Short Communication

Changes in Glucosinolates, Sinapine and Flavonols during Seed Development of *Camelina sativa* (L.) Crantz

ABSTRACT

Camelina sativa (L.) Crantz is an oilseed crop whose oil is used as biofuel and the meal as animal feed. While the seed composition is widely documented, little is known about variations in the concentration of some compounds during seed development of camelina. The aim of this study was to characterize changes in the content of glucosinolates, sinapine, sinapic acid and flavonols (quercetin and koempferol) at different stages of seed maturation. Glucosinolates are accumulated in camelina seed in the first 4 weeks after pollination, while the other substances were transiently accumulated during development and may play a role in seed development.

Keywords: Camelina sativa; flavonols; glucosinolates; koempferol; quercetin; seed development; sinapic acid; sinapine.

1. INTRODUCTION

Camelina sativa (L.) Crantz is an ancient culture (known since the Bronze Age) whose oil in previous centuries was used for lighting the lamps. Camelina or false flax is a crop of temperate zones that is becoming important specially in North America and Europe. The interest for this oilseed crop has risen because it has several agrotechnical advantages: cultivation is environmentally friendly (application of pesticides/herbicides is not needed), the life cycle is less than 4 months (85–100 days), the plant grows well even in low fertile soils and it is resistant to drought and cold [1-4]. From the seed, a very appreciated oil is obtained to produce jetfuels (biokerosin) [5]. After oil extraction, the remaining meal (by-product of oil production) is a source of protein (30-40%) of good quality for animal feed [6-7]. Due to its high omega-3 content (30% of oil), it could also be used as a food ingredient in the future.

Camelina meal is a significant source of phenols (antioxidants like flavonols) but also contains some anti-nutritional compounds. Of the latter, two types of substances deserve attention: 1) glucosinolates (GSLs) that by action of the enzyme myrosinase produce a spectrum of toxic substances (isothiocyanates, thiocyanates, nitriles and epithionitriles); 2) Sinapine (choline ester of sinapic acid) that has several undesirable properties as a constituent in animal feeds (bitter tasting, a fishy odour or taste in the eggs) [8-9].

The composition of camelina seed is well known [6], but little is known about the changes in the composition of the seed during development. The present study was undertaken for give information on the variations of some specific compounds (glucosinolates, sinapine and flavonols) at different stages of seed development.

2. MATERIALS AND METHODS

Camelina sativa (L.) Crantz var. Calena was sown in spring in a hilly area of Lombardy (Casazza - 45°45′N - 9°54′E; 450 m AMSL). After pollination, immature seeds were weekly collected at different stages of development from 7 days until maturity (49 days after pollination, DAP). A lot of care has been taken in seed harvesting to consider plants with a similar stage of development. Three different samples for each stage were collected.

Seeds were ground in a mortar and flour defatted with hexane (1:10, w/v). The solution was vigorously shaken for 30 minutes. After centrifugation, the supernatant was collected and the extraction procedure repeated. The defatted flour was used for analyses.

GSL extraction and assay was basically according to the official method (ISO9167-1) but with the HPLC separation adapted to camelina GSLs [10]. After extraction with 70% hot ethanol and centrifugation for 15 min at 15,000 rpm, the supernatant was loaded onto a DEAE-Sephadex

A-25 column (100 mg) in formate form. The unbound compounds were washed twice with 1 mL of 20 mM Na acetate (pH 4.0). The retained GSLs were desulfated overnight by addition of 50 µL of sulfatase (500U). Desulfo-GSLs were eluted from the column with 1 mL of ethanol and the samples dried at 65˚C. The samples were resuspended in 20% ethanol and filtered with 0.22 µm Costar Spin-X Centrifuge Tube Filter (Corning Incorporated, NY, USA) before HPLC analysis. HPLC separation of desulfo-GSLs was according to Russo et al. [11] and detection was at 229 nm.

Sinapine and sinapic acid were extracted from defatted flours with 70% methanol for 30 min at 75˚C [12]. The samples were then centrifuged for 10 min at 15,000 rpm and the supernatant diluted 1:1 with HPLC grade water. Before analysis, the samples were filtered with 0.22 µm Costar Spin-X Centrifuge Tube Filter (Corning Incorporated, NY, USA). The HPLC analysis was according to Clausen et al. [13], but slightly modified by us [9]. Sinapine and sinapic acid were separated by isocratic HPLC with a mobile phase consisting of 13.5% acetonitrile in 10 mM Na acetate (pH 4.0) and detection at 330 nm. The compounds were separated on a 100 × 2.1 mm Waters Atlantis T3 C18 column (2.6 µm) at a flow rate of 0.275 mL min^{-1}. The peaks of sinapine and sinapic acid eluted at 4.5 and 8.7 minutes.

Flavonols were determined on the same alcoholic samples used for the analysis of GSLs. These compounds were separated according to Kumar et al. [14]. The run was carried out on a Phenomenex Kinetex C18 column (250 x 4.6 mm, 5 µm), at a flow rate of 1 ml min^{-1} and at 35°C. Mobile phase were water (A) and acetonitrile with 0.02% trifluoroacetic acid (B), respectively. The program started with 20% B for 5 min, then 40% B in 8 min and 50% B in 12 min. The column was then returned to the initial conditions in 10 min. Phenols were detected at 280 nm and peaks of quercetin and koempferol were identified by comparing their retention times (14.8 and 16.4 min, respectively) with those of standards.

All samples were analyzed in triplicate and mean values and standard errors were reported.

3. RESULTS AND DISCUSSION

3.1 Glucosinolates at Different Developmental Stages

In Fig. 1 are shown the levels of the three GSLs present in camelina (GSL1: 9-methyl-sulfinyl-nonyl-GSL; GSL2: 10-methyl-sulfinyl-decyl-GSL; GSL3: 11-methyl- sulfinyl-undecyl-GSL) [10]. As can be seen, the highest levels of GSLs are reached between 21-28 DAP and then their content decreased. If we consider that even after 28 days from pollination the seed continues to synthesize oil and protein (protein synthesis is maximum between 21-42 DAP) [15], it is likely that the translocation of GSLs from plant to seed occurs mainly in the first month of seed development. As a consequence of this, on the dry weight (DW) basis, the content of GSLs decreased after 28 DAP.

3.2 Sinapine and Sinapic Acid During Seed Development

Sinapine is considered a source of choline and sinapic acid for the young seedlings [16]. It is well known that sinapic acid in brassicaceous plants (like camelina) may be converted into many O-ester conjugates (sinapine is a member of this family) which are typical of a certain stage of development [17]. During the development of camelina seed (Fig. 2), there was a large increase in sinapine and sinapic acid soon after pollination (14 DAP). The content of these two substances then decreased with the development of the seed. It is really significant that at maturity, only a residual fraction of sinapine is still present (about one fifth). The decrease of sinapic acid after 14 days after pollination could be due to the synthesis of lignin (of which it is a precursor) that at maturity represents 5-6% of the dry seed [6].

3.2 Flavonols during Development of Camelina Seed

Like sinapic acid, also flavonols derive from the phenylpropanoid pathway. These compounds are considered developmental regulators and/or signaling molecules [18]. As shown in Fig. 3, the levels of quercetin and koempferol increased many times up to 21 DAP and resulted very low after 35 DAP. At maturity, only quercetin was detected and this is in agreement with Terpinic et al. [19] and Rahman et al. [20] who found only quercetin in camelina meal. The peak of flavonols at 21 days after pollination coincides with the beginning of the maximum accumulation of storage compounds (max increase in seed weight). It is interesting to note that in *Arabidopsis* (a specie very close to camelina), flavonols localized in the nucleus can regulate the transcription of genes required for growth and development [21]. This could suggest an involvement of these compounds in seed development rather than in avoiding the generation of reactive species of oxygen (a

scavenging role). Another possible role of flavonols is their control on the movement of the auxin [18], a hormone that could control the filling of the seed.

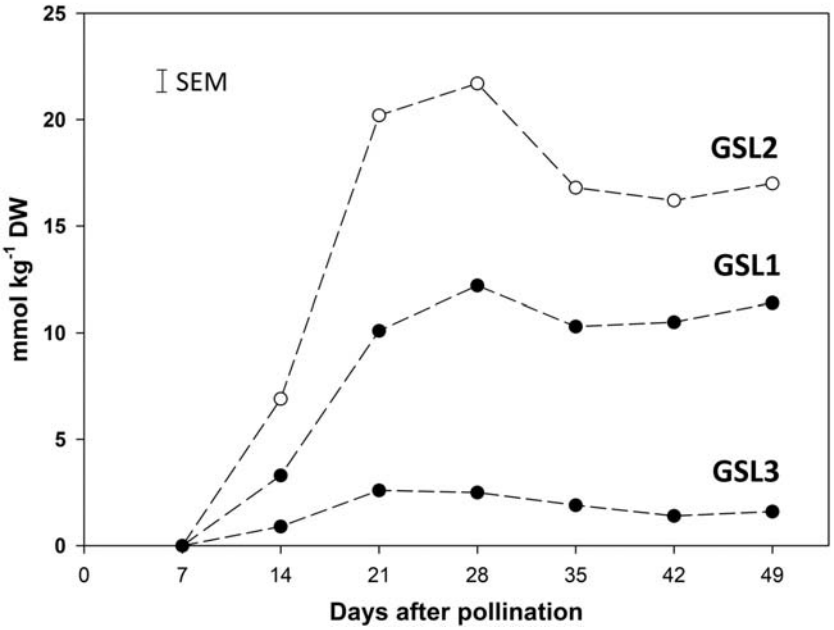

Fig. 1. Glucosinolate (GSL1, GSL2 and GSL3) contents during the seed development of *Camelina sativa*. Data are expressed on a dry weight (DW) basis

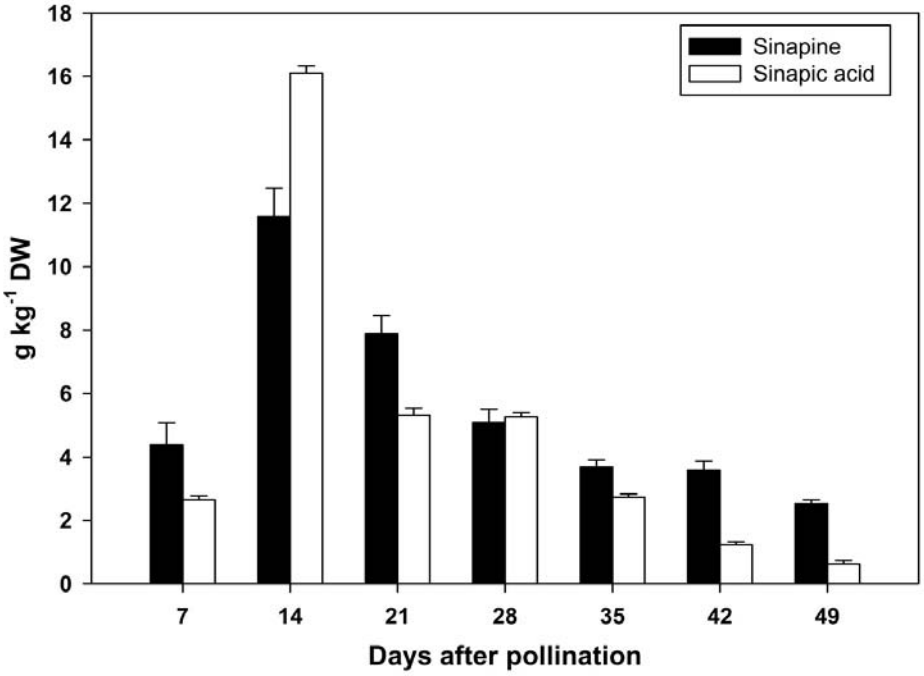

Fig. 2. Sinapine and sinapic acid contents during the seed development of *Camelina sativa*. Data are expressed on a dry weight (DW) basis

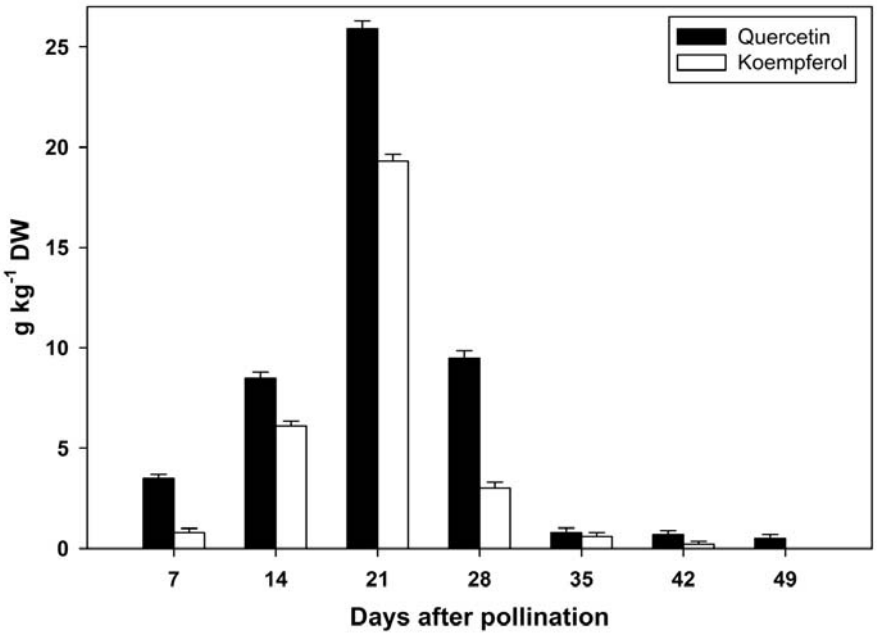

Fig. 3. Content of flavonols (quercetin and koempferol) during the seed development of *Camelina sativa*. Data are expressed on a dry weight (DW) basis.

4. CONCLUSION

Analysis of the content of these substances during the seed development of camelina showed that: 1) GSLs are storage compounds that are accumulated very early during seed development; 2) sinapine, sinapic acid and flavonols showed a transient accumulation that suggests a specific role early during seed development. Even if sinapine is considered an unwanted substance, however, after ripening its concentration is relatively low.

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