouping together pinoresinol (lariciresinol) reductases (P(L)R), isoflavone reductases (IFR) and phenylcoumaran benzylic ether reductases (PCBER) (Gang et al. 1999). Members of this family are present as a small multigene family in plant genomes (Persson and Kallberg 2013). Interestingly, Huis et al. (2012) have described the expression of a third PLR in flax stem accumulating lariciresinol. Therefore, it is highly plausible that at least a third PLR present in flax genome could act as a pinoresinol reductase and explain lariciresinol accumulation. Here, the differences in accumulation profiles and kinetics of SDG and LDG in our flax cultures strongly suggest (i) a differential expression regulation profiles of the several PLRs involved in their biosynthesis and (ii) a possible different physiological role(s) for these compounds.

Conclusion

In the current study, different concentrations of YE were used to elicit flax cell cultures. Among studied concentrations, 200 mg/L YE favored optimal elicitation. Flax cell culture grown on Y200 showed enhanced biomass accumulation, total phenolic and flavonoid content than control. Moreover, this concentration of YE also enhanced the biosynthesis of SDG, LDG and GGCG. These results suggested that YE could be an effective elicitor to enhance the secondary metabolites profile of flax on both pilot and commercial scale.

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Author contributions MN performed experiments, analyzed and compiled data and prepare manuscript. AZ and WA assisted with experiments and analyses of TPC and TFC. BHA conceived the idea, provided platform to complete this research, reviewed the manuscript. LG, SD and CH contributed to HPLC phytochemical analysis. NGG and CH contributed to critical reading of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declares that they have no conflict of interest.

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