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Physiochemical and Molecular Analysis of *Brassica napus* Seeds of Different Varieties

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Abstract: This research was conducted to characterize *B. napus* seeds and to compare seeds of different varieties for their compositions. The seeds of ten different *Brassica napus* varieties namely Cyclon, Star, Vangard, MLCP-048, Bullet, Altex, Con-III, Deffender, Hyola and Dunkled were analyzed for parameters like, pH, electrical conductivity and thousand seed mass. Minerals (Na^+ and K^+), vitamin C and protein contents were also analyzed. Na^+ and K^+ were determined by Flame photometer and proteins were analyzed by SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis). The amount of sodium ranges from 0.020-0.039%. The lowest value of sodium was found in Vangard and the highest in Cyclone. While potassium ranges from 1.14-1.42% with the lowest value for Bullet and the highest for Star. Vitamin C was found in all the varieties in range of 30-96 mg/100 g. The maximum amount of vitamin C was found in Bullet variety while minimum concentration in MLCP-048. Maximum value of pH (6.933) was recorded for MLCP-048, while minimum value for Altex 6.632. The thousand seed mass for cyclone was 5.52 g that was maximum and the lowest value was for bullet variety (3.63 g). Electrical conductivity for different varieties was estimated after different time intervals (20, 40, 60, 80, 100 and 120 min). The overall electrical conductivity determined by conductometer ranged from 0.9-10.4 μ sec. Seeds proteins were analyzed by SDS-PAGE and different numbers of bands showed that variation existed among the different varieties.

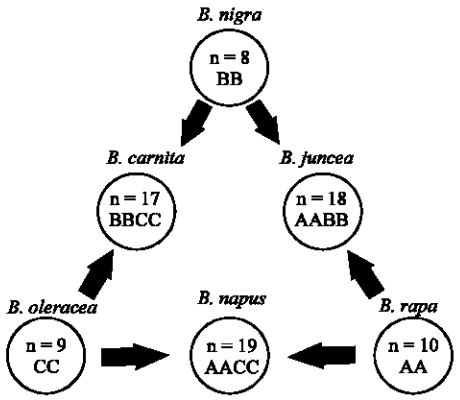
Key words: *Brassica napus* seeds, varieties, SDS-PAGE, vitamin C, minerals Na^+ and K^+

INTRODUCTION

Brassica napus is also known as Canola. In strict sense canola is that whose oil must contain less than 2% erucic acid and the solid component of the seed must contain less than 30 μM of any one or a mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate and 2-hydroxy-4-pentenyl glucosinolate per gram of air dry, oil free solid (Cardoza and Stewart, 2002). Rapeseed/canola (*Brassica napus* L. and *B. campestris* L.) recently moved up to the world's third most important edible oil source after soybean and palm (Downey, 1990).

The given triangle indicates the genetic relationships among the economically most important Brassica species, *B. napus* (2n = 38, AACC), *B. juncea* (2n = 36, AABB) and *B. carinata* (2n = 34, BBCC) species resulting from combining chromosome sets of the low chromosome number species *B. nigra* (2n = 16, BB), *B. oleracea* (2n = 18, CC) and *B. rapa* (2n = 20, AA) (Morinaga, 1934; U.N., 1935).

B. napus contain 35% crude protein basis, having amino acids like Alanine, Histidine, Arginine, Isoleucine, Aspartate, Leucine, Cystine, Lysine, Glutamate, Methionine, Glycine, Methionine+cystine, Phenylalanine, Tryptophan, Proline, Tyrosine, Serine, Valine and Threonine (Anderson *et al*., 1993; Bell *et al*., 2000).



Brassica napus contains Calcium (0.63%), Phosphorus (1.08%), Sodium (0.10%), Chlorine (0.10%), Potassium (1.22%), Sulphur (0.85%), Magnesium (0.54%), Copper (5.8 mg kg^{-1}), Iron (166 mg kg^{-1}), Manganese (52 mg kg^{-1}), Molybdenum (1.4 mg kg^{-1}), Zinc (58 mg kg^{-1}) and Selenium (1.1 mg kg^{-1}) (Bell and Keith, 1991; Bell *et al*., 1999).

Acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate has become one of the most widely used techniques to separate and characterize proteins (Laemmli, 1970). This technique offers two distinct advantages. Polypeptides migrate according to molecular weight on SDS gels so that molecular weight of polypeptides may be easily and rapidly estimated. At the

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same time many insoluble proteins are solubilized by SDS so that SDS gel electrophoresis has become the technique of choice for resolving mixtures of insoluble proteins, especially membrane proteins. Electrophoresis is the migration of charged molecules in solution in response to an electric field. Their rate of migration depends on the strength of the field; on the net charge, size and shape of the molecules and also on the ionic strength, viscosity and temperature of the medium in which the molecules are moving. The gel electrophoresis used support matrices such as paper, cellulose acetate, starch gel, agarose or polyacrylamide gel separating the molecules by size. Sodium Dodecyl Sulphate (SDS) is an anionic detergent which denatures proteins by wrapping around the polypeptide backbone and SDS binds to proteins fairly specifically in a mass ratio of 1.4:1. In so doing, SDS confers a negative charge to the polypeptide in proportion to its length i.e., the denatured polypeptides become rods of negative charge cloud with equal charge or charge densities per unit length. It is usually necessary to reduce disulphide bridges in proteins before they adopt the random-coil configuration necessary for separation by size. In denaturing SDS-PAGE separations therefore, migration is determined not by intrinsic electrical charge of the polypeptide, but by molecular weight. Aim of this research was to characterize *B. napus* seeds and to compare seeds of different varieties for their compositions.

MATERIALS AND METHODS

The seeds of ten different varieties of *Brassica napus* namely Cyclon, Star, Vangard, MLCP-048, Bullet, Altex, Con-III, Deffender, Hyola and Dunkled were obtained from Agriculture Research Station North Swat NWFP and National Agriculture Research Center (NARC) Islamabad and were processed for analysis of different parameters like, pH, electrical conductivity, thousand seed mass, sodium, potassium and vitamin C contents, proteins were analyzed by SDS-PAGE. This study was conducted in Feb. 2007 in Department of Biotechnology University of Malakand.

Na^+ and K^+ was determined by flame photometer using acid digestion method (Jones *et al*., 1991) the instrument was standardized by standard sodium and potassium solutions respectively (Sabir *et al*., 2005).

Electrical conductivity of seeds was determined by imbibitions method. Two gram of seeds were taken in beaker and with the help of calibrated conductive meter, electrical conductivity was determined after 20, 40, 60, 80, 100 and 120 min.

Thousand seeds were counted and their average mass was measured by electronic balance.

Ascorbic acid also called vitamin C was determined by redox titration method with iodine solution using starch as indicator. Two gram of seeds was dissolved in 100 mL of distilled water, from this 10 mL was taken and titrated against standard iodine solution. From this weight vitamin C was determined by formula.

$$\text{Weight of Vitamin C} = \frac{N \times V \times \text{equivalent weight}}{1000}$$

For pH values two grams of seeds were taken in powder form, dissolved in 100 mL of distilled water and placed in shaker for 24 h, then filtered and pH was determined by pH meter.

Brassica seed proteins were analyzed by SDS-PAGE (Sodium dodecyl sulfate Polyacrylamide Gel Electrophoresis) using method describe by (Laemmli, 1970). For the preparation of 100 mL Protein extraction buffer, Tris (0.6057 g), Sodium Dodecyl Sulfate (SDS) 0.2 g and Urea (30 g) were dissolved in distilled water. The pH was adjusted to 8 with concentrated HCl, 1 mL marcaptoethanol was added and then diluted to 100 mL with distilled water. A little Bromophenol Blue (BPB) was added and stored in the refrigerator at 5°C. A 500 mL electrode buffer was prepared by dissolving 15.15 g Tris, 0.5 g SDS and 7.2 g glycine in distilled water then diluted to 500 mL and stored at room temperature. Staining solution was prepared by mixing methanol 440 mL, acetic acid (glacial) 60 mL and Coomassie Brilliant Blue (CBB; R-250) 2.25 g in distilled water and diluted to 1 L. The solution was stirred for 30 min, filtered and stored at room temperature. Destaining solution was prepared by mixing methanol, acetic acid and distilled water at a ratio of 20: 5: 75 mL, respectively. For extraction of proteins, grains were ground to fine powder with pestle and mortar. A 400 μL Protein Extraction Buffer (PEB) was added to 0. 01 g of seed flour of *Brassica napus* and vertex thoroughly to homogenize, kept overnight at 40°C. In order to purify the homogenate, samples were centrifuged at 13000 rpm for 10 min at room temperature. The supernatant contained extracted, that was transferred to new 1.5 mL eppendrof tubes and stored at 4°C until they were run on the polyacrylamide gel. After electrophoresis the gel was transferred to tray containing staining solution shake gently for 40 min, followed by destaining until the background of gel disappeared. The picture was taken by gel documentation system with white light illuminator.

Data analysis: The molecular weight of protein subunits was measured by comparing sample bands to the standard protein molecular weight marker bands in the electrophorogram.

RESULTS

Sodium and Potassium concentration (%) was found different for each variety, Table 1 shows maximum Na^+ concentration in seeds of Cyclon and minimum in

Vangard, while potassium was minimum in Bullet variety and maximum (1.42%) in seeds of two varieties Star and Con-III as compared to other varieties.

Analysis of Ascorbic acid (Vitamin C) showed maximum value in seeds of Bullet variety while minimum value of ascorbic acid was found in seeds of MLCP-048 variety (Table 1).

pH is an important parameter to detect the acidic or basic nature of sample. The pH of juice from 2 g of seeds for variety was determined with pH meter, the pH value for Altex was found minimum (6.63) while maximum pH (6.93) was shown by MLCP-048 and HYOLA as compared to other varieties. Table 1 shows maximum thousand seeds mass for Cyclon (5.52 g) while minimum for BULLET variety as compared to other varieties.

The property of a sample to pass electric current due to the oozing of ions from sample material in the solution is known as Electrical Conductivity (EC). Electrical conductivity of *Brassica napus* seeds was determined by conductometer after different time intervals i.e., 20, 40, 60, 80, 100 and 120 min. The data regarding electrical conductivity is show in Table 2. Maximum EC value was shown by seeds of Vangard variety after each interval of time.

Table 1: The presence of Na^+, K^+, Ascorbic Acid concentration in seeds of *B. napus* varieties and also shows pH values and thousand seeds mass

Varieties	Mean values of Na^+ Conc. in (%)	Mean values of K^+ Conc. (%)	Mean values of ascorbic acid (mg/100 g)	pH	Thousand seeds mass
Cyclon	0.039	1.34	60	6.81	5.52
Star	0.032	1.42	50	6.91	3.72
Vangard	0.020	1.31	60	6.85	4.50
MLCP-048	0.023	1.28	30	6.93	3.93
Bullet	0.026	1.14	96	6.70	3.63
Altex	0.033	1.40	56	6.63	4.10
Con-III	0.025	1.42	70	6.64	3.98
Deffender	0.036	1.32	83	6.73	4.05
Hyola	0.028	1.35	78	6.93	4.78
Dunkled	0.035	1.27	85	6.88	4.71

Table 2: Electrical conductivity (μ sec) mean values for seeds of different varieties of *Brassica napus*

	Time (min)					
Varieties	20	40	60	80	100	120
Cyclon	0.9	2.9	3.2	3.9	4.5	5.1
Star	1.6	2.1	2.5	2.7	3.3	3.6
Vangard	4.4	6.2	7.7	8.9	9.6	10.4
MLCP-048	1.4	1.7	2.1	2.5	3.1	3.7
Bullet	2.7	3.4	4.3	5.0	6.1	6.3
Altex	0.9	1.1	1.3	1.6	2.1	2.6
Con-III	2.5	2.7	3.7	5.6	6.6	7.6
Deffender	2.1	2.9	3.1	3.9	4.7	6.2
Hyola	1.7	1.9	2.1	2.8	3.6	4.8
Dunkled	3.1	3.4	4.2	4.9	5.9	6.6

Table 3: Molecular weight, banding range, Number of protein bands and total number of bands for different varieties of *Brassica napus*

Varieties	Mol. wt. range (kDa)	No. of protein bands	Total bands	Varieties	Mol. wt. range (kDa)	No. of protein bands	Total bands
Cyclon	10-40	5			10-40	6	
	40-85	4	12	Altex	50-100	4	11
	100-120	2			120-200	1	
	150-200	1					
Star	10-25	4			10-50	7	
	30-40	3	14	Con-III	60-100	3	11
	60-100	4			120-200	1	
	120-200	3					
Vangard	10-25	3			10-60	8	
	30-50	5	14	Deffender	60-100	4	12
	60-100	4			120-200	1	
	150-200	2					
MLCP-048	10-25	2			10-50	8	
	25-50	5	12	Hyola	60-120	4	13
	60-120	4			120-200	1	
	120-200	1					
Bullet	10-50	8			10-50	8	
	60-120	5	12	Dunkled	60-100	4	13
	150-20	2			120-200	1	

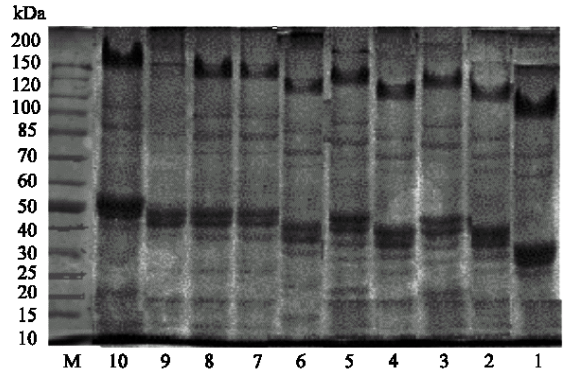

Fig. 1: Protein banding pattern of different varieties of *Brassica napus* seeds separated by SDS-PAGE

Molecular characterization of proteins from seeds of ten different varieties of *Brassica napus* was determined using SDS-PAGE. Molecular weight and their corresponding band numbers of each variety of *Brassica napus* have been shown in Table 3 and Fig. 1. Banding pattern shows variation in molecular weights for proteins among different varieties. Protein ladder (molecular marker) is a mixture of highly purified proteins designed for precise sizing of proteins by SDS-PAGE. The proteins resolved into clearly identifiable sharp bands from 10 to 200 kDa when analyzed by SDS-PAGE.

The digits 01, 02, 03, 04, 05, 06, 07, 08, 09 and 10 represent the *Brassica napus* varieties Cyclone, Star, Vangard, MLCP- 048, Bullet, Altex, Con-III, Deffender, Hyola and Dunkled, respectively.

DISCUSSION

This study was carried out to evaluate the physicochemical, elemental and molecular characterization of seeds of ten different varieties of *Brassica napus*. The seeds were analyzed for various physiochemical parameters such as, pH, Thousand Seeds Mass (TSM), electrical conductivity, mineral composition (Sodium/Potassium) and vitamin C. Molecular characterization of seeds was also carried out by studying the protein-banding pattern through SDS-PAGE.

The amount of sodium ranges from 0.020-0.039% in the present study while using ten varieties of *Brassica napus*. The lowest value was 0.020% for Vangard and highest value was 0.039% for Cyclone variety. Similar results have also been found by Najib and Al-Khateeb (2004) by using *Brassica napus* (canola) in their experiment and found that the amount was 0.02%. The results of the present study also correlate with Leeson *et al.* (1991) who worked on Brassica napus (canola) and found that the sodium amount was 0.01%. However the present results have deviation from Bell and Keith (1991) and Bell *et al.* (1999) who found sodium level of 0.1%. This deviation may be due to difference in varieties used.

The minimum value 1.14% of Potassium was found in Bullet while the maximum value 1.42% for Con-III. Same results were documented by Bell *et al.* (1999) who found that potassium level in canola was 1.22% that correlates with the present study. Leeson *et al.* (1991) also found that the amount of potassium in canola was 0.81%, which shows narrows resemblance with the study conducted. This slight variation may be due to differences in species or varieties used. Najib and Al-Khateeb (2004) observed that the amount of potassium found in canola was 0.71%, which does not satisfy the present results variation may be due to difference in varieties used.

Vitamin C amount in seeds was found in the present study ranges from 30-96 mg/100 g. The maximum amount was found in Bullet variety while the lowest amount was found in MLCP-048. Similar results have also been found by Goldoni *et al.* (1983). They used *Brassica olaracea* as an experimental plant and observed that the amount ranged from 12-112.5 mg/100 g that satisfy the present study.

MLCP-048 pH value was the highest as it was 6.933 while the lowest pH value was found for Altex which was 6.632. The pH value of 6-6.8 was observed by the Husted and Schjoerring (1995) in *Brassica napus*, which shows complete correlation with the present study.

Thousand Seeds Mass (TSM) values showed variation due to different varieties of *Brassica napus* used. In present study the high value of TSM was 5.52 g for Cyclone variety and the low value range for Bullet variety was 3.63 g. TSM values reported by Laoniste *et al.* (2004) in the oil seed rape variety, ranging from 3.5-4.5 g, satisfying the present results.

The overall electrical conductivity of Brassica napus seeds in present work ranged from 0.9-10.4 μ sec. Electrical conductivity reported by Stephen *et al.* (2001) was 1.2 μ sec for nutrients seeds of *Brassica napus*, which shows resemblance to the present study. In contrast to nutrient seeds when saline water was used, the value recorded was 11.2-24.9 μ sec. The variation was due to increased ion concentration. The conductivity of *Brassica napus* seeds studied by Boem *et al.* (1997) ranges from 5-8 μsec, which justifies the present study.

SDS-PAGE (Sodium Deodecyl Sulfate Polyacrylamide Gel Electrophoresis) analysis of total seed protein of ten different varieties is shown in Table 3. Maximum number of bands were found between the range of 10-50 kDa in majority of the varieties. Minimum number of bands were found in the range of 120-200 kDa. In all varieties the number of bands were different. Maximum number of

bands were (15) for Bullet while minimum number of bands (11) were found for Altex and Con-III. In the present study variation was found among protein banding pattern of sees from different varieties through SDS-PAGE. Similar results were documented by Benmoussa *et al*. (2000).

CONCLUSION

The lowest value of sodium was 0.020% for Vangard and the highest 0.039% for Cyclone. While the potassium having lowest value for Bullet 1.14% and the highest of 1.42% for Star. The highest amount of vitamin C (96 mg/100 g) was found in Bullet variety while the lowest concentration (30 mg/100 g) was found in MLCP-048.The pH values for all the varieties were observed to be in the range of 6.632-6.933. The thousand seed mass determined were 3.63-5.52 g. The overall electrical conductivity of *Brassica napus* seeds were ranged from 0.9-10.4 μ sec. *Brassica napus* seed proteins were analyzed by SDS-PAGE. The different numbers of bands of the gel picture showed that variation exists among the different varieties.

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