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Original Paper

Osmotic stress induced alterations in fatty acid composition and other metabolic responses in seedlings of *Sinapis alba*

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Abstract

Sinapis alba L. (white mustard), an annual member of Brassicaceae, is an important oilseed crop with significant agronomic traits including tolerance to abiotic stress. The present study was aimed to assess the changes in biochemical attributes of *S. alba*, mainly the composition of lipids and fatty acids, in response to osmotic stress. Seedlings were subjected to stress condition by growing them on media supplemented with three different concentrations of polyethylene glycol (PEG 6000) solutions, respectively with –0.04, –0.16 and –0.64 MPa osmotic potential. Compatible solute content, lipid content, and lipid peroxidation of the seedlings were measured. Total fatty acid composition was analysed by gas chromatography mass spectrometry. Overall negative impact of PEG-mediated osmotic stress was apparent from seedling morphology. Total lipid content decreased in stressed plants together with increased level of triacylglycerol. Increase of lipid peroxidation in stressed condition indicated suppression of membrane integrity. The key outcome was the significant increase of unsaturation level of fatty acids due to higher accumulation of linoleic acid, α-linolenic acid and erucic acid in the seedlings exposed to higher osmotic stress. The results indicate that, in white mustard, the biochemical changes induced by osmotic stress mainly depend on changes in lipid composition and fatty acid unsaturation level, which maintain membrane stability during the early stage of seedling development.

Key words: fatty acids, lipid peroxidation, lipids, osmotic stress, proline, *Sinapis alba*, sugars, triacylglycerol.
Abbreviations: DAG, diacylglycerol; DW, dry weight; FAME, fatty acid methyl esthers; FFA, free fatty acids; FW, fresh weight; GC-MS, gas chromatography-mass spectrometry; MDA, malondialdehyde; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; RWC, relative water content; SFA, saturated fatty acids; TAG, triacylglycerol; TLC, thin layer chromatograhpy; TW, turgid weight.

Introduction

Drought is certainly a deleterious abiotic factor that has become a major concern worldwide, as it considerably reduces the quality and productivity of crop plants (Hanson, Hitz 1982). Moderate to severe drought conditions, created due to a prolonged period of water shortage, lead to osmotic stress by accumulation of salts and ions in the upper layer of soil (Ma et al. 2020). Seed germination and the period of seedling establishment in any crop species are the most sensitive phases to osmotic stress (Mickky, Aldesuquy 2017). Seedlings exposed to osmotic stress generate several complex molecular responses at the cellular level to regulate osmotic pressure and turgor (Bray 1997; Bavaro et al. 2007). One of the most significant of these responses is alteration of composition and properties of lipids (Navari-Izzo et al. 1993). Although the responses are genetically mediated, they can effectively result due to a stress condition (Bellaloui et al. 2015). Osmotic stress invariably reduces total lipid content, and alters the composition of polar lipids and their fatty acids (Gigon et al. 2004). Hyperosmotic stress also leads to oxidative damage in cells by enhancing the accumulation of reactive oxygen species, which results in lipid peroxidation, suppression of lipid biosynthesis (Monteiro de Paula et al. 1990), and stimulation of lipolytic and peroxidative activity (Matos et al. 2001).

In oilseed producing crops, mobilization and distribution of lipid resources largely determine the tolerance ability of plants (Bewley 2001). The degree of unsaturation of acyl residues of polar lipids determines physical features of membranes and affects functions of membrane proteins (Quinn et al. 1989). Several reports suggest that in higher plants, unsaturation degree of fatty acids changes in response to osmotic stress for maintaining membrane stability, thereby acclimatizing to the adverse condition (Aziz, Larher 1998). The change in unsaturation level is mainly achieved by the regulation of fatty acid desaturase activity (Upchurch 2008).

Sinapis alba L. (white mustard), an annual member of the family Brassicaseae, is available worldwide as weed, and is also one of the earliest cultivated oilseed crops (Cserhalmi et al. 2000). It is extensively used as vegetable and source of condiments for years in different regions of India. Besides having a number of cropping applications, this plant has significant agronomic value due to its high oil content (Blake, Diosady 2000). Recently, the seed oil is receiving more interest for its potential to be used as feedstock for production of biodiesel (Mitrovic et al. 2020). However, this plant also has some important agronomic traits such as its higher tolerance to water deficit condition, compared to the other oilseed crops (Downey et al. 1975; Ciubota-Rosie et al. 2013). Thus, it could be important to investigate the physiological and biochemical responses of this plant when exposed to osmotic stress. The present study was conducted to evaluate the effect of osmotic stress on the early development of *S. alba* seedlings along with their metabolic responses to the stress condition. The primary objective was to characterize the changes that occur in lipid and fatty acid composition when exposed to osmotic stress.

Materials and methods

Plant material and experimental design

Fresh seeds of *Sinapis alba* L. were surface sterilized with 5% sodium hypochlorite and were washed thoroughly. Viable and imbibed seeds were placed for germination in Petri dishes (10 cm) on Whatmann filter papers moistened with solutions of polyethylene glycol (PEG) with water potentials of –0.04 MPa, –0.16 MPa and –0.64 MPa. The PEG (PEG 6000, E. Merck, India) solutions were prepared following the formula of Michel and Kaufman (1973):

$$\Psi s = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, CT + (8.39 \times 10^{-7})\, C^2T,$$

where Ψs is osmotic pressure (Bar), C is concentration (g L^{-1} PEG 6000 in water), and T is room temperature (°C).

The control treatment (0 MPa) contained distilled water as growth medium. For each treatment, twenty seeds were allowed to germinate on each Petri dish, and were kept in dark and humid conditions for 48 h in a germinator at 20 ± 2 °C. Then the seeds were exposed to a 16 h photoperiod (260 μmol m^{-2} s^{-1} photon flux density of photosynthetically active radiation) after emergence of radicles. The seedlings were harvested after six days of growth.

Developmental parameters (shoot length, root length, fresh weight, and dry weight of seedlings) from each experiment set were measured. Lengths of root, shoot and fresh weights were measured immediately after harvest. Dry weight was determined after drying of seedlings at 60 to 80 °C for 48 h to a constant weight.

Analysis of relative water content

Fresh weight of leaf discs of about 1 cm was measured. Turgid weight was measured after incubating leaf discs for 4 h in distilled water (Weatherley 1950). The leaves were subsequently dried in an oven at 65 °C for 13 h. Relative water content (RWC) was calculated using the following formula:

$$RWC = [(FW - DW) / (TW - DW) \times 100,$$

where FW is fresh weight, DW is dry weight, and TW is turgid weight.

Estimation of total soluble sugar content

Total soluble sugars were extracted by homogenizing 200 mg seedlings of each experiment set in 2.5 N hydrochloric acid followed by boiling in water bath for 3 h. Then the extracts were cooled at room temperature and were neutralized with sodium carbonate. Extracts were centrifuged at 10000 rpm for 10 min. Total sugar content of the supernatants were determined following the method of Yemm and Willis (1954). A volume of 1 mL of supernatant was added to 2 mL 75% H_2SO_4 and 4 mL chilled anthrone (0.2% w/v in 75% H_2SO_4), and was incubated at 95 °C for 10 to 15 min till development of a green colour. Absorbance of the developed colour was measured at 620 nm by a spectrophotometer. The result was plotted on a calibration curve (R^2 = 0.995) prepared using different concentrations of standard glucose solution and the content was expressed as mg g^{-1} fresh weight.

Estimation of proline content

Free proline is considered as an osmoprotectant and abiotic stress marker. The standard protocol of Bates et al. (1973) was followed for measurement of free proline. Extraction was performed with a 500 mg plant sample by homogenizing in 10 mL of 3% aqueous sulphosalicylic acid (v/v) followed by centrifugation of homogenate at 5000 rpm for 10 min. A volume of 2 mL supernatant was added to 2 mL of glacial acetic acid and 2mL acid-ninhydrin solution. The mixture was heated in a boiling water bath for 1 h. The reaction was terminated by placing the reaction mixtures in an ice bath. Then, 4 mL toluene was added to the reaction mixture and was stirred well for 20 to 30 s. The intensity of the colour developed in the toluene layer was measured at 520 nm. The proline content of the test sample was calculated by plotting the absorbance on a calibration curve (R^2 = 0.9601), which was prepared using a known amount of proline using a similar experimental method. Proline content was calculated using the following formula:

$$\text{Proline } (\mu mol\ g^{-1}\ FW) = [(\mu g\ \text{proline}\ mL^{-1} \times V) / 115.5] \times [5 / W],$$

where 115.5 is the molecular weight of proline; V is volume of toluene in mL, and W is weight of sample in g.

Analysis of storage lipids

Total lipids were extracted following the phase separation method of Bligh and Dyer (1959), using a mixture of chloroform, methanol and water having a ratio of 2:2.3:2.3 (v/v). Total lipid content was estimated by gravimetric

method using the chloroform phase. Separation of storage lipids was performed by thin layer chromatography (TLC) using silica gel 60F 254 TLC plates (E. Merck, Germany) as the stationary phase. A volume of 500 µL of chloroform extract was spotted on a pre-heated TLC plate and the lipid classes were separated on the stationary phase using a solvent mixture of *n*-hexane: diethyl ether: glacial acetic acid (80:20:1.5 v/v) as the mobile phase. Bands of lipid classes were visualized by placing the TLC plates inside a chamber saturated with iodine vapour and were identified by comparing with the R_f values of standard triacylglycerol (TAG), diacylglycerol (DAG) and free fatty acids (FFA).

Analysis of fatty acids by gas chromatography

Fatty acids of total lipids were derivatized to fatty acid methyl esters (FAME) for analysis using gas chromatography-mass spectrometry (GC-MS). For derivatization, the chloroform phase was evaporated to dryness followed by the addition of methanol: benzene: sulfuric acid (86:10:4, v/v) (Christie 1993). The solution was heated at 85 °C for 8 h. Total saponifiable derivatives were eluted to 1 mL *n*-hexane, which was subjected to GC-MS (Agilent 7890A, Agilent Technologies Inc., Santa Clara, USA) analysis using an HP5-MS column (30 m × 0.25 mm × 0.25 µm), preceded by a 10 m Duraguard capillary column and coupled to a triple axis mass detector (Agilent MS-5975C), operated at source temperature 230 °C, auxiliary heater temperature 280 °C, quadrupole temperature 150 °C, electron energy 70 eV and source pressure $2.21e^{-0.5}$ torr. Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. The quantitation of FAMEs was performed by GC (Varian CP 3800) with a VF-1MS capillary column (FactorfourTM, WCOT, 15 m × 0.25 mm × 0.25 µm; Varian, UK) and a flame ionization detector. Nitrogen was used as the carrier gas with a flow rate of 1 mL min^{-1}. The injector and detector temperatures were maintained at 250 °C and 260 °C, respectively. For both GC-MS and GC-FID analyses, the column temperature was programmed from 70 °C (1 min hold) to 260 °C (10 min hold) with a ramping rate of 5 °C min^{-1}. The FAME were identified by comparing their mass spectra with those from the NIST library (2010), co-chromatography with 37 components of a standard FAME mixture (Supelco, Bellefonte, PA, USA), by calculating RRt with respect to a particular FA, and by logRt carbon chain length calculation.

Lipid peroxidation

Total malondialdehyde (MDA) content was estimated as lipid peroxidation following the method of Heath and Packer (1968). Extraction of MDA was performed using 0.1% trichloroacetic acid followed by centrifugation of the homogenate at 10 000 rpm for 10 min. A volume of 1 mL of supernatant was added to 3 mL of 0.5% thiobarbitaric acid in 20% trichloroacetic acid and the mixture was incubated for 25 min at 95 °C. The reaction was terminated by keeping the mixture in ice water. Absorbance of the developed colour was measured at 532 nm and 600 nm. Lipid peroxidation activity was calculated by the following formula:

$$[(A_{532} - A_{600}) \times 1000 \times 4] / 155,$$

and was expressed as nmoles of malondialdehyde mg^{-1} plant tissue.

Statistical analysis

The total experimental setup with all four treatment conditions was replicated thrice at a ten-day interval. For each treatment, a Petri dish containing twenty seedlings of each set-up was considered as a biological replicate. All biochemical analyses were independently performed three times, each after harvesting of seedlings from each set-up. For the biochemical parameters of each biological replica, three technical replications were performed, and the mean values were calculated. The final results of biochemical parameters were computed as the mean values of three biological replicates along with the standard deviations. The comparisons of data were statistically tested by one-way analysis of variance (ANOVA). Significant differences between the experimental treatments were determined by the Tukey's honestly significant difference test with a significance level of 0.05 ($p < 0.05$). Statistical analysis was done using the PAST 3.20 software package (Hammer et al. 2001).

Results

Plant growth, biomass and relative water content

The tested developmental attributes clearly showed negative impact of osmotic stress on the early growth of *Sinapis alba* seedlings. PEG treatment at –0.04 MPa did not significantly affect the development of seedlings, but the adverse effects were more prominent in seedlings exposed to PEG solutions of higher concentration. Germination of seeds was delayed, and the shoot growth was restricted in the –0.64 MPa PEG treatment. PEG treatment significantly lowered dry weight of six days old seedlings (Fig. 1). Treatments of –0.16 MPa and –0.64 MPa solutions resulted in 51.13 and 75.53% reduction of dry weight, respectively, compared to the control (0 MPa).

Relative water content (RWC) was lower in seedlings exposed to –0.16 MPa PEG treatment (71.3%), compared to the control plants (85.04%) at the significance level of $p = 0.005$ (Fig. 1). The difference was not significant for the –0.04 MPa PEG treatment. Due to unavailability of suitable leaves, the parameter was not measured for the –0.64 MPa treatment.

Compatible solutes

No significant change in total soluble sugar content was observed in seedlings in –0.04 MPa and –0.16 MPa PEG treatments compared to the control. High osmotic stress at

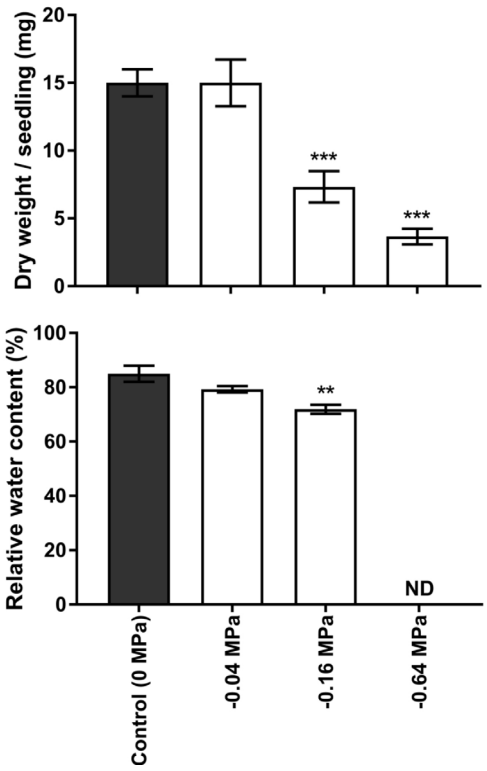

Fig. 1. Dry weight and relative water content of seedlings grown in different treatment conditions. Error bars represent SD values of three biological replicates. Asterisks on error bars indicate significant differences compared to the control set (0 MPa) at the significance level of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). ND, not determined due to unavailability of proper leaf sample.

–0.64 MPa resulted in a significant increase of total soluble sugar content ($p < 0.01$) (Fig. 2). Similarly, proline content, a key indicator of stress, increased with greater osmotic stress ($p < 0.001$ for all sets). Proline content reached to 33.77 μmol g^{-1} FW due to –0.64 MPa PEG treatment, which was only 8.04 μmol g^{-1} FW for control plants. Elevation of the value was consistent with the increase in concentration of PEG solution.

Total lipid content and lipid peroxidation

Total lipid content decreased significantly in the seedlings exposed to high osmotic stress (Fig. 2). The –0.16 MPa and –0.64 MPa treatments caused a 33.93 and 49.11% decrease of total lipid content in seedlings compared to the control, which had total lipid content of 112 mg g^{-1} biomass ($p < 0.001$). However, low concentration of PEG solution did not result in any considerable change in lipid content. Qualitative analysis of neutral lipids revealed the presence of triacylclycerols (TAG) and free fatty acids (FFA) as the major storage forms. Intensity and area of spots of detected lipids predicted higher accumulation of TAG in the lipids extracted from seedlings grown in –0.16 MPa or under higher stress (Fig. 3).

Malondialdehyde content increased considerably with increase of PEG concentration, indicating higher rate of lipid peroxidation due to osmotic stress. Although the change was not significant for seedlings exposed to –0.04 MPa treatment, –0.16 MPa or lower osmotic potential resulted in a significant increase of malondialdehyde content compared to the control ($p < 0.001$) (Fig. 2).

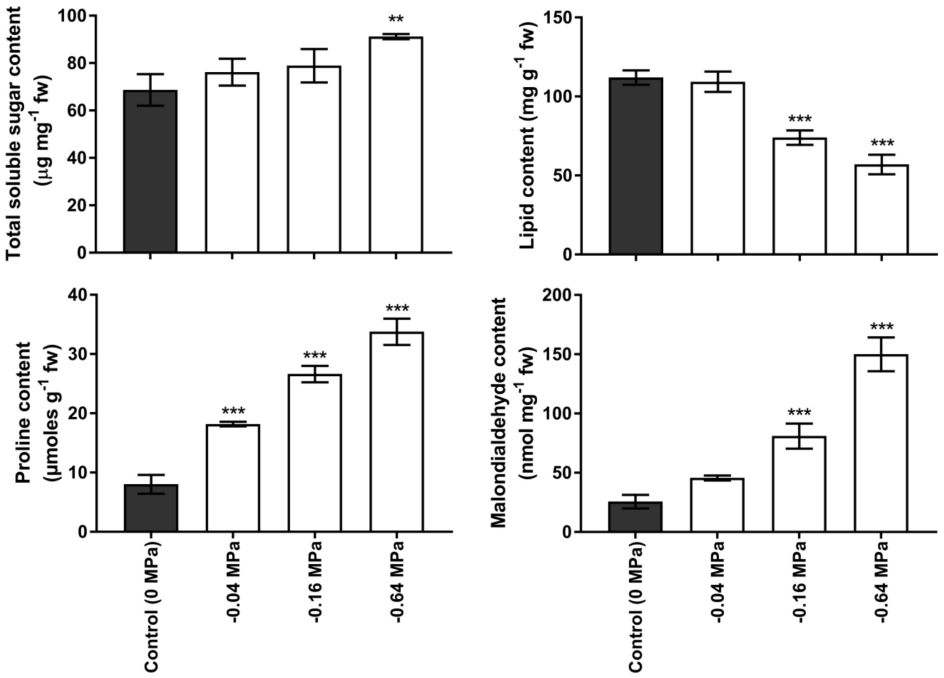

Fig. 2. Total soluble sugars, proline, total lipids and malondialdehyde contents of seedlings grown in different treatment conditions. Error bars represent SD values of three biological replicates. Asterisks on error bars indicate significant differences compared to the control set (0 MPa) at the significance level of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

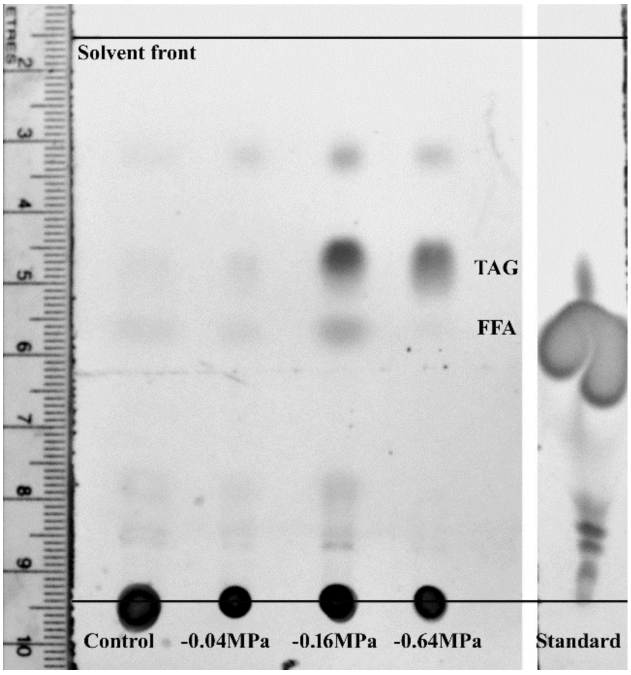

Fig. 3. Thin layer chromatogram showing the spots of triacylglycerol (TAG) and free fatty acids (FFA) of lipid extracts as visualized after keeping the plate within chamber containing iodine vapour.

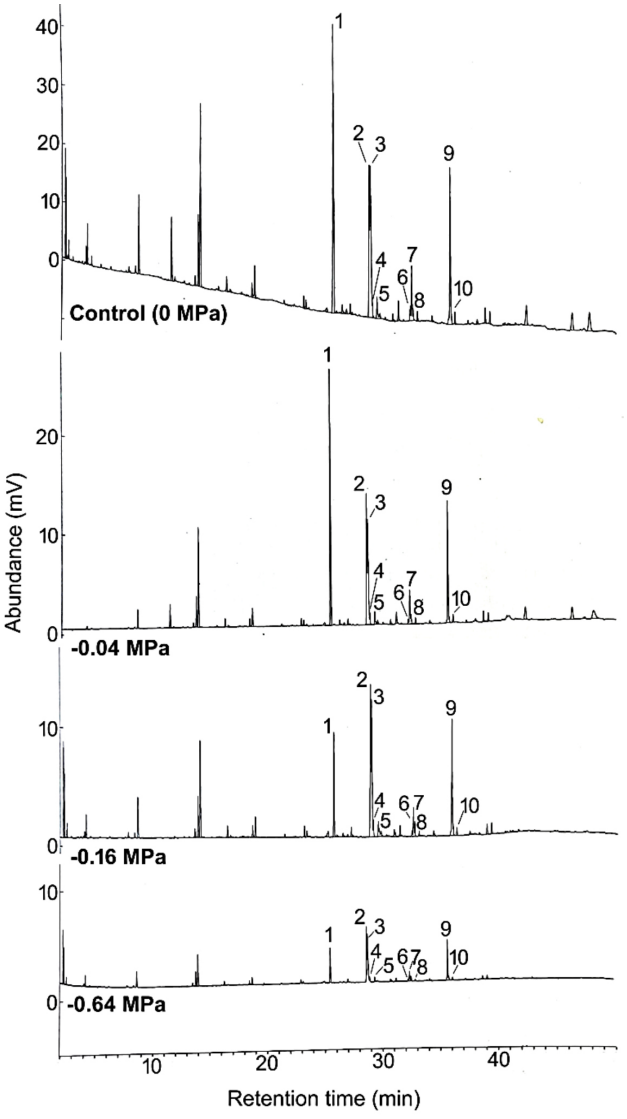

Fig. 4. Gas chromatograms of fatty acid methyl esters of total lipids extracted from seedlings growing in four treatment conditions. Peak numbers represent methyl esters of major identified fatty acids: 1, C16:0; 2, C18:2n6; 3, C18:3n3; 4, C18:1n9; 5, C18:0; 6, C20:2n6; 7, C20:1n9; 8, C20:0; 9, C22:1n9; 10, C22:0.

Fatty acid profile

Fatty acid profiles exhibited the occurrence of a number of saturated, monounsaturated and polyunsaturated fatty acids with varying quantities. Even carbon chain saturated fatty acids (SFA) with length from C12 to C24, viz dodecanoic acid (C12:0), tetradecanoic acid (C14:0), hexadecanoic acid (C16:0), octadcanoic acid (C18:0), eicosanoic acid (C20:0), docosanoic acid (C22:0) and tetracosanoic acid (C24:0) were detected. C16:0 was the predominant fatty acid in all treatments. Four monounsaturated fatty acids (MUFA) from C16 to C22 were identified. 13(Z)-docosenoic acid was the major MUFA in seedlings of all treatments. However, 7(Z)-hexadecenoic acid (C16:1n9), 9(Z)-octadecenoic acid (C18:1n9) and 11(Z)-eicosenoic acids (C20:1n9) were available in low quantities. Among the polyunsaturated fatty acids (PUFA), 9,12(Z,Z)-octadecadienoic acid (C18:2n6) and 9,12,15(Z,Z,Z)-octadecatrienoic acid (C18:3n3) were predominantly available (Fig. 4).

Osmotic stress significantly reduced the total SFA content, the major portion of which was contributed by C16:0 (Fig. 4). The total amount of SFA in seedlings of the control was 42.82% of total identified fatty acids. PEG treatment of –0.16 MPa and –0.64 MPa resulted in 43.11 and 44.93 % reduction of SFA content, respectively ($p < 0.01$) (Table 1). C16:0 content was considerably lower in these two treatments with 14.55 and 14.54% of total identified fatty acids respectively, compared to the control with 38.26% of this fatty acid ($p < 0.001$). However, low osmotic stress did not produce any considerable effect on total SFA content. Total MUFA content was slightly elevated due to high osmotic stress but this difference was not significant (Table 1). C22:1n9 represented the major MUFA contributing 14.91 ± 2.03 % of total identified fatty acids in the control. Interestingly, C22:1n9 content was significantly higher in seedlings exposed to –0.16 MPa and –0.64 MPa PEG solutions ($p < 0.05$ for -0.16 MPa, $p < 0.01$ for –0.64 MPa), in sharp contrast with a decrease in C18:1n9 content ($p < 0.05$ for –0.04 MPa and -0.16 MPa, $p < 0.001$ for –0.64 MPa treatments) (Fig. 5).

PUFA content significantly differed between treatments, where the two major PUFAs, C18:2n6 and C18:3n3 had higher content in stressed conditions. Total PUFA content

Table 1. Total SFA, MUFA and PUFA contents among the experimental sets. Relative changes in treated sets (RC) = [(value of treated sets – value of control) / value of control] ×100; Asterisks indicate significant differences as compared to the control set at the significance level of $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

Experimental sets	SFAs		MUFAs		PUFAs	
	Mean ± SD	RC (± %)	Mean ± SD	RC (%)	Mean ± SD	RC (%)
Control (0 MPa)	42.82 ± 5.93	–	24.12 ± 2.53	–	33.06 ± 4.36	–
PEG –0.04 MPa	38.38 ± 2.41	–10.37	22.72 ± 2.69	–5.80	38.90 ± 0.95	+17.66
PEG –0.16 MPa	24.36 ± 0.77**	–43.11	27.43 ± 1.80	–13.72	48.21 ± 2.34**	+45.83
PEG –0.64 MPa	23.58 ± 4.34**	–44.93	28.09 ± 0.57	–16.46	48.33 ± 4.90**	+46.19

was significantly higher in –0.16 MPa (48.21%) and –0.64 MPa (48.33%) PEG treatments compared to the control (33.06%) ($p < 0.01$). The control set contained 18.27% C18:2n6 and 13.09% C18:3n3, which increased to 27.49 and 19.13%, respectively, in the –0.64 MPa PEG solution treatment ($p < 0.05$ for C18:3n3, $p < 0.01$ for C18:2n6) (Fig. 5).

Discussion

In the present study, PEG effectively created a stressed condition, which adversely affected early seedling development and altered the overall fatty acid composition of seedlings. Generally, PEG mediated osmotic stress imitated a severe water deficit condition (Muscolo et al. 2014). PEG molecules are chemically inert, non-ionic and do not enter the apoplast of plant tissue (Ranjbarfordoei et al. 2000). When PEG solution is added to the hydroponic medium, it reduces the water potential causing outward flow of water from plant tissue (Meneses et al. 2011). PEG 6000 solutions of low water potentials were also previously used for in-vitro screening of stress for creation of a controlled water deficit condition (Toosi et al. 2014).

Negative impact of osmotic stress on the growth of seedlings was apparent from the morphological parameters. High concentration of PEG (–0.64 MPa) effectively restricted the growth of shoots. Generally, changes in root growth along with number and density of root hairs are common adaptive features in drought tolerant varieties, which maintain effective water uptake even under stress (Ehdaie et al. 2012). However, reduction in growth of roots of *S. alba* along with density of root hairs in the present study, especially at high concentration of PEG, indicates the detrimental effect of osmotic stress.

Relative water content (RWC) is a key trait demonstrating water relations and physiological conditions of a plant (Hemmati et al. 2018). Reduction of dry weight and RWC of seedlings grown in PEG solutions indicated negative impact of the solution on roots and cotyledons, which can result from a lower absorption rate. Effect of osmotic stress on RWC was previously demonstrated (Halder, Burrage 2003). Deficiency of water restricts overall growth of seedlings, which can be predicted from developmental parameters.

Accumulation of osmoregulators in cytosol is a response mechanism for increasing osmotic potential in cells in a stressed condition (Marcinska et al. 2013). Thus, turgor of the cells is maintained, which restricts the loss of

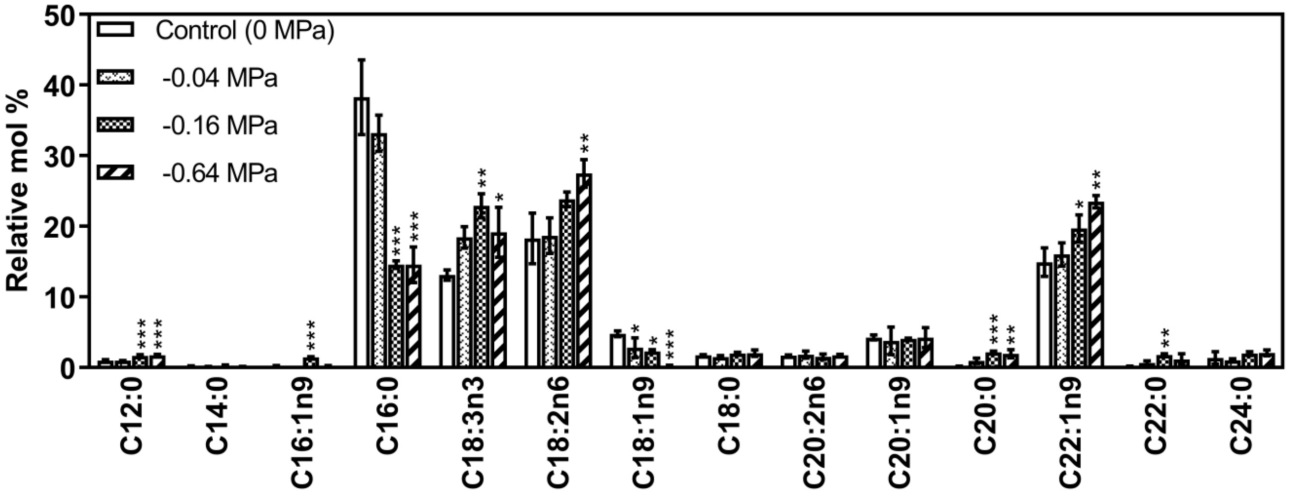

Fig. 5. Relative percentages of the fatty acids of total lipids extracted from seedlings grown in different treatment conditions. Error bars represent SD values of three biological replicates. Asterisks on error bars indicate significant differences compared to the control set (0 MPa) at the significance level of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

water (Rhodes, Samaras 1994). Compatible solutes such as soluble sugars and prolines have key roles in regulating the water potential and mediating cellular membrane integrity under stress. In water deficit condition, sugar metabolism is modulated by changes in enzymatic activities (Darko et al. 2019). Proline also accumulates in response to osmotic stress, which contributes towards osmotic adjustment (Yoshiba et al. 1997). Higher content of proline was also observed in stress tolerant cultivars of *Brassica*, due to enhanced activity of proline biosynthetic enzyme (Phutela et al. 2000). Increase of these metabolites in the present study seems to be a way to acclimatize the stressed condition for improvement of plant function. They can also help in stress tolerance by protecting and stabilizing membranes and enhancing water utilization efficiency during stress (Blum 2005).

Osmotic stress invariably stimulates generation of reactive oxygen species leading to oxidative stress (Mickky, Aldesuquy 2017). The reactive oxygen species can subsequently peroxygenate the membrane fatty acids leading to higher accumulation of malondialdehyde (Montillet et al. 2002). Acceleration of lipid peroxidation in seedlings grown under osmotic stressed has been reported (Guo et al. 2018). Therefore, in the present study, plants were also exposed to oxidative stress resulted from osmotic stress, as predicted from the higher content of malondialdehyde.

Utilization of lipid resources during germination and early seedling development is a well-documented fact (Offem et al. 1993). In oilseed crops, changes in lipid composition are important parameters to evaluate the biochemical status of plants. In present study, total lipid content of the plants was significantly lower with increase in PEG concentration. Decrease of lipid content by abiotic stress was also observed in a previous report on another Brassicaceae member, *Arabidopsis thaliana* (Gigon et al. 2004; Sanchez-Martin et al. 2012). Water deficiency hinders lipid biosynthesis and activates lipolytic and peroxidative activity leading to a decrease in membrane lipid content (Matos et al. 2001).

Thin layer chromatogram of neutral lipid classes showed higher accumulation of triacylglycerols (TAG) under stress. Such an increase of neutral lipids, specifically TAG, has been previously reported in other plants (Hubac et al. 1989; Dakhma et al. 1995). Free fatty acids can be stored in triacylglycerols under water deficit to avoid oxidation by free radicals (He, Ding 2020). Higher storage lipid content in the form of TAG can be considered as a defence response of plants against abiotic stress (Hubac et al. 1989). Moreover, oxidative reactions mostly act on membrane lipids and free fatty acids, and to a lesser extent on storage lipids (Galliard 1979).

Significant quantitative variation of the fatty acid saturation level in stressed plants was a key observation in our study. The increased unsaturation level was mainly in the form of higher C18 PUFA content, such as for C18:2n6 and C18:3n3. Such changes were also observed in previous studies on other Brassicaceae members as a response to stress condition (Dakhma et al. 1995; Bouchereau et al. 1996). Generally, plants acquire stress resistance through changing membrane fluidity in order to maintain membrane integrity and functionality of membrane proteins (Iba 2002). Adjusting the membrane fluidity by altering levels of unsaturation of fatty acids is a common acclimatization strategy of plants to abiotic stress, which is regulated by fatty acid desaturases (Zhang et al. 2005). The inherent ability of adjusting unsaturation level of fatty acids is considered as a tolerance mechanism of plants to any abiotic stress (Gigon et al. 2004).

Sinapis alba is considered to be a rich source of C22:1n9, indicating their possible additional industrial applications (Yaniv et al. 1994). The present study also demonstrated that the content of C22:1n9 was highest among the MUFAs in the young seedlings. Content of this fatty acid was higher in seedlings subjected to higher osmotic stress, in concurrence with the previous reports (Feizabadi et al. 2020). In general, C22:1n9 content gradually decreases in developing seedlings, suggesting the fatty acid to be a reverse source of energy, forming C18 fatty acids that are stored in green tissues (Liu, Brown 1996). Therefore, higher content of C22:1n9 in stressed condition indicates lesser utilization of C22:1n9 during development of seedlings.

A decrease of C18:1n9 content in response to abiotic stress was also previously reported (Dornbos, Mullen 1992). Higher content of C18:1n9 compared to lower C18:2n6 is desirable for stability of lipids (Bellaloui et al. 2015). Therefore, the lower lipid content under osmotic stress is corroborative with the decrease in C18:1n9 content, indicating lesser stability of lipids. Furthermore, C18:1n9 was negatively correlated with C22:1n9 and C18:2n6 content. C18:1n9 acts as the precursor for separate pathways, one leading to the formation of higher carbon MUFAs such as C22:1n9 catalyzed by elongases, and another converting to C18:2n6 by desaturase activity (Krzymanski, Downey 1969).Therefore, the present result suggests partial control of the metabolic process under osmotic stress.

Additionally, increase of C18 PUFAs such as C18:2n6 and C18:3n3 is also considered to be associated with a decrease of C16:0 (Toumi et al. 2008). Therefore, reduction of C16:0 in the present study, along with concomitant accumulation of C18:2n6 and C18:3n3 can be explained. Such a negative correlation was also observed between C16:0 and C22:1n9, in accordance to previous studies on some *Brassica* species (Sharafi et al. 2015). Enhanced desaturase and elongase activity causes higher accumulation of MUFAs and PUFAs (Agarwal, Stumpf 1985), even under water stress (Sobrino et al. 2003). Therefore, the overall changes of fatty acid composition in the form of an elevated unsaturation level in *Sinapis alba* can be an adaptive measure to adjust

membrane integrity in order to overcome the oxidative damage generated due to osmotic stress.

Different biochemical attributes clearly showed that the PEG mediated osmotic stress induces shifting of the metabolic responses in *S. alba* during early seedling development. Enhanced generation of osmolytes was the key metabolic response, which was primarily for osmotic adjustment and minimization of the effects of oxidative damage created by osmotic stress. Moreover, the enhanced lipid peroxidation induces modifications in the form of an enhanced unsaturation level for maintaining fluidity and permeability of the membrane, thereby influencing the whole metabolic process. Alteration of lipid and fatty acid composition seems to be the major element for the structural stability and protection of seedlings. Thus, the inherent level of fatty acid unsaturation or the ability to adjust unsaturation level indicates tolerance ability of *S. alba* seedlings to moderate osmotic stress, at least to some extent. Therefore, the preliminary study will help plant breeders and future researchers in work on oil quality of *S. alba* under stress condition.

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