

DIFFERENCES IN SOME CONSTITUENTS, ENZYMES ACTIVITY AND ELECTROPHORETIC CHARACTERIZATION OF DIFFERENT RAPESEED (*Brassica napus* L.) CULTIVARS

Hossam Saad EL-BELTAGI*, Amal Amin MOHAMED**, Bahaa El-Din Bastawy MEKKI***

* Biochemistry Dept., Faculty of Agriculture, Cairo University- Cairo, Egypt

** Plant Biochemistry Dept., National Research Centre, Dokki, Cairo- Egypt

*** Field Crops Research Dept., National Research Centre, Dokki, Cairo- Egypt

Corresponding author: Amal A. Mohamed, Plant Biochemistry Dept., National Research Centre, El Behouth St., P.O. Box 12311, Dokki, Cairo- Egypt, phone: (+202) 33361225, Mobile: (+2) 0106216110, Fax: (+202) 37610850, e-mail: amin_amal@yahoo.com

Abstract. Rapeseed is one of the most important oilseed crops cultivated in many parts of the world and used as a source of edible oil for human consumption. The present work reports some phytochemical and nutritional potentials of different rapeseed cultivars with a view to exploit their benefits for human nutrition. Five rapeseed cultivars namely; pactol, silvo, topas, serw 4 and serw 6 were analyzed for their phytochemical constituents (such as glutathione, ascorbate, phenolic, flavonoid contents) and some antioxidant enzymes activity (i.e. GPX; guaiacol peroxidase, APX; ascorbate peroxidase, CAT; catalase, SOD; superoxide dismutase) together with their protein profile. Among all cultivars significant variations in glutathione, ascorbate, phenolic and flavonoid contents were observed. The highest DPPH radical scavenging activity of the extract was observed in topas cultivar. Minor variations were noticed in SDS-PAGE protein profile. The results of the study suggest the phytochemical and nutritional potentials of *B. napus* seeds for human and other animal uses due to their extensive consumption in the world.

Keywords: Antioxidant enzymes, DPPH, Phytochemical compounds, Rapeseed, SDS-PAGE, Total phenolic.

Abbreviations: BSA: bovine serum albumin, DPPH: 2,2-diphenyl-1-picrylhydrazyl, PAGE: polyacrylamide gel electrophoresis, PVP: polyvinylpyrrolidone, SDS: sodium dodecyl sulphate, NBT: nitro blue tetrazolium, GSH: reduced glutathione.

INTRODUCTION

Most vegetable oils are edible and have been used in food preparation to make it more palatable and nutritious. Vegetable oils contain a mix of monounsaturated, and polyunsaturated fatty acids which decrease the low-density lipoprotein (LDL) cholesterol in the blood and thus prevent heart complications such as angina [17]. Rapeseed (*Brassica napus* L.) is one of the most important oilseed crops and considered as the most promising oil crops characterized with high seed oil content (40-45%). Cultivation of rapeseed in Egypt may provide an opportunity to overcome some of the local deficit of crop edible oil production [14, 48]. When compared to other edible oils, the rapeseed oil has the lowest amount of harmful saturated fatty acids. It also contains adequate amounts of the two essential fatty acids, linoleic and linolenic, which are not present in many other edible oils [36]. Rapeseed oil contains both omega-6 and omega-3 fatty acids in a ratio of 2:1, they are quite helpful in protecting against fatal heart disease and have anti-inflammatory effects [23]. Recently special attention has been paid towards *Cruciferous* or *Brassica* vegetables, especially those that are rich in secondary metabolites (frequently called phytochemicals) and there is now increasing interest in antioxidant activity of such phytochemicals present in the diet [40]. These naturally occurring compounds present in vegetable oils are believed to have strong anti-oxidative properties, act by scavenging harmful reactive oxygen species (ROS). These incomplete reduced oxygen species are toxic by-products, generated at low levels in non-stressed plant cells in chloroplasts and mitochondria [35]. However, plants possess enzymatic systems that protect them against H₂O₂ and other harmful ROS. These include guaiacol

peroxidase (GPX; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11), superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6). SOD converts superoxide radicals to hydrogen peroxide and, APX, GPX, CAT converts H₂O₂ to water and oxygen. Plants also contain non-enzymatic antioxidant compounds, e.g. ascorbic acid (AsA), reduced glutathione (GSH), α -tocopherol, phenolic and flavonoids [58]. These compounds may exert their activity by suppressing the production of harmful active species. Recent reports suggest that *Brassica* vegetables act as good source of natural antioxidants due to high levels of carotenoids, tocopherol and ascorbic acid [19]. Phenolic compounds exist widely in plants and play an important role as defense compounds. However, some trials aim to include the rapeseed sprouts in human nutrition because of their content of ascorbic acid [60], tocopherols [62], reduced glutathione [63], dietary fiber [61], and higher total antioxidant status [26] when compared to the ungerminated rapeseeds. In general, genetic improvement of crops can be accelerated when broad genetic diversity and the information of these genetic resources are available. Research on *Brassica* germplasm could enhance the edible oil production and nutritional benefits of these crops. At the same time it is necessary to develop better methods of characterization and evaluation of germplasm collections, to increase the utilization of plant genetic resources. In this concern, fatty acids, total tocopherol and phenolic contents could be used as selection criteria for developing genotypes with modified seed quality in *Brassica napus* [10]. Rapeseed is a rich source of protein (between 30 and 45%), and therefore defatted rapeseed meal may constitute a good source of protein for humans [13]. Protein isolates obtained from defatted rapeseed meal, free of anti-nutritional components such

as glucosinolate, phenols or phytic acid may be used for the fortification of foods [7, 59]. The electrophoresis of seed storage protein is a method to investigate genetic variation and to classify plant varieties [24]. Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable and could be important supplemental method for cultivars identification [54]. Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice [55]. However, the information on the SDS-PAGE on different species of Brassica for genetic diversity is still limited [43]. Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding. Therefore, the aims of this study were to (a) determine the chemical composition of five cultivars of rapeseeds and (b) assess the protein polymorphisms.

MATERIALS AND METHODS

Plant materials

Five cultivars of rapeseed (*B. napus*) were used in this study namely, pactol, silvo, topas (French origin cultivars), serw 4 and serw 6 (Egyptian origin cultivars). Seeds were obtained from Egyptian Ministry of Agriculture, and cultivated under Egyptian environmental conditions at the experimental farm of National Research Centre, Egypt. The practices of field preparation, optimum fertilizer applications and irrigation were standard for rapeseed culture as described previously [34]. When seeds matured, the plants were harvested and the immature and the mature seeds were dried in the sun light and stored in refrigerator (4°C) until further use.

Preparation of seed flour

After removing the immature and damaged seeds, the mature seeds of five different accessions of rapeseeds were dried in the open sunlight for 2 days. A known weight of samples from each cultivar was air dried seeds and ground to fine powdered in a Wiley Mill. The fine seed powder was stored in screw-cap bottles until further use.

Determination of reduced glutathione (GSH)

Seeds powder was added to 2 ml ice-cold 5% (w/v) sulphosalicylic acid solution. The mixture centrifuged for 30 min at 10000 ×g then the supernatants were collected and immediately assayed. Glutathione was measured with Ellman's reagent [49]. 300 µl of the supernatant was mixed with 1.2 ml of 0.1 M phosphate buffer solution (pH 7.6). After a stable absorbance reading of 412 nm was obtained, 25 µM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added, and the increase in absorbance at 412 nm was monitored ($\Sigma 412 = 13.6 \text{ mM}^{-1} \text{ cm}^{-1}$).

Determination of ascorbic acid (AsA)

Levels of AsA were measured according to the procedure described [51]. Seeds powder was added to 3 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 18000 ×g for 15 min. AsA was determined in a reaction mixture consisting of 0.2 ml of supernatant, 0.5 ml of 150 mM phosphate buffer (pH 7.4,

containing 5 mM EDTA) and 0.2 ml of deionized water. Color was developed in reaction mixture containing 0.4 ml of 10% (w/v) TCA, 0.4 ml of 44% (v/v) phosphoric acid, 0.4 ml of α,α -dipyridyl in 70% (v/v) ethanol and 0.2 ml of 3% (w/v) FeCl_3 . The reaction mixtures were incubated at 40°C for 40 min. and the absorbance was read at 532 nm.

Preparation of methanolic extracts

The extracts were prepared according to [32]. Briefly, 5.0 g of the dried powder from each cultivar was refluxed with 50 ml methanol 80% in a water bath at 45°C for 3 h. The extracts were filtered and dried under vacuum at 45°C using a rotary evaporator, and the extraction was repeated twice. The resulting residue was re-dissolved in methanol 80% and used for the determination of total phenolic, flavonoid and antioxidant activity.

Determination of total phenolic content

Total phenolic content of each extracts was determined by using the Folin-Ciocalteu reagent [52]. Methanolic extract (0.5 ml), 0.5 ml of Folin-Ciocalteu reagent, 10 ml of 7.5% sodium carbonate and deionized water were added to a final volume of 25 ml. After 1 h, the absorbance of the sample was measured at 725 nm against a blank by spectrophotometer. Gallic acid was used as the standard for preparing the calibration curve. The results were expressed as mg of gallic acid equivalent per gram of dried samples.

Determination of flavonoids

Colorimetric aluminum chloride method was used for flavonoid determination [37]. The methanolic extract (250 µl) was mixed with 1.25 ml of distilled H_2O and 75 µl of 5% NaNO_2 solution. After 5 min, 150 µl of 10% AlCl_3 solution was added and filtered. Then 500 µl of 1.0 M NaOH and 275 µl of distilled H_2O were added to the mixture. The absorbance of the sample was measured at 510 nm against a blank by spectrophotometer. The results were expressed as mg of quercetin equivalent per gram of dried samples.

Determination of free radical scavenging activity (DPPH assay)

Quantitative measurement of radical scavenging properties of different rapeseed cultivars was carried out [30]. Briefly, 0.1 mM solution of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) in methanol was prepared and 1 ml of this solution was added to 3 ml of each methanolic extract at one concentration (150 µg/mL). Butylated hydroxytoluene (BHT) was used as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements were taken at least in triplicate. The capacity to scavenge the DPPH· radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{ADPPH} - \text{AS}}{\text{ADPPH}} \times 100$$

Where, ADPPH is the absorbance of the DPPH solution and AS is the absorbance of the solution when the sample extract is added.

Enzymes extraction and activity assay

The seeds were ground and defatted by using hexane in order to remove most of the fat from the

seeds material before enzymes extraction. The resulting defatted rapeseed flour (0.5 g) was extracted with 4 ml of ice cold extraction buffer (250 mM sucrose and 25 mM tris, pH 7.2). The homogenate was centrifuged at 16000 x g for 20 min at 4°C and supernatant was used to determine the activity of GPX, APX, CAT and SOD. Guaiacol peroxidase activity (GPX; EC 1.11.1.7) activity was assayed by monitoring the increase in absorbance at 470 nm due to the oxidation of guaiacol (extinction coefficient = 26.6 mM⁻¹ cm⁻¹) [20]. The reaction mixture contained 50 mM sodium-acetate buffer (pH 5), 20 mM guaiacol, 40 mM H₂O₂, and 0.05 ml enzyme extract diluted 6 times. Ascorbate peroxidase activity (APX, EC 1.11.1.11) was measured by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM⁻¹ cm⁻¹). Enzyme activity was determined by the decrease in absorbance of ascorbate at 290 nm [38]. The reaction mixture consisted of enzymatic extract, 50 mmol l⁻¹ sodium phosphate buffer, pH 7, 0.5 mmol l⁻¹ ascorbate, 0.1 mmol l⁻¹ hydrogen peroxide and 0.1 mmol l⁻¹ EDTA, in a 0.3 ml final volume. The reaction started after the hydrogen peroxide addition. The molar extinction coefficient (2.8 mmol⁻¹ cm⁻¹) was used to calculate ascorbate peroxidase activity. Catalase activity (CAT; EC 1.11.1.6) was determined as H₂O₂ consumption measured as the decrease in absorbance at 240 nm [16]. The assay contained 50 mM phosphate buffer (pH 7), 10 mM H₂O₂ in phosphate buffer, 0.1 ml enzyme extract. Extinction coefficient of 40 mM⁻¹cm⁻¹ was used to calculate catalase activity. Superoxide dismutase activity (SOD; EC 1.15.1.1) was measured by the photochemical method as described by SOD activity assay [15]. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction measured at 560 nm in the presence of riboflavin and light. The reaction mixture contained 45 mM potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 13 mM methionine, 0.17 mM NBT in ethanol, 0.007 mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15 min. The protein concentration of the supernatant was determined according to the Bradford method using bovine serum albumin as standard [4].

Protein extraction for SDS-PAGE

For SDS-PAGE, leaf tissues of each clones were ground to powder under liquid nitrogen and melted in ice-cold extraction buffer (50 mM Tris-HCl, pH 8, 10

mM NaCl, 1% SDS, 5% 2-mercaptoethanol, 0.1 mM PMSF, 0.1 mM DTT), followed by centrifugation at 10000 ×g at 4 °C for 15 min. Protein content of the clear supernatants obtained after centrifugation were stored at -20 °C until used.

One-dimensional SDS-PAGE

Proteins, 30 µg of each sample, were separated by SDS-PAGE according to the method of Laemmli [29]. The separation was performed with a 10% separating gel and a 4% stacking gel using protein vertical electrophoresis unit (Hoefer Scientific Instruments). Electrophoresis was started at 10 mA constant current until the tracking dye entered the separating gel and continued at 25 mA until the tracking dye reached the end of the gel. Protein subunit bands were stained with coomassie blue R-250 by standard techniques. The protein marker from Sigma was used. The molecular weight of standard protein (in KD) as follows: 116, 66.2, 45, 35, 25, and 18.4 KD (6 bands).

Statistical analysis

All data are reported as mean ± standard deviation (±SE) for the three independent samples (n=3). Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package [53]. The least significant difference (LSD) at P≤ 0.05 level was calculated.

RESULTS

Phytochemical composition

Since oil seeds are high in antioxidants, a diet high in these oils should prevent oxidative stress, and may therefore help prevent chronic disease and slow aging. Glutathione (GSH) content of rapeseed was measured and data presented in Table (1). The results showed that there was a significant difference in the content of GSH among all rapeseed cultivars and wide variations in GSH content were observed. The highest content was recorded in topas cultivar (29.74±0.20 mg/g dw) followed by pactol (25.20±0.35 mg/g dw), while the lowest content was recorded in serw 6 (15.38±0.33 mg/g d.w). Similar trend was observed in ascorbate (AsA) content, for example; topas and pactol cultivar recorded the highest values (17.66 ± 0.28 and 14.50 ± 0.38 mg/g dw) while silvo and serw 6 recorded the lowest values (7.33± 0.18 and 3.18±0.17 mg/g dw). The results concerning phenolic contents of five varieties of *B. napus* are presented also in Table I. Significant (p<0.05) variations were found among the five cultivars, with respect to their phenolic content.

Table 1. Glutathione, ascorbate, phenolic and flavonoid contents of different rapeseed (*Brassica napus* L.) cultivars.

Rapeseed cultivars	Glutathione (mg/g DW)	Ascorbate (mg/g DW)	Phenolic (mg/g DW)	Flavonoid (mg/g DW)
Pactol	25.20 ± 0.35 ^b	14.50 ± 0.38 ^b	34.5 ± 0.59 ^b	29.50 ± 0.46 ^b
Silvo	19.43 ± 0.25 ^d	7.33 ± 0.18 ^d	28.4 ± 0.43 ^d	22.42 ± 0.36 ^d
Topas	29.74 ± 0.20 ^a	17.66 ± 0.28 ^a	35.4 ± 0.45 ^a	32.57 ± 0.43 ^a
Serw 4	22.34 ± 0.30 ^c	10.33 ± 0.22 ^c	33.4 ± 0.23 ^c	26.28 ± 0.24 ^c
Serw 6	15.38 ± 0.33 ^c	3.18 ± 0.17 ^c	28.0 ± 0.20 ^c	18.14 ± 0.22 ^c
LSD (0.05)	0.527	0.470	0.84	0.645

^{a,b,c,....}Mean values followed by the different letters are significantly different at P<0.05. Values are expressed as the means ± SD of three independent assays.

The highest phenolic content was recorded in topas cultivar (35.4 ± 0.45 mg/g dw) followed by pactol cultivar (34.5 ± 0.59 mg/g dw). Whereas, the lowest phenolic content was recorded in serw 6 cultivar (28.0 ± 0.20 mg/g dw) followed by silvo cultivar (28.4 ± 0.43 mg/g dw). There was significant difference among all cultivars in total flavonoid content and the values ranged from 18.14 to 32.57 mg/g dw. The highest flavonoid content was recorded in topas cultivar (32.57 ± 0.43 mg/g dw) followed by pactol cultivar (29.50 ± 0.46 mg/g dw).

Free radical scavenging activity (DPPH)

The rapeseed extracts showed strong antioxidant activity, by measuring their capacity to scavenge

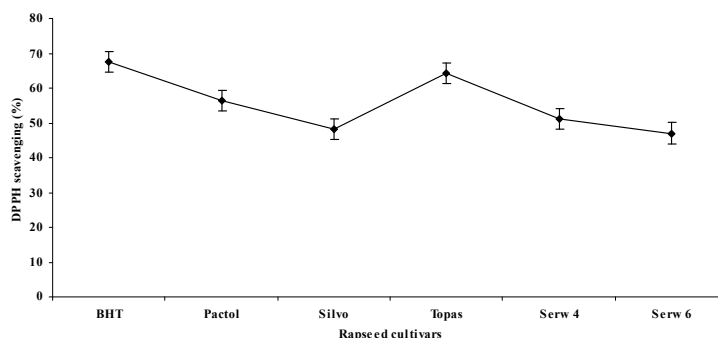


Figure 1. Free radical scavenging activity of methanolic extract (150 µg/ml) of different rapeseeds (*Brassica napus* L.) cultivars and BHT by DPPH radicals. Values show mean \pm SD from three triplicates.

Activities of antioxidant enzymes

The antioxidant enzyme defense system consists of hundreds of different substances and mechanisms. Antioxidant enzymes have the capacity to lower the free radical generation and neutralize excess free radicals created by stress conditions. The rapeseed cultivars differed significantly in their antioxidant enzymes activities. Topas cultivar had the highest enzymes activity followed by pactol. Topas had GPX 71.72 ± 0.70 unit /mg protein, APX of 16.82 ± 0.75 unit /mg protein, CAT of 17.66 ± 0.63 µ mol/mg protein/min, and SOD of 56.84 ± 0.62 unit /mg protein (Table 2). Among all cultivars tested in this study, serw

DPPH[•] radical against the rate of peroxide formation (Fig. 1). We used BHT as standard, the scavenging effects of methanolic extracts from each rapeseeds cultivars and standard compound on the DPPH[•] radical decreased in the order of, BHT > pactol > silvo > topas > serw 4 > serw 6 which were 67.55, 56.3, 48.2, 64.2, 51.06, and 47.03 % at the concentration of 150 µg/ml, respectively. The highest radical scavenging activity was found in topas cultivar and the lowest value was found cv. serw 6. These results may be attributed to (or paralleled with) the high content of phenolic content in the same cultivar (topas).

6 cultivar had lowest antioxidant enzyme activities (Table 2).

Polypeptide profile

The separated polypeptide fragments were photographed and presented in Fig. 2. The results showed that the protein profiles were represented by 7 major and common distinct bands with molecular weight of 42.0, 40.0, 37.0, 33.0, 30.0, 18.4 and 12.0 KDa and one common band with molecular weight of 116 KDa. The electrophoretic analysis of rapeseeds protein shows minor changes between serw 4 (lane 4) and serw 6 (lane 5).

Table 2. Antioxidant enzymes activities of; guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) in different rapeseed (*Brassica napus* L.) cultivars.

Rapeseed cultivars	GPX (Unit/mg pro./min)	APX (Unit/mg pro./min)	CAT (µ mol/mg pro./min)	SOD (Unit/mg pro./min)
Pactol	64.71 ± 0.57^b	12.69 ± 0.67^b	13.69 ± 0.55^b	50.21 ± 0.74^b
Silvo	50.63 ± 0.56^d	5.55 ± 0.45^d	6.53 ± 0.45^d	42.88 ± 0.95^d
Topas	71.72 ± 0.70^a	16.82 ± 0.75^a	17.66 ± 0.63^a	56.84 ± 0.62^a
Serw4	57.79 ± 0.44^c	8.48 ± 0.40^c	9.62 ± 0.51^c	44.59 ± 0.56^c
Serw6	44.53 ± 0.37^e	2.69 ± 0.27^e	4.28 ± 0.35^e	38.42 ± 0.39^e
LSD (0.05)	0.983	0.978	0.916	1.233

^{a,b,c,.....} Mean values followed by the different letters are significantly different at $P < 0.05$. Values are expressed as the means \pm SD of three independent assays.

DISCUSSIONS

Differences in some biochemical composition among different genotypes of rape seeds, including GSH, Ascorbate and phenolic compounds, have generated remarkable interest based on positive reports of their antioxidant properties and ability to serve as free radical scavengers. Significant differences were found in glutathione, ascorbate, phenolic and flavonoid

contents among different cultivars of rape seeds (Table 1). GSH a disulfide reductant that protects thiols group of enzymes, regenerates ascorbate (AsA) level and reacts with singlet oxygen, hydrogen peroxide and hydroxyl radicals. Therefore, GSH plays a central role in protecting plants from the active oxygen species [27]. The antioxidants compounds such as ascorbate (AsA) and glutathione (GSH) are involved in scavenging of harmful ROS species ($\cdot O_2$, H_2O_2 , $\cdot OH$)

[21]. Recent experimental evidence has indicated that the levels of GSH and AsA could be used to sense the environmental changes and trigger an up-regulation of mechanisms involved in co-regulation of AsA, and GSH pools during environmental stress [31, 33]. The

potential protective role of *Brassica* vegetables such as phenolic and flavonoids has been extensively studied since these phytochemicals may help in breeding programs to develop new germplasm with a high content of such useful phytochemicals.

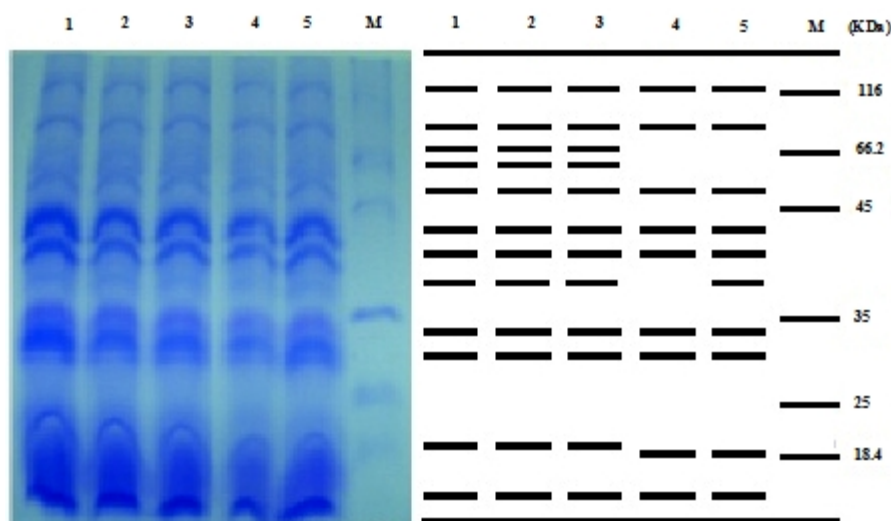


Figure 2. SDS-PAGE protein patterns of different rapeseed (*Brassica napus* L.) cultivars. M: refer to protein marker, lane 1: pactol, lane 2: silvo, lane 3: topas, lane 4: serw 4, lane 5: serw 6.

Phenolic compounds also play an important role in the interaction between the plant and the surrounding environment. They can protect plants against the biotic (microbiological or herbivorous vermin) or abiotic (polluted air, ions of heavy metals, UV-B radiation) stress [3]. Rapeseed meal has a high content of phenolic acid esters, mainly sinapate esters, which have been shown to cause a dark color and a bitter taste in rapeseed meal and derived protein products.

On the basis of the obtained results, it was found that Topas cultivar had the highest total phenolic content (35.4 mg/g d.w.), which is shown table 1. Phenolic compounds content in rapeseed meal at the level of 1080.2 – 1807 mg/100g [47]. Whereas, it obtained 17.2 to 22.9 mg/g total phenolic compounds in rapeseed meal depending on the temperature and time of extraction using 70% and 100% methanol [5]. Phenolic acids are natural hydrophilic antioxidants, which occur ubiquitously in vegetables oils, spices and aromatic herbs [50]. The most active rapeseed meal phenolic fraction contained several classes of phenolic compounds including phenolic acids, flavones and flavonols [25]. Potential genotypes with desired phenolic levels may play an important role in the future for the development of new and improved cultivars delivering potential health benefits. Total flavonoid in oil seeds is indicative of the total antioxidative activity due to the availability of the phenolic hydrogens, as hydrogen-donating radical scavengers [39, 22]. In this concern, seeds of five different flax cultivars named (Sakha 1, Sakha 2, Giza 8, Lithuania and Aryana) were tested to assess their nutritional value using different parameters such as total phenolics and total flavonoids contents [11]. The authors found that Sakha 1, Sakha 2 and Aryana had lower total flavonoids (16.10, 14.43 and 12.94 mg/100 g d.w.)

than Giza 8 and Lithuania (19.98 and 19.51 mg/100 g d.w.) respectively. Additionally, previous studies have shown that some flavonoid components such as quercetin had anticancer activities and were able to inhibit cancer cell growth [9, 44]. Hence, the results of this research showed that phenolic and flavonoid are important components of rapeseeds, and some of its pharmacological effects could be attributed to the presence of these valuable constituents as stated by [1]. Also, both genetic and environmental effects create a significant variation in the amount and quality of each of these constituents.

Sinapic acid is the most common phenolic acid in rapeseed, and a powerful scavenger of free radicals [41, 57]. While the great stability of rapeseed oils, in conditions of oxidation, is due to the presence of an elevated rate of natural antioxidants, most of which important are phenolics. These components are essential for protection of poly unsaturated fatty acids in plants and animals deterioration [46]. Finally, these results provide useful and important information for researchers in order to understand the antioxidant capacity and functional value of rapeseeds for the food and nutraceutical industries.

The content antioxidant activity using DPPH method in *Brassica* seeds analyzed varies from 64.2 to 47.03%. The maximum activity was found for Topas at the 150 µg/ml as shown in Fig 1. The present work suggests that the highest antioxidant compounds of rape seeds may be due to the presence of different antioxidant components such as phenolic compounds.

The ability of plants to overcome oxidative stress partly relies on the induction of SOD activity and subsequently on the up-regulation of other downstream antioxidant enzymes. In our experiment, the results showed significantly differences in antioxidant

enzymes activity among rape seeds (Table 2). Our results are in agreement with El-Beltagi et al., [12] who found remarkable differences in the activities of (GPX, APX, CAT and SOD) at three flax cultivars (Sakha 1, Sakha 2 and Giza 8). Additionally, significant different in the activities of (GPX, CAT and SOD) at four cultivars canola was found (Dunkled, CON-III, Rainbow and Cyclone) [2]. Antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) are the systems designed to minimize the concentrations of superoxide and hydrogen peroxide. Superoxide-dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. (H_2O_2) is eliminated by catalase and peroxidases, which include both enzymic and non-enzymic H_2O_2 degradation [42].

The results obtained from SDS-PAGE electrophoresis (Fig. 2) showed that the method provides a powerful tool for reliable variety identification based on genetic differences in seed storage protein composition among different varieties of *Brassica napus*. Generally, the electrophoretically separated protein in rapeseeds cultivars revealed (i) quantitative decline in certain proteins, (ii) rise in levels of other proteins, (iii) some proteins remained unchanged, and (iv) *de novo* induction of specific proteins. These alterations ranged in molecular weight from as low as 12 KDa to as high as 116 KDa. From the general picture of seeds protein emerging from this work, one point is noteworthy, less protein alterations were scored among cultivars, and it is possible that this alteration reflect their relative sensitivities to both genetic and environmental effects.

No report on SDS-PAGE of seed protein of Brassica varieties of Egypt is available, so it seems to say that SDS-PAGE technique has proven to be a useful tool in supporting classical taxonomy studies [56]. It was possible to distinguish certain genotypes based on seed protein. Landraces are a useful source of genetic variation and the greater the variation, the greater the chances of a landrace possessing genes of gene combinations of interest to plant breeders [6]. The high stability of seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants [28].

Similarly, a similar electrophoretic pattern of protein among the cultivars was reported in sunflower and lettuce, respectively [45, 7]. Polypeptide expression varies depending upon the different developmental stages due to differential gene expression of concerned structural or regulatory genes, which are under the control of master gene. Polypeptides, polymorphic for presence or absence could be potentially used as marker to decipher the differentiation pathway and selection of organogenic potential callus or tissue [18]. The protein expression differences may be caused by certain biochemical

alterations at the cellular level of the studied callus cultures.

The present work concluded that, significant variations among rapeseeds cultivars in glutathione, ascorbate, and total phenolic, flavonoid contents were observed. The extract of topas cultivar recorded the highest antioxidant activity, while the lowest value was detected in serw 6. Also, the highest value of antioxidant enzymes activity (GPX, APX, CAT and SOD) was found in topas cultivar while the lowest activities were detected in serw 6. Different variation among rapeseed cultivars were noticed in the protein profile. Thus, our results could be used for developing rapeseed cultivars with increased health promoting compounds due to its extensive consumption in the world.

REFERENCES

- [1] Aleksander, S., Malgorzata, N.K., (2010): Studies on influence of rapeseed vegetation stages on level of phenolic compounds. *Journal of Oilseed Brassica*, 1(1): 12-18.
- [2] Ashraf, M., Ali, Q., (2008): Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). *Environmental and Experimental Botany*, 63: 266-273.
- [3] Bennett, R.C., Wallsgrove, R.M., (1994): Secondary metabolites in plant defense mechanisms. *New Phytologist*, 127: 617-633.
- [4] Bradford, M.M., (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.
- [5] Cai, R., Arntfield, S.D., 2001. A rapid high-performance liquid chromatographic method for the determination of sinapine and sinapic acid in canola seed and meal. *Journal of American Oil Chemists' Society*, 78: 903-910.
- [6] Damania, A.B., Porceddu, E., Jackson, M.T., (1983): A rapid method for the evaluation of variation in germplasm collections of cereals using polyacrylamide gel electrophoresis. *Euphytica*, 32: 877-883.
- [7] De-Vries, I.M., (1996): Characterization and identification of *Lactuca sativa* cultivars and wild relatives with SDS-electrophoresis (*Lactuca sect. Lactuca, Compositae*). *Genetic Resources and Crop Evolution*, 43: 193-202.
- [8] Dev, D.K., Mukherjee, K.D., (1986): Functional properties of rapeseed protein products with varying phytic acid contents. *Journal of Agriculture and Food Chemistry*, 34: 775-780.
- [9] Elattar, T.M., Virji, A.S., (2000): The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells *in vitro*. *Anticancer Research*, 20: 1733-1738.
- [10] El-Beltagi, H.E.S., Mohamed, A.A., (2010): Variations in fatty acid composition, glucosinolate profile and some phytochemical contents in selected oil seed rape (*Brassica napus* L.) cultivars. *Grasas y Aceites*, 61(2): 143-150.
- [11] El-Beltagi, H.S., Salama, Z.A, El-Hariri, D.M., (2007): Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars (*Linum usitatissimum* L.). *General Applied and Plant Physiology*, (3-4): 187-202.

- [12] El-Beltagi, H.S., Salama, Z.A., El-Hariri, D.M., (2008): Some Biochemical Markers for Evaluation of Flax Cultivars under Salt stress conditions. *Journal of Natural Fibers*, 5(4): 316-330.
- [13] Finlayson, A.J., (1976): The Seed Protein Contents of some Cruciferae. In Vaughan, J.G., McLeod, A.J. and Jones, B.M.G. (Eds.). *The Biology and Chemistry of the Cruciferae*. Academic Press, London.
- [14] Ghallab, K.H., Sharaan, A.N., (2002): Selection in canola (*Brassica napus* L.) germplasm under conditions of newly reclaimed land. II. Salt tolerant selections. *Egyptian Journal of Plant Breeding*, 6(2): 15-30.
- [15] Giannopolitis, C.N., Ries, S.K., (1977): Superoxide dismutases. I. Occurrence in higher plants. *Plant Physiology*, 59: 309-314.
- [16] Goel, A., Goel, A.K., Sheoran, I.S., (2003): Changes in oxidative stress enzymes during artificial agein in cotton (*Gossypium hirsutum* L.) seeds. *Journal of Plant Physiology*, 160: 1093-1100.
- [17] Grundy, M.D., (1987): Monounsaturated fatty acids, plasma cholesterol and coronary heart disease. *American Journal of Clinical Nutrition*, 45: 1168-1175.
- [18] Hakman, I., (1993): Embryology in Norway spruce (*Picea abies*). An analysis of the composition of seed storage proteins and deposition of storage reserves during seed development and somatic embryogenesis. *Physiologia Plantarum*, 87: 148-159.
- [19] Heinonen, M.I., Ollilainen, V., Linkola, E.K., Varo, P.T., Koivistoinen, P.E., (1989): Carotenoids in finnish foods: vegetables, fruits, and berries. *Journal of Agriculture and Food Chemistry*, 37: 655-659.
- [20] Hemedi, H.M., Klein, B.P., (1990): Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *Journal of Food Science*, 55: 184-185.
- [21] Horemans, N., Foyer, C.H., Potters, G., Asard, H., (2000): Ascorbate function and associated transport systems in plants. *Plant Physiology and Biochemistry*, 38: 531-540.
- [22] Hudson, B., Lewis, J., (1983): Polyhydroxy flavonoid anti-oxidant for edible oils - phospholipids as synergists. *Food Chemistry*, 10: 111-120.
- [23] Ion, G., Akinsete, J.A., Hardman, W.E., (2010): Maternal consumption of canola oil suppressed mammary gland tumorigenesis in C3 (1) TA_g mice offspring. *BMC Cancer*, 10(81): 1-12.
- [24] Isemura, T., Chiyo, N., Shigeyuki, M., Yamashita, M., Nakanishi, H., Inoue, M., Kamijima, O., (2001): Genetic variation and geographical distribution of Azuki bean (*Vigna angularis*) landraces based on the electrophoregram of seed storage proteins. *Breeding Science*, 51: 225-230.
- [25] Koski, A., Pekkarinen, S., Hopia, A., Wähälä, K., Heinonen, M., (2003): Processing of rapeseed oil: Effects on sinapic acid derivative content and oxidative stability. *European Food Research and Technology*, 217: 110-114.
- [26] Kozłowska, H., Zieliński, H., Bucin'ski, A., Piskula, M.K., (2003): Bioactive compounds in rapeseed sprouts. *Oilseed Crops*, XXIV: 31-39.
- [27] Kunert, K.J., Foyer, C.H., (1994): Thiol/disulphide exchange in plants. In De Kok, L.J., Stulen, I., Rennenberg, H., Brunhold, C., Rausen, W. (Eds.): *Sulfur Nutrition and Assimilation in Higher Plants: Regulatory, Agricultural and Environmental Aspects*. SPB Academic Publishers, The Hague, pp. 132-164.
- [28] Ladizinsky, G., Hymowitz, T., (1979): Seed protein electrophoresis in taxonomic and evolutionary studies. *Theoretical and Applied Genetics*, 54: 145-151.
- [29] Laemmli, U.K., (1970): Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature*, 227: 680-685.
- [30] Lee, S.K., Zakaria, H.M., Cheng, H.S., Luyengi, L., Gamez, E.J.C., Mehta, R., Kinghorn, A.D., Pezzuto, J.M., (1998): Evaluation of the antioxidant potential of natural products. *Combinatory Chemistry & High-Throughput Screening*, 1: 35-46.
- [31] Maggio, A., Migazaki, S.P., Veronese, T., Fujita, H.I., Ibeas, B., Damsz, M.L., Navasimhan, P.M., Joly, R.A., Bressan, R.A., (2002): Does proline accumulation play an active role in stress induced growth reduction? *Plant Journal*, 31: 169-712.
- [32] Matkowschi, A., Piotrowska, M., (2006): Antioxidant and free radical scavenging activities of some medicinal plants from the *Lamiaceae*. *Fitoterapia*, 77: 346-353.
- [33] May, M.J., Vernoux, T., Leaver, C., Montagu, M.V., Inze, D., (1998): Glutathione homeostasis in plants: implications for environmental sensing and plant development. *Journal of Experimental Botany*, 49: 649-667.
- [34] Mekki, B.B., (2003): Proceeding of the 11th International Rapeseed Congress 6-10 July. The Royal Veterinary and Agric. Univ., Copenhagen, Denmark, 3: 915-917.
- [35] Michalak, A., (2006): Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, 15(4): 523-530.
- [36] Muhammad, S., Khalil, I.A., Khan, S., (1991): Fatty acid composition of rape and mustard oilseed cultivars. *Scientific Khyber*, 4: 29-36.
- [37] Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F., Hamidinia, A., Bekhradnia, A.R., (2008): Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica*. *Mey Pharmacologyonline*, 2: 560-567.
- [38] Nakano, Y., Asada, K., (1981): Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, 22: 860-867.
- [39] Nieto, S., Garrido, A., Sanhueza, J., Loyola, L., Morales, G., Leighton, F., Valenzuela, A., (1993): Flavonoids as stabilizers of fish-oil – an alternative to synthetic antioxidants. *Journal of the American Oil Chemists' Society*, 70: 773-778.
- [40] Ou, B., Huang, D., Hampsch-Woodil, M., Flanagan, J.A., Deemer, E.K., (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agriculture and Food Chemistry*, 50(11): 3122-3128.
- [41] Pekkarinen, S.S., Stockmann, H., Schwarz, K., Heinonen, M., Hopia, A., (1999): Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. *Journal of Agriculture and Food Chemistry*, 47: 3036-3043.
- [42] Peltzer, D., Dreyer, E., Polle, A., (2002): Differential temperature dependencies of antioxidative enzymes in two contrasting species: *Fagus sylvatica* and *Coleus blumei*. *Plant Physiology and Biochemistry*, 40: 141-150.
- [43] Rahman, M.M., Hirata, Y., (2004): Genetic diversity in Brassica species using SDS-PAGE analysis. *Journal of Biological Science*, 4(2): 234-238.

- [44] Ranelletti, F.O., Maggiano, N., Serra, F.G., (1999): Quercetin inhibits p21-ras expression in human colon cancer cell lines and in primary colorectal tumors. *International Journal of Cancer*, 85: 438-445.
- [45] Raymond, J., Inquello, V., Azanza, J.L., (1991): The seed proteins of sunflower: comparative studies of cultivars. *Phytochemistry*, 30: 2849-2856.
- [46] Sebei, K., Inoubli, M.H., Boussiga, H., Tlig, S., Alouani, R., Boujamaoui, M., (2007): Seismic stratigraphy, tectonics and depositional history in the Halk El Menzel region, NE Tunisia. *Journal of African Earth Science*, 47(1): 9-29.
- [47] Shahidi, F., Naczk, M. (1992). An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *JAOCS*, 69: 917-924.
- [48] Sharaan, A.N., Ghallab, K.H., Yousif, K.M., (2002): Performance and water relations of some rapeseed genotypes grown in sandy loam soils under irrigation regimes. *Annals of Agriculture Science Moshtohor*, 40(2): 751-767.
- [49] Silber, R., Farber, M., Papopoulos, E., Nervla, D., Liebes, L., Bruch, M., Bron, R., (1992): Glutathione depletion in chronic lymphocytic leukemia β -lymphocytes. *Blood*, 80: 2038-2040.
- [50] Silva, F., Borges, F., Guimaraes, C., Lima, J., Matos, C., Reis, S., (2000): Phenolics and derivatives: studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. *Journal of Agriculture and Food Chemistry*, 48: 2122-2126.
- [51] Singh, N., Ma, L.Q., Srivastava, M., Rathinasabapathi, B., (2006): Metabolic adaptations to arsenic-induced oxidative stress in *Pteris vittata* L. and *Pteris ensiformis* L.. *Plant Science*, 170(2): 274-282.
- [52] Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.
- [53] Snedecor, G.W., Cochran, G.W., (1980): *Statistical Methods*, 7th edition. The Iowa State, University Press, Ames, Iowa.
- [54] Tanksley, S.D., Jones, R.A., (1981): Application of alcohol dehydrogenase allozymes in testing the genetic purity of F1 hybrids of tomato. *Horticulture Science*, 16: 179-181.
- [55] Thanh, V.O.C., Hirata, Y., (2002): Seed storage protein diversity of three rice species in the Mekong Delta. *Biosphere Conservation*, 4: 59-67.
- [56] Thanh, V.C., Nguyen, T.N., Hirata, Y., Thuong, N.V., (2003): Antenna protein diversity of prawns (*Macrobrachium*) in the Mekong Delta. *Biosphere Conservation*, 5: 11-17.
- [57] Thiyam, U., Kuhlmann, A., Stöckmann, H., Schwarz, K., (2004): Prospects of rapeseed oil by-products with respect to antioxidative potential. *Comptes rendus Chimie*, 7: 611-616.
- [58] Wingsle, G., Karpinski, S, Hallgren, J.E., (1999): Low temperature, high light stress and antioxidant defence mechanisms in higher plants. *Phyton (Austria) Special issue: "Eurosilva"*; 39: 253-268.
- [59] Zhou, B., He, Z.Q., Yu, H.M., Mukherjee, K.D., (1990): Proteins from double-zero rapeseed. *Journal of Agriculture and Food Chemistry*, 38: 690-694.
- [60] Zielin'ski, H., Bucin'ski, A., Kozłowska, H., (2002): Monitoring of the vitamin C content in germinating cruciferae seeds by HPLC. *Polish Journal of Food and Nutrition Science*, 11/52(1): 142-146.
- [61] Zielin'ski, H., Frias, J., Piskula, M.K., Kozłowska, H., Vidal-Valverde, C., (2005): Vitamin B1 and B2, dietary fiber and minerals content of Cruciferae sprouts. *European Food Research and Technology*, 221: 78-83.
- [62] Zielin'ski, H., Kozłowska, H., (2003): Content of tocopherols in cruciferae sprouts. *Polish Journal of Food and Nutrition Science*, 12/53(4): 25-31.
- [63] Zielin'ski, H., Mudway, I., Kozłowska, H., Kelly, F.J., (2002): Impact of germination on glutathione content in cruciferous seeds. *Polish Journal of Food and Nutrition Science*, 11/52(SI 1): 68-72.

Received: 3 February 2011

Accepted: 4 April 2011

Published Online: 12 April 2011

Analele Universității din Oradea – Fascicula Biologie

<http://www.bioresearch.ro/revistaen.html>

Print-ISSN: 1224-5119

e-ISSN: 1844-7589

CD-ISSN: 1842-6433