Pak. J. Bot., 53(5): 1851-1857, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-5(38)

EVALUATION OF COTTON SEED OF ADVANCED LINES FOR NUTRITIONAL QUALITY THROUGH BIOCHEMICAL ANALYSIS

SHAHWAR FATIMA[1], SAJJAD HAIDAR[2*] AND NOREEN BIBI[2]

[1]*Department of Biological Sciences, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad*; *Constituent College of Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad,*
[2]*Plant Breeding and Genetics Division, Nuclear Institute for Agriculture & Biology (NIAB-C), Faisalabad, Pakistan*
**Corresponding Author's Email: Sajjadhaidar_pk@yahoo.com*

Abstract

This paper presents a study to find an advanced line of cotton which has desirable yield and quality parameters with better nutritional constituents and low amount of gossypol contents in the seed. The randomized complete block design (RCBD) with three replications was used for this experiment during 2017-18 at NIAB. Seed biochemical composition was analyzed for gossypol contents, total soluble sugars, protein fractions, amino acids, crude fibers and micro/macro nutrients. Analysis of gossypol contents revealed highly significant differences (p<0.01) among the genotypes. Gossypol content in all of advanced lines was lower than standard checks. Total amino acids were statistically highly significant among all the genotypes. For instance, the content of total free amino acids was highest (0.1686 µg/g FW) in NIAB-4-44-3 while lowest (0.0219 µg/g FW) in NIAB-4-54. There were no significant differences among advanced lines and standard checks for the contents of total soluble sugars. However, significant variations were observed for the contents of glucose and sucrose with the amount of maximum glucose in NIAB-4-54 and highest sucrose in NIAB-4-79-2. Among four protein fractions (albumin, globulin, glutelin, prolamin), albumin was abundantly present in all genotypes. Water soluble albumin fraction ranged from 3.35 mg/g fresh weight (FW) to 3.82 mg/g FW being maximum in NIAB-14-43-19 while lowest in NIAB-4-79-2. Crude fiber, crude protein, ash and other micro nutrients were also high in NIAB-4-44-3. From these results it is concluded that NIAB-4-44-3 has better nutritional quality with higher contents of amino acid and crude fiber while lowest amount of gossypol, hence, can be a better source for animal feed and human consumption.

Key words: *Gossypium hirsutum,* Gossypol, Advanced lines, Nutrient analysis.

Introduction

Cotton (*Gossypium* spp.) is the principal natural fiber crop and plays a dynamic role in the country economy as cash crop (Song *et al*., 2007). Cotton is grown commercially in the moderate and tropical areas of more than 50 countries (Smith *et al*., 1999; Smith *et al*., 2014). There are ~ 50 cotton species documented in the world from which 4 are cultivated for cotton fiber. India, China, United States, Pakistan, Brazil, Australia and Uzbekistan are the major exporters of cotton and together they contribute ~75% of the world's cotton production (Khadi *et al*., 1970). Cotton is chiefly cultivated for fiber used in textile industry (Guedes & Soto Balanco, 2010; Cheng *et al*., 2016) but after ginning about 1.65 kg of cotton seeds is produced as byproduct for each kilogram of cotton fiber. The cotton products are used in different forms, for human as cotton seed oil, for animal as cotton seed meal and cake and in textile industries (Gao *et al*., 2010; He *et al*., 2014). Besides, cotton seed also contain proteins, fats, dietary fibers and minerals. Cotton seed kernels hold 28.24–44.05% oil and 27.83–45.60% protein (Shikang *et al*., 1987). Kouser *et al*., 2015 reported the amount of oil and protein in the tested seeds varied from 15.06 to 18.35% and 20.42 and 27.03%, respectively. Two types of amino acids, essential and non-essential amino acids are present in the world (Edmunds *et al*., 2013) and essential amino acids are abundantly found in cotton proteins, which meet the recommendations of many health specialists (Chen *et al*., 1986). Cotton seed is becoming a major source of edible oil and contribute more than 20% in the edible oil (Abid *et al*., 2011; Cheng *et al*., 2016; Haidar *et al*., 2012; Khan *et al*., 2007, 2009, 2010). Cotton seed oil added 70% in global output during 1995 to 2003 (Song *et al*., 2007) and in many countries, soybean oil has been effectively replaced by cotton seed oil because of its high protein percentage (Barros *et al*., 2002). Cotton seed oil used as a food complement for human consumption as it contains high-grade proteins (De Buckle *et al.*, 1979). According to an estimate, global cotton seed production can provide the yearly protein requirements for a billion people (Sunilkumar *et al*., 2006). So, cotton seed is equally good for livestock as protein source in the form of cotton seed meal (defatted cotton seeds) and for human beings as cooking oil (Dowd *et al*., 2010; Wanapat *et al*., 2013a). Cotton seed is fed to dairy cows to provide effective fiber as well as energy and protein (Broderick *et al*., 2013). Cotton seed meal can increase the milk yield in dairy cows and hence improve farmer's income (Wanapat *et al*., 2013b).

With the quick increase in world population, cotton seeds became more valuable in research due to their superior nutritive properties. However, the presence of anti-nutritional compounds in cotton seed cannot be overlooked. Cotton and related species have pigment glands and these glands contain a compound called gossypol (Karishma *et al*., 2016). Gossypol is a pigmented phenolic compound present throughout the cotton plant. The poisonous effect of gossypol has been reported in many species like pigs (Haschek *et al*., 1989), dogs (West, 1940), sheep (Morgan *et al*., 1988), broiler chicks (Henry *et al*., 2001) and goats (East *et al*., 1994). Gossypol causes many harmful effects to non-ruminant animals such as pigs and poultry (De Peyster *et al*., 1993). It can cause reduction in bull fertility. However, ruminant such as cattle have the capability to detoxify the dangerous effect of gossypol because of the microbes within the rumen bind it so it cannot be absorbed (Myer *et al*., 2003). Toxic effect of gossypol limits the use of cottonseed meal as a feed ingredient (Alexander *et al*.,

2008). In this context, screening of a genotype with better nutritional quality in terms of higher contents of oil, amino acids, micro/ macro elements, dietary fiber, ash %, protein, carbohydrates, while lowest quantity of gossypol would be valuable choice for oil and animal feed industries for effective utilization of cotton seed.

Materials and Methods

Cottonseeds are by-products of cotton fiber production and obtained after separation of lint through ginning. These are rich in oil and proteins and are therefore used for cottonseed oil production and as a feed supplement as feed cake (Alexander *et al*., 2008). Cottn plant/varieties varies for presence of gossypol glands at differett parts of plant. Hence, six newly developed lines of cotton developed at NIAB were included in this study.

Plant material: Cotton lines namely NIAB-4-100-5, NIAB-4-79-2, NIAB-4-54, NIAB-1-1, NIAB-14-43-19, NAIB-4-44-3 and two standard varieties FH-Lalazar and MNH-886 were used as experimental material. The experiment was laid out in a randomized complete block design (RCBD) with three replications at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The plot size was 23 x 20 Ft and seeds were sown using seed dibbler. There were five rows for each genotype with row to row distance 75cm and 30 cm plant to plant distance. Existing culture practices of cotton cultivation were exploited to have a good crop stand.

Estimation of gossypol contents: Gossypol content was quantified from the de-hulled seed through spectrophotometer by modified procedure of the American Oil Chemist Society (AOCS) 1989 (Nawab *et al*., 2014; Pettigrew *et al*., 2014).

Complex reagent preparation: Complex mixture was prepared with 10 ml glacial acetic acid, 2 ml of 3-amino-1-propanol and made up to 100 ml volume with dimethyl foramide.

Sample gossypol extraction method: About one gram seeds were crushed in pestle mortal. Then 0.3 g seed powder was taken in test tube. One drop of 70% aqueous acetone and one drop of acetic acid was added in it after that add 1.0 ml of complex reagent in it. The reagent blank contained only 1.0 ml of complex reagent. Seed samples and blank solution were heated in water bath at 95°C-100°C for half an hour and let cool at room temperature. Samples and blank were diluted to 5.0 ml with hexane-isopropyl alcohol mixture (40:60). Test tubes were shaken well and sample extract was filtered. Two aliquots of sample filtrate (2.0 ml each) and blank solution were poured into test tubes. One set of sample and blank aliquot was diluted to 3.0 ml volume with the isopropyl alcohol-hexane mixture and kept as reference solutions for absorbance measurement. About 1.0 ml of aniline was added to the other set of the sample and the blank aliquot. The tubes in this set were heated in water bath (95-100°C) for half an hour and cooled at room temperature. Tubes were again diluted with 3.5 ml of isopropyl alcohol-hexane mixture. Tubes were homogenized well and allowed to stand for 60 minutes at room temperature before determining the absorbance. Standard samples were also prepared using gossypol in a similar way to draw standard curve. The OD of blank solution and the standard aliquots was determined on the spectrophotometer (Hitachi u-2800) at 440 nm. The optical density of the sample reacted with aniline was determined using diluted sample without aniline as reference solution.

Corrected absorbance (mg) = OD of sample aliquot - OD of reagent blank

Determination of total free amino acids: Total free amino acids were determined according to protocol suggested by *Hamilton* and *Van Slyke* (Hamilton *et al*., 1943). De-hulled seeds (0.2 g) were extracted with phosphate buffer pH 7.0. About 1.0 ml of extract was taken in test tubes. Then 1.0 ml of 10% pyridine and 1.0 ml of 2.0 % ninhydrine solution was mixed with seed extract in each tube. After that the tubes were heated for half an hour in boiling water bath then the volume was made up to15.0 ml with distilled water to dilute the solution. The absorbance of the colored solutions was measured at 570 nm.

Estimation of sugars: Total soluble sugars, glucose and sucrose were estimated by using method of (Raizi *et al.*, 1985) with some adjustments (Yemm & Willis, 1954). About 0.1 g of de-hulled seeds powder was ground in 1ml of 80 % ethanol (v/v). Samples were vortexed vigorously then spun in centrifuge machine at 10, 000 rpm for 15 minutes. For the estimation of sugars, the supernatants were collected in fresh tubes. For total soluble sugars, 0.1 ml sample was taken in test tube then3.0 ml of freshly prepared anthrone was added. Tubes were then placed in water bath for 10 minutes at 97°C and immediately cooled on ice. The optical density was measured at 625 nm by using spectrophotometer.

For glucose content, 0.3 ml of sample was poured in the test tube. After that 2.5ml of o-tolidine was added. After that the tubes were placed in hot water bath set at 97°C for 15 minutes. Tubes were then immediately placed on ice to stop the reaction and readings were measured at 630 nm using spectrophotometer.

For sucrose content, 0.1 ml of ethanol seed extract was taken in test tubes followed with addition of 0.1 ml of 5.4 N KOH. After that samples were kept on hot plate for 10 minutes at 97°C. After that tubes were immediately cooled in ice cold water. Then 3.0 ml of anthrone was pipetted in the tubes and tubes were again warmed in the water bath at 97°C for 5 minutes and placed in ice cold water. Samples were then kept at room for 20 minutes for the measurement of OD at 620 nm using spectrophotometer.

Estimation of seed protein fractions: A systematic method was used to extract different proteins fractions of the cotton seed according to their solubility in various solvents by Osborne method (Gandhi *et al*., 2017).

Cotton seeds were de-hulled and ground into fine powder using pestle and mortar. About 0.1g of the grounded seed powder was taken in 2.0 ml tubes and followed by the addition of 1.0 ml of n-hexane. Tubes were vortexed for 2.0 hours and centrifuged at 10,000 rpm for 15 min. About 1.0 ml water was added and

vortexed for 2.0 hours to obtain albumin fraction. Centrifuged for 15 minutes at 10,000 rpm and the supernatant were saved in another tube. Added 1.0 ml of chilled acetone and again centrifuged for 10 minutes. The supernatant was discarded and pellet was dissolved in 1.0 ml of phosphate buffer pH 7.0. Again the residue after soluble albumin removal was then extracted sequentially with 70 % ethanol, salt (usually NaCl) solution and alkali (Borate buffer) solution to obtain prolamin, globulin and glutelin respectively. The procedure was repeated for all the remaining protein fractions as for albumin fraction.

Estimation of crude fibers and crude proteins: Crude fibers and crude proteins were estimated according to AOAC (1990) method (He *et al*., 2013). About 0.5 g de-hulled seed powder was taken in 250 ml flask. Then 50 ml of 1.25 % H_2SO_4 was added and boiled for 30 minutes. Samples were cooled and filtered. The procedure was repeated three times. Again the filtrate was taken and 50 ml of 1.25% of NaOH was added and boiled on hot plate for 30 minutes. Samples were again cooled and residue was filtered. The procedure was repeated thrice. The residue was finally air dried and weighed. The filtrate was burned in high temperature muffle furnace at 600°C. The ash was weighed and the ash% and crude fiber was calculated by given formula:

$$\text{Ash (\%)} = \frac{\text{wt. of Ash}}{\text{wt. of original sample}} \times 100$$

$$\text{Crude fiber (\%)} = \frac{\text{wt. of residue- wt. of ash}}{\text{wt. of the sample}} \times 100$$

Estimation of nutrients

Sample preparation: Dried ground seed samples (0.5 g) were taken in the digestion flasks. About 5.0 ml strenuous H_2SO_4 (65%) as added. All the flasks were incubated overnight at room temperature. Then 2.0 ml of 35 % H_2O_2 was added. Flasks were transferred to the digestion block and heated at 350°C for 30 minutes up to the production of fumes. Then flasks were removed from hot plate and cooled for 5.0 minutes. Again 2.0 ml of 35% H_2O_2 was added and placed the flasks back to the hot plate. This step was repeated until the color of digested material was colorless. Extract was sieved and make the volume of extract up to 50 ml with distilled water in a volumetric flask and used for the estimation of sodium, potassium, phosphorus and nitrogen.

Sodium potassium determination: Sodium potassium were measured by using the flame photometer (Jenway PFP 7). The instrument was standardized with standard solution of Na^+ and K^+ using NaCl salt and KCl salt respectively. Samples were diluted with 50 ml of distilled water to get appropriate concentrations of Na^+ and K^+. The mgL^{-1} of Na^+ and K^+ were noted from the standard curve and divided by its equivalent weight (23) to ge meL^{-1}.

Determination of phosphorus: Spectrophotometer (Hitachi u-2800) was used for the determination of phosphorus content. For this, 1.0 ml of sample aliquot was mixed with 1.0 ml of barton's reagent and then final volume was adjusted to 50.0 ml. Samples were kept at room temperature for 30 minutes and OD was taken at 470 nm (Wolf *et al*., 1985).

Determination of total nitrogen: Nitrogen is assessed by micro-Kjeldhal's method by using UDK 132 Semiautomatic Distillation Unit (Allen *et al*., 1986; Bremner, 1965). For this, 5.0 ml of digested sample was poured in Kjeldhal's tube. About 25 ml of 40% NaOH was added in the conical flask. Then take 5.0 ml of 2 % boric acid solution in the conical flask with few drops of mixed indicator (Bromocresol green = 0.009 g + Methyl red = 0.066 g + Ethanol = 100 ml). The tube was then placed on Kjeldhal ammonia distillation unit. After two minutes, the tube was removed from operator and cooled the distillate. Finally it was titrated with standardized 0.01 N H_2SO_4 and stop as the solution turned pink. For the accuracy of the procedure, blank solution was also run along with the sample solution.

$$\text{N \% age} = ((V2\text{-}V1) \times N \times 1.4)/W$$

Here, "V2" volume H_2SO_4 for the sample solution, "V1" volume of H_2SO_4 for the blank solution, 1.4 acid factor, "N" normality of H_2SO_4, whereas "W" weight of sample.

Results

Cottonseed and its derived products can be used as human food, animal feed, and industrial raw material. Chemical composition of cottonseed is one of the critical parameters for evaluating its quality and potential end use specially the presence of gossypol contents. Protein and dietary fibers are also desirable nutritional properties of cottonseed. In this study, we determined the specifically gossypol contents in cottonseed and other nutritionally important nutrients. Information derived will provide nutritional value and digestibility of cottonseed of selected cotton lines.

Gossypol content in cotton seed: Gossypol is an orally-active polyphenolic aldehyde with potential antineoplastic activity. Analysis of gossypol contents revealed major variances ($p<0.01$) among the advanced lines. Interestingly, gossypol content in all of our advanced lines was lower than standard checks as depicted in the OD values plotted in graph (Fig. 1). For instance, NIAB-1-1 have gossypol content i.e. 90 % lower than standard check FH-Lalazar, NIAB-14-43-19 has 89 %, NIAB-4-44-3 87%, 78 % in NIAB-4-100-5, 75 % in NIAB-4-54 and 72% in NIAB-4-79-2.

Total free amino acids (TFA): Significant variances were observed for TFA among the genotypes studied. Among the genotypes, NIAB-4-44-3 has highest total free amino acid content and followed by MNH-886, FH-Lalazar, NIAB-14-43-19, NIAB-1-1, NIAB-4-79-2, NIAB-4-100-5 and NIAB-4-54. However, if we compare the magnitude of difference from advanced lines is much higher than standard checks. For instance, the content of total free amino acids was highest (0.1686) in NIAB-4-44-3 while lowest (0.0219) in NIAB-4-54. The results of amino acids are shown in (Fig. 2).

Estimation of sugars: Results revealed no significant differences among advanced lines and standard checks for the contents of total soluble sugars (Fig. 3). However, significant variations were observed for the contents of glucose and sucrose. The amount of glucose was maximum in NIAB-4-54 and was not only higher than the standard checks but also greater than other advanced lines and the NIAB-4-79-2 indicates the elevated level of sucrose.

Protein fractions: We observed remarkable differences among the cotton genotypes for the contents of protein fraction. Among four fractions, albumin was abundantly present in all genotypes. Water soluble albumin fraction ranged from 3.35 mg/g FW to 3.82 mg/g FW being maximum in NIAB-14-43-19 while lowest in NIAB-4-79-2. Second most abundant fraction was globulin that ranged from 1.02 mg/g FW to 2.69 mg/g FW. Highest globulin content was found in NIAB-4-54 while lowest in NIAB-1-1. Glutelin and prolamin behaved differently in different genotypes. Glutelin ranged from 0.42 mg/g FW to 1.17 mg/g FW as glutelin was more in FH-Lalazar and lowest in NIAB-4-79-2. Prolamin fraction ranged from 0.72 mg/g FW which is NIAB-4-54 to 1.19mg/g FW in NIAB-14-43-19. Results of protein fractions are given in (Fig. 4).

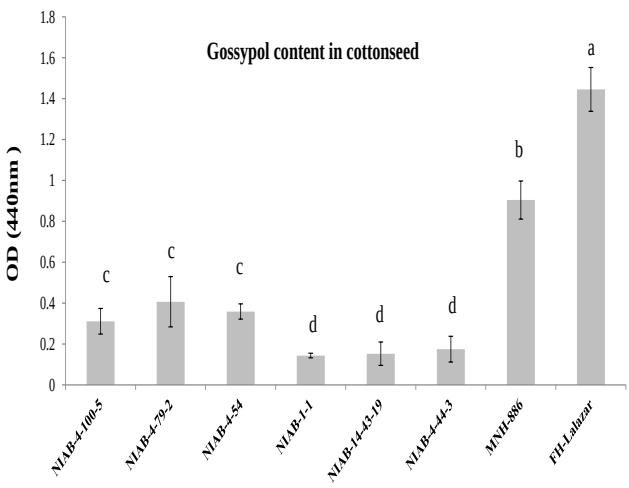

Fig. 1. Quantification of gossypol content in de-hulled cotton seeds.

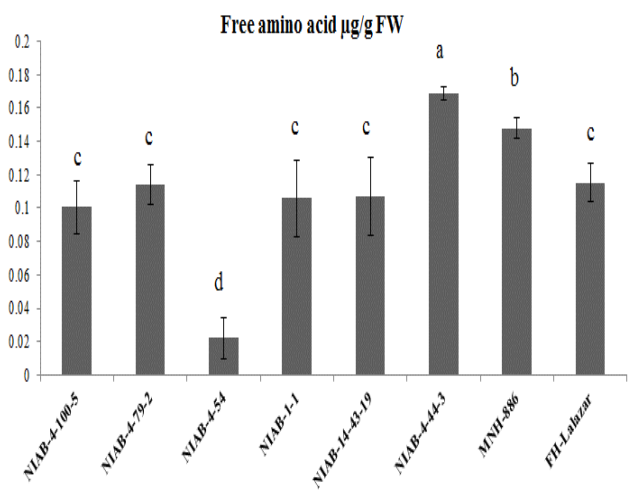

Fig. 2. Estimation of total free amino acids of advanced lines of cotton developed at NIAB with two standard varieties.

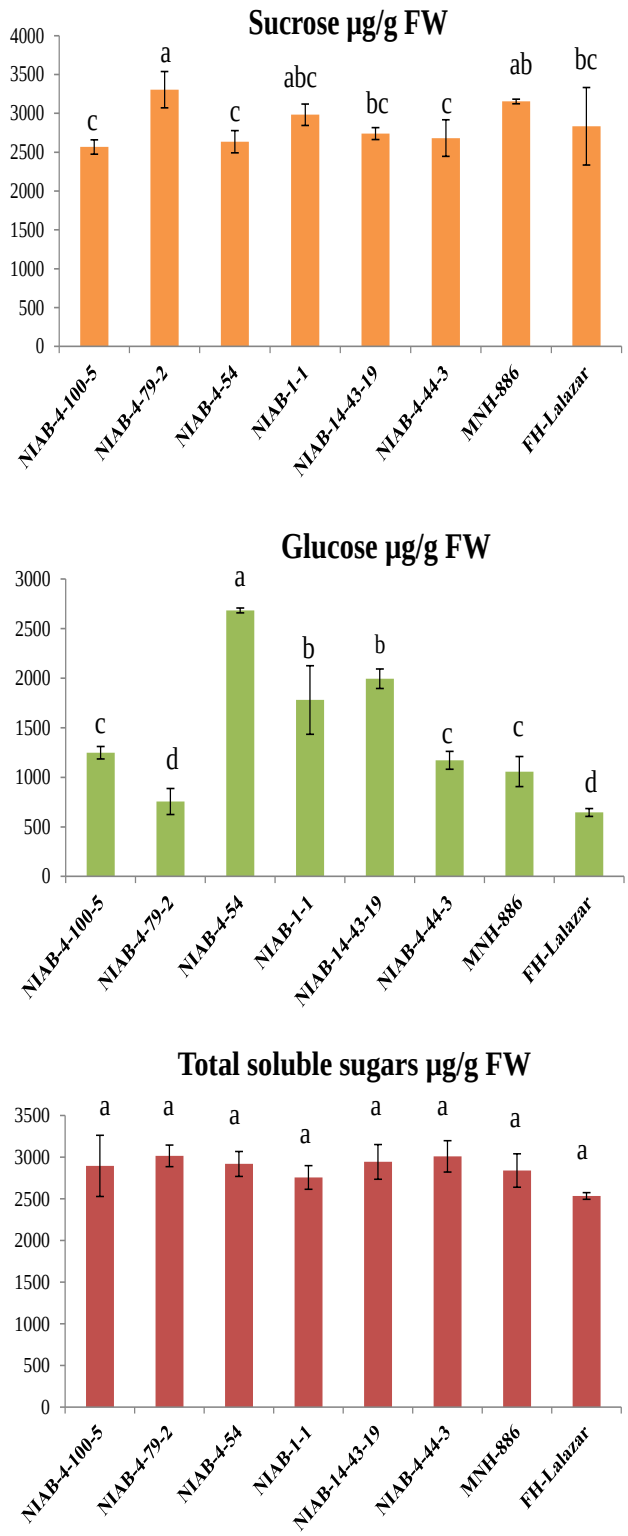

Fig. 3. Estimation of total soluble sugars, sucrose and glucose of advanced lines of cotton developed at NIAB with two standard varieties.

Nutrient analysis: Nutrient analysis was done to determine the concentration of different macro and micro nutrients in all the genotypes. Data revealed that NIAB-4-44-3 had the maximum amount of potassium, phosphorus and nitrogen when compared with the standard checks. Crude fiber and crude protein was also high in NIAB-4-44-3 (Table 1).

Table 1. Elemental analysis of cotton seed.

Genotypes	Sodium (Na^+ ppm)	Potassium (K^+ ppm)	Phosphorus (P ppm)	Nitrogen (N%)	Ash (%)	Crude fiber (%)	Crude protein (%)
NIAB-4-100-5	983	8283	7350.00	3.51	10.38	24.20	21.93
NIAB-4-79-2	1050	7567	5316.67	3.34	5.37	22.07	20.88
NIAB-4-54	1100	9100	6516.67	3.90	6.79	21.73	24.38
NIAB-1-1	1083	6800	5050.00	2.74	11.60	23.40	17.15
NIAB-14-43-19	1100	9400	1716.67	3.55	11.63	20.60	22.17
NIAB-4-44-3	1167	12366	8816.67	4.59	8.00	27.00	28.70
MNH-886	1200	7500	7716.67	3.97	6.57	14.33	24.79
FH-Lalazar	1100	9467	6283.33	4.15	8.79	24.33	25.96

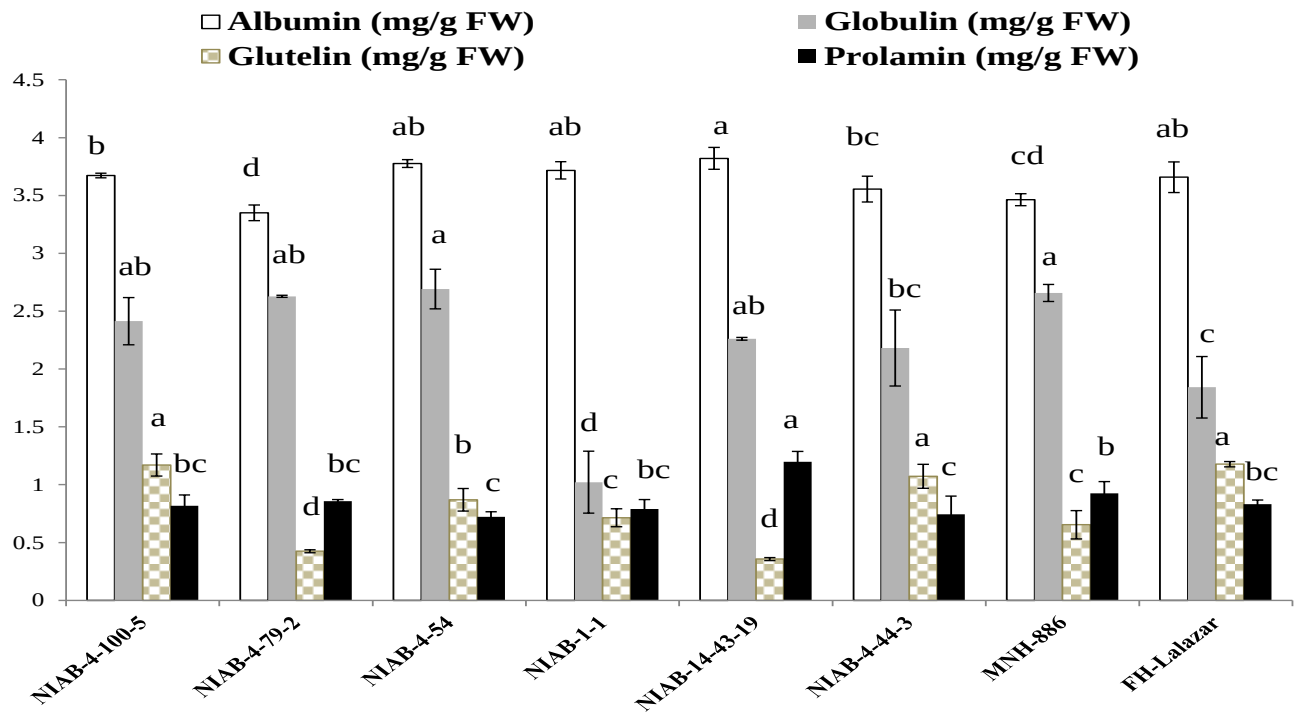

Fig. 4. Extraction of seed proteins fractionsof advanced lines of cotton developed at NIAB with two standard varieties.

Discussion

The recent genetic development of low gossypol cotton seed has the potential to alter both the processes used to extract the oil and the use of the seed protein. Seed biochemical composition is one of the decisive factor for the evaluation of the seed nutritional quality (He *et al*., 2015). Protein, amino acids, carbohydrates and crude fibers contents are the most desired characteristics of cottonseed for human and animal feed (He *et al*., 2014). The nutritional value of cottonseed protein is dependent on the level of essential amino acids and total protein content (Bertrand *et al*., 2005). Cotton storage protein accounts up to 80% of total protein. The proportion of proteins in cotton seed is different in different varieties due to the genetic deposition of variety. Hence, the objective of the current study was to evaluate the protein fractions in cotton seed from different genotypes. Cotton seeds showed the same results as observed in wheat germ contain 4.6, 10.6, 15.6 and 34.5% of prolamin, glutelin, globulin and albumin respectively (Zhu *et al*., 2006). Experimental evidence has been demonstrated that the maximum amino acid content and crude fiber and lowest amount of gossypol was observed in NIAB-4-44-3 when we compared it with all other genotypes. So, NIAB-4-44-3 can be used for animal feed and human consumption as it has the lower gossypol content in cotton seed. We have to be taken into account that the MNH-886 also performed best in contest of the total amino acids. The carbohydrate concentration can be either reduced or increased by varying the concentration of macro or micro elements (Steven, 1985). Changes in the amount of nitrogen in seed germination can alter the carbohydrate and protein content of seeds (He *et al*., 2014; Shikang *et al*., 1987). Our results showed the same hypothesis that NIAB-14-43-19, NIAB-4-44-3 and NIAB-4-54 was the best lines in contest of albumin globulin protein fractions along with the high value of nitrogen and crude fibers. Any genotype which is found to have higher contents of oil, amino acids, micro macro elements, dietary fiber, ash %, protein content, carbohydrates, and lowest quantity of gossypol can be utilized efficiently in oil and animal feed industries. Fortunately, among six advanced lines of NIAB, NIAB-4-44-3 was found that fulfill all the requirements.

Conclusions

It is concluded that scientists can select better cotton lines having high output in term of lint yield as well as of seed having better nutritional quality. Genotype which is found to have higher yield of lint and higher contents of oil, essential nutrients and lowest quantity of gossypol can be utilized efficiently for economic gain from fiber, oil and animal feed industry. The result showed that one line identified in this study may be given more emphasis for release as variety and use as genetic source for creation of new genetic variability in seed nutritional quality.

References

Abid, M., M. Ashfaq, S. Hassan and N. Fatima. 2011. A resource use efficiency analysis of small Bt cotton farmers in Punjab, Pakistan. *Pak. J. Agri. Sci.,* 48(1): 65-71.

Alexander, J., H.C. Andersson, A. Bernhoft, L. Brimer, B. Cottrill, J. Fink-Gremmels, J. Jaroszewski and H. Sørensen. 2008. Gossypol as undesirable substance in animal feed: scientific opinion of the panel on contaminants in the food chain. *EFSA J.*, 908: 1-55.

Allen, S.E., H.M. Grimshaw and A.P. Rowlan. 1986. Chemical Analysis. In: *Methods of Plant Ecology,* (Eds.): P.D. Moore and S.B. Chapman, Blackwell, Oxford, 285-344.

Barros, M,. M., C. Lim and P.H. Klesius. 2002. Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of Channel Catfish (*Ictalurus puctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture*, 207: 263-279.

Bertrand, J.A., T.Q. Sudduth, A. Condon, T.C. Jenkins and M. Calhoun. 2005. Nutrient content of whole cottonseed. *J. Dairy Sci.*, 88(4): 1470-1477.

Bremner, J.M. 1965. Total Nitrogen: Methods of soil analysis; Chemical and microbiological properties: Ed.: .A.G. Norman., American Society of Agronomy, Inc, Agrnonomy Monographs, 1149-1178.

Broderick, G.A, T.M. Kerkman, H.M. Sullivan, M.K. Dowd and P.A. Funk. 2013. Effect of replacing soybean meal protein with protein from upland cottonseed, Pima cottonseed, or extruded Pima cottonseed on production of lactating dairy cows. *J. Dairy Sci.*, 96(4): 2374-2386.

Chen, Z.F., Z.W. Zhang and H.L. Cheng. 1986. The analysis of upland cotton quality. *Acta Agron. Sinica*, 12: 195-200.

Cheng, H., C. Lu, Z.Y. John, C. Zou, Y. Zhang, Q. Wang, J. Huang, X. Feng, P. Jiang and W. Yang.2016. Fine mapping and candidate gene analysis of the dominant glandless gene Gl2e in cotton (*Gossypium* spp.). *Theor. & Appl. Gene.*, 129: 1347-1355.

De Buckle, T., L. Zapata, G. Silva, J. Cabrera, A. De Sandoval, I. Ben-Gera, H. Riveros and I. Shomer. 1979. Two food applications of cottonseed flours and meals. *J. Amer. Oil Chem. Soci.*, 56: 297-299.

De Peyster, A. and Y.Y. Wang. 1993. Genetic toxicity studies of gossypol. *Mut. Res./Rev. in Genetic Toxicol.*, 297(3): 293-312.

Dowd, M, P.J. Wakelyn and R. Chaudhry. 2010. Cottonseed current and future utilization. Cotton: technology for the 21st century. ICAC Press, Washington, DC: 437-460.

East, N.E., M. Anderson and L. Lowenstine. 1994. Apparent gossypol-induced toxicosis in adult dairy goats. *J. Amer. Vet. Med. Asso.*, 204: 642-643.

Edmunds, B., K.H., Südekum, R. Bennett, A. Schröder, H. Spiekers and F. Schwarz. 2013. The amino acid composition of rumen-undegradable protein: A comparison between forages. *J. Dairy Sci.*, 96: 4568-4577.

Gandhi, K, N. Litoriya, A. Shah and J. Talati. 2017. Extraction, Fractionation and Characterization of Cotton (*Gossypium herbaceum* L.) Seed Proteins. *Ind. J. Agri. Biochem.*, 30(1): 21-26.

Gao, D., Y. Cao and H. Li. 2010. Antioxidant activity of peptide fractions derived from cottonseed protein hydrolysate. *J. Sci. Food Agric.*, 90: 1855-1860.

Guedes, F.C.B. and B. Soto Blanco. 2010. Sperm quality of sheep fed cottonseed cake. *Acta Scientiae Veterinariae*, 38(4): 415-418.

Haidar, S., M. Aslam, M. Hassan, H.M. Hassan and A. Ditta. 2012. Genetic diversity among upland cotton genotypes for different economic traits and response to cotton leaf curl virus (CLCV) disease. *Pak. J. Bot.,* 44(5): 1779-1784.

Hamilton, P.B., D.D Van Slyke and S. Lemish. 1943. The gasometric determination of free amino acids in blood filtrates by the ninhydrin-carbon dioxide method. *J. Biol. Chem*, 150: 231-250.

Haschek, W., V.R. Beasley, W.B. Buck and J.H. Finnell. 1989. Cottonseed meal (gossypol) toxicosis in a swine herd. *J. Amer. Vet. Med. Asso.*, 195(5): 613-615.

He, Z., H. Cao, H.N. Cheng, H. Zou and J.F. Hunt. 2013. Effects of vigorous blending on yield and quality of protein isolates extracted from cottonseed and soy flours. *Mod. Appl. Sci.*, 7(10): 79-88.

He, Z., H. Zhang and D.C. Olk. 2015. Chemical composition of defatted cottonseed and soy meal products. *PloS One,* 10(6): e0129933; https://doi.org/10.1371/journal.pone.0129933.

He, Z., H. Zhang, D.C. Olk, M. Shankle, T.R. Way and H. Tewolde. 2014. Protein and fiber profiles of cottonseed from upland cotton with different fertilizations. *Mod. Appl. Sci.*, 8(4): 97-105.

Henry, M.H., G.M. Pesti and T.P. Brown. 2001. Pathology and histopathology of gossypol toxicity in broiler chicks. *Avian Dise.*, 54(3): 598-604.

Karishma, R., U. Lakshmi Sahithya, P. Suneetha, V. Chinna Bbabu Naik and M.S.R. Krishna. 2016. Determination of total gossypol and free gossypol content in different varieties of Bt and Non Bt Cotton seed extracts by High-Performance Liquid Chromatography (HPLC). *Res. J. Biotech.* 11(2): 70-74.

Khadi, B, M., S. Venoor and M. Yadav. 1970. Cotton: An Introduction. In Book pp1-14; U.B. Zehr (Ed.), Cotton, Biotechnology in Agriculture and Forestry 65, DOI 10.1007/978-3-642-04796-1_1, # Springer-Verlag Berlin Heidelberg

Khan, N.U., G. Hassan, K.B. Marwat, M. Kumbhar, I. Khan, Z. Somro, M.J. Baloch and M.M.Z. Khan. 2009. Legacy study of cotton seed traits in upland cotton using griffing's combining ability model. *Pak. J. Bot*., 41(1): 131-142.

Khan, N.U., G. Hassan, M. Kumbhar, A. Parveen, UM-E Aiman, W. Ahmad, S. Shah and S. Ahmad. 2007. Gene action of seed traits and oil content in upland cotton (*Gossypium hirsutum* L.). *SABRAO J. Breed. & Gen.*, 39(1): 17-29.

Khan, N.U., K. B. Marwat, G. Hassan and Farhatullah. 2010. Genetic variation and heritability for cotton seed, fiber and oil traits in *Gossypium hirsutum* L. *Pak. J. Bot.,* 42(1): 615-625.

Kouser, S., K. Mahmood and F. Anwar. 2015. Variations in physicochemical attributes of seed oil among different varieties of cotton (*Gossypium hirsutum* L.). *Pak. J. Bot.,* 47(2): 723-729.

Morgan, S., E.L. Stair, T. Martin, W.C. Edwards and G. Morgan. 1988. Clinical, clinicopathologic, pathologic, and toxicologic alterations associated with gossypol toxicosis in feeder lambs. *Amer. J. Vet. Res.*, 49: 493-499.

Myer, R.O. and L.R. McDowell. 2003. Potential for gossypol toxicity when feeding whole cottonseed to beef cattle. *Fla. Coop. Ext. Service, Inst. of Food and Agric. Sci.* AN130.

Nawab, N.N. and A.A. Khan. 2014. Categorization and scaling of distinct gossypol classes with respect to gossypol content in cotton (*Gossypium hirsutum* L.), *J. Environ. & Earth Sci.*, 4(5): 45-53.

Pettigrew, W.T. and M.K. Dowd. 2014. Nitrogen fertility and irrigation effects on cottonseed composition. *J. Cotton Sci*, 18: 410-419.

Riazi, A., K. Matsuda and A. Arslan. 1985. Water-stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. *J. Exp. Bot.*, 36(11): 1716-1725.

Shikang, S.S.C.J.X and W. Shoujun .1987. Study on the nutritional quality of cotton seeds. *Scientia Agri. Sinica*, 5: 2.

Smith, C.W. and J.T. Cothren. 1999. Cotton: origin, history, technology, and production. John Wiley & Sons Inc., 605 Third Avenue, New York, NY.

Smith, J.S.C., B.W. Diers, J.E. Specht and B.F. Carver. 2014. Yield gains in major US field crops. *Spec. Publ. 33. CSSA, Madison, WI. p.* 125-171.

Song, X.L. and T.Z. Zhang. 2007. Identification of quantitative trait loci controlling seed physical and nutrient traits in cotton. *Seed Sci. Res.*, 17(4): 243-251.

Steven, H.C. 1985. Role of potassium in photosynthesis and respiration. Agricultural Research Service, USDA. North Carolina State, https://doi.org/10.2134/1985.potassium.c15.

Sunilkumar, G., L.M. Campbell, L. Puckhaber, R.D. Stipanovic and K.S. Rathore. 2006. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. PNAS (*Proceedings of the National Academy of Sciences)*, 103 (48): 18054-18059.

Wanapat, M., N. Anantasook, P. Rowlinson, R. Pilajun and P. Gunun. 2013a. Effect of carbohydrate sources and levels of cotton seed meal in concentrate on feed intake, nutrient digestibility, rumen fermentation and microbial protein synthesis in young dairy bulls. *Asian-Australasian J. Anim. Sci.*, 26: 529-536.

Wanapat, M., R. Pilajun and P. Rowlinson. 2013b. Effect of carbohydrate source and cottonseed meal level in the concentrate: IV. Feed intake, rumen fermentation and milk production in milking cows. *Trop Anim. Health Prod*., 45: 447-453.

West, J. 1940. Lesions of gossypol poisoning in the dog. *J. Amer. Vet. Med. Associ.,* 96: 74-76.

Wolf, A.M., D.E. Baker, H.B. Pionke and H.M. Kunishi. 1985. Soil tests for estimating labile, soluble, and algae-available phosphorus in agricultural soils. *J. Environ. Qual.*, 14: 341-348.

Yemm, E.W. and A.J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.*, 57(3): 508-514.

Zhu, K.X., H.M. Zhou and H.F. Qian. 2006. Proteins extracted from defatted wheat germ: Nutritional and structural properties. *Cereal Chem.*, 83(1): 69-75.

(Received for publication 8 September 2019)