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Short Communication

Lipid content and fatty acid change in the developing silique wall of mustard (*Brassica juncea* L.)

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ABSTRACT

The present study describes changes in the lipid and fatty acid contents of developing silique wall of mustard (*Brassica juncea* L.). Initially, at 20 days after flowering (DAF), we observed a rapid accumulation of total lipids (75.8 mg/g dry weight [wt.]), comprising 51.4 ± 0.66 and 23.7 ± 0.12 mg/g dry wt. of polar and non-polar lipids, respectively. A gradual decline in lipids in the silique wall was observed until maturity. During seed development, the fatty acid profile of the silique wall varied significantly among all stages of maturity. Seed development resulted mainly in an increase in linoleic acid and a decrease in linolenic and palmitic acids. At full maturity, the main fatty acids present were linolenic (30.1 ± 0.09%), linoleic (24.2 ± 0.60%), palmitic (19.5 ± 0.63%), oleic (16.8 ± 0.66%), and stearic (8.1 ± 0.38%) acids.

1. Introduction

In India, Indian mustard *Brassica juncea* (L.) Czern and Coss is an important oilseed crop, cultivated in over approximately 6.32 million ha with an average production of 6.12 million tons (Mandal et al., 2010). *B. juncea* is also the major crop in India, occupying around 80% of the area cultivated for oilseed *Brassica* (Singh and Singh, 2013). India hosts approximately 25.6% of the world area under oilseed Brassica, contributing to 14.7% of the production (Vittal et al., 2004).

Traditional rapeseed oil contains 25–45% of erucic acid that is considered unsuitable for human consumption. Canola is distinct from traditional rapeseed due to its reduced levels of erucic acid and glucosinolates (Iqbal et al., 2008). However, through extensive breeding programmes mustard has been developed into an edible oil crop with a fatty acid profile that is very similar to canola containing zero erucic acid (Kirk and Oram, 1981), 55–60% oleic acid, 6.5% linolenic acid, 31–33% linoleic acid and glucosinolate concentration 0–20 μmol/g (Gunasekera et al., 2006).

B. juncea is a potential edible oil crop having various advantages over *B. napus*, including more vigorous seedling growth, quicker ground covering ability, less seed pod shattering, comparatively higher percentage of oil and protein, greater tolerance to different stresses, and enhanced resistance to the blackleg fungus *Leptosphaeria maculans* (Woods et al., 1991; Iqbal et al., 2008).

Several studies have already shown lipid biosynthesis in *Brassica* seeds (Munshi et al., 1990; Perry and Harwood, 1993; Chhokar et al., 2008), but no attempt has been made so far to determine the fatty acid composition of the various lipid classes in the silique wall of *B. juncea* during seed development. The siliqua or silique (oilseed pod) consists of two carpels separated by a replum, containing the main vascular tissues of the pod. A layer of 2–3 parenchymatous cells between the carpel edges and the replum forms the separation or dehiscence zone. Separation of the cells in the dehiscence zone takes place about 7 weeks after anthesis, immediately before moisture loss is complete (Child et al. 1998). Du and Halkier (1998) reported synthesis of glucosinolates in the developing silique walls and seeds of *Sinapis alba.* They concluded that initially glucosinolates are synthesized in the silique wall and further translocated to the seeds.Thus, the present investigation was undertaken to study the changes in various lipid classes and their fatty acid compositions of developing siliqua wall in *B. juncea.*

2. Materials and methods

Mustard crop (*Brassica juncea* L. CV RH-30) was raised in the earthenware pots filled with 5 kg of sandy loam soil in a naturally lit screen house and the pots were lined with polyethylene bags to avoid contamination. A recommended dose of nitrogen (60 ppm) and phosphorous was also given in the form of urea and potassium

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dihydrogen orthophosphate. A basal dose of micronutrient Zn, Mn, Cu, and Fe at the rate of 5, 2.5, 2.5, and 10 ppm, respectively was also supplied.

After the emergence of seedling, three uniform plants/pot was retained. About three hundred plants were tagged at the initiation of flowering and silique were collected after 20, 40, and 60 days after flowering and at maturity. The silique was further separated into silique wall and seed samples. Silique walls were dried in a hot air oven maintained at 70 °C.

Total lipids were extracted according to the methods of Folch et al. (1957). Five hundred mg of sample containing 0.5 g anhydrous sodium sulfate was crushed thoroughly and mixed with 100 ml of chloroform: methanol (2:1 v/v). The contents was shaken for one hour and filtered through the glass sintered funnel. The solvent was distilled off under vacuum. To this, 20 volumes of chloroform: methanol (2:1 v/v) and 5 volumes of 1 percent sodium chloride solution was added. The content was transferred to separating funnel, shaken and allowed to stand for 5 min. The pure lipids fraction came in the lower chloroform layer, while soaps glycerols and other water soluble impurities remained in the upper layer. The lower phase was recovered and the upper phase was again treated with 5–10 ml of chloroform: methanol mixture to obtain the residual lipids if any. To remove moisture, anhydrous sodium sulfate was added and the excess solvent was removed under vacuum.

Total lipids extracted were separated into polar and non-polar lipid fraction using the method of Nichols (1964). A known weight of lipid sample was dissolved in 3 volumes (w/v) of petroleum ether (boiling range 80–100 °C) pre-equilibrated with 95% methanol. This solution was extracted thrice with equal volumes of 95% methanol (saturated with petroleum ether). All the methanol extracts were combined and back extracted with one third volume of petroleum ether. The polar lipids were obtained by evaporation of methanol under vacuum. Similarly, non-polar lipids were obtained by evaporating petroleum ether and their amounts were determined gravimetrically.

Total phospholipids and glycolipids were estimated by the method of Brockhuyse (1968) and Trevelyan and Harrison (1952) respectively. Total, non-polar, and polar lipid fractions were methylated (Luddy et al. 1968), and fatty acids were estimated in Hewlett Packard (model no-5730 A) gas chromatography equipment with a flame ionization detector. A stainless steel column (305 cm × 3.175 mm) packed with 20% diethylene glycol succinate (DEGS) on 60–80 mesh chromosorb was used. The temperature of 185 °C and a nitrogen (carrier gas) flow rate of 35 ml/min were maintained. The individual peak was identified by comparison of their retention times with those of the standard fatty acid methyl esters. The area under the individual peak was calculated and converted directly into relative percentage.

Table 1
Lipid composition (mg/g) of mustard (*Brassica juncea. L*) silique wall during development (n=3).

Lipid composition	Stage	Mean (mg/g)	Std. error	F-value	Sig.
Total lipids	20 DAF	75.8	0.25		
	40 DAF	63.9	0.63	4031.2	0.001
	60 DAF	37.6	0.14		
	Maturity	28.5	0.08		
Polar lipids	20 DAF	51.4	0.38		
	40 DAF	44.7	0.14	2426.2	0.001
	60 DAF	25.8	0.45		
	Maturity	19.1	0.13		
Non-polar lipids	20 DAF	23.7	0.07		
	40 DAF	18.3	0.05	6738.2	0.001
	60 DAF	10.6	0.10		
	Maturity	7.2	0.12		
Phospholipids	20 DAF	18.7	0.02		
	40 DAF	13.1	0.47	364.7	0.001
	60 DAF	9.2	0.08		
	Maturity	8.5	0.10		
Glycolipids	20 DAF	32.3	0.64		
	40 DAF	29.4	0.20	829.7	0.001
	60 DAF	16.9	0.23		
	Maturity	10.6	0.11		

Maturity period varies from 80–90 DAF.

3. Statistical analysis

Triplicate experiments were performed and means were compared using SPSS statistical software, Release version 20 (IBM SPSS, Inc., 2009, Chicago, IL, USA, www.spss.com). One-way ANOVA was used to analyze the differences between means. All statements reported in this study are at the $P < 0.05$ levels.

4. Results and discussion

4.1. Lipid composition of mustard silique wall during seed development

Table 1 shows the lipid composition of mustard silique wall during seed development. One-way ANOVA revealed significant effect of seed development on lipid composition of silique wall. Initially, at 20 days after flowering (DAF), there was a rapid accumulation of total lipids (75.8 mg/g dry wt.) followed by a constant decline until maturity. Among the total lipids, polar lipids (51.4 mg/g dry wt.) were found to be the major components, while non-polar lipids (23.7 mg/g dry wt.) were present in comparatively small amounts. When phospholipids and glycolipids were observed, glycolipids (32.3 mg/g dry wt.) were found to be the major component, showing maximum accumulation at 20 DAF followed by a gradual decline until maturity. The rapid accumulation of polar lipids at 20 DAF may be because polar lipids are the major constituents of cell membrane lipids.

Fig. 1 clearly shows that during seed development, there was a slow accumulation of total lipids in the seeds up to 20 DAF, followed by a rapid accumulation. In contrast, the silique wall showed maximum lipid levels at 20 DAF, which represents a period of rapid oil accumulation (Turnham & Northcote, 1983), followed by a gradual decline in lipids as seed development progresses. Similar trend has been reported previously for glucosinolates found in Cruciferae. Toroser et al. (1995) reported that a continuous increase in the accumulation of 35 S-labeled glucosinolates in the *Brassica napus* L. seed was correlated with a progressive decrease in labeled glucosinolates in the silique wall. Zhao et al. (1993) demonstrated that allylglucosinolates added exogenously to the intact silique were rapidly translocated from the wall to the seeds. Other studies on oilseed rape have reported that the silique wall is the major site for biosynthesis of the glucosinolates found in the seeds, indicating that the glucosinolates synthesized in the silique wall are subsequently translocated to the seeds (Du and Halkier, 1998).

4.2. Fatty acid composition of total lipids in developing silique wall of mustard

Table 2 shows the fatty acid profile of mustard silique wall during seed development. The results are found to be statistical significant with a p value of 0.001 and the F value ranging from 21.1 to 537.9. Initially, at 20 DAF, linolenate (43.3%) and palmitic acid (22.2%) were found to be the major fatty acids among the total lipids. This may be because these fatty acids are considered components of chloroplast membrane lipids in mustard seed (Sukhija et al., 1983 and Gupta et al., 1991). Stearic acid was not found to be a major contributor throughout development, with a maximum level of 8.2% at maturity. These results are not in