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Comparing and Correlating some Morphological and Physicochemical properties of the Natural (Non-transgenic), and some Genetically Modified (Transgenic) Cottonseeds (*Gossypium hir-. Sutum L*)

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Abstract
This study aims to compare some morphological features and physicochemical constituents of some natural cottonseeds (Gossypium hir sutum) (non- transgenic), and some genetically modified (GM) (transgenic) cotton seeds. The non- transgenic cottonseeds samples were collected from local markets in Sudan, while GM (transgenic) seeds were collected from some governmental and private research centers, seeds were imported from China and some other countries. Phenotypic features and proximate parameters of the two varieties were found somewhat similar, but some variations in the color, moisture, and ash content were noticed. The weight of 1000 seeds was also reported. Different colors of the GM cottonseeds had reported. The carbohydrate % content of non- transgenic seeds was 27.00±0.40, and it was 26.70±1.00 for the transgenic seeds; moisture % contents were 10.87±0.10, and 7.90±1.10, respectively; ash contents % were 3.55±0.06, and 5.19±1.06. Comparing these characterizations will help the farmers and the small agricultural companies to differentiate between organic and GM cottonseeds by simple and more applicable methods. Although the research article is specific to a local Sudanese samples, it gives good indications about the necessity of the subject which can be generalized world-wise.

Keywords: cottonseeds, genetic modifications, non-transgenic, morphological, physicochemical analysis

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the essential commercial annual crops widely cultivated in most regions of the world [1]. Sudan was one of the major players in the world cotton trade for many years, mainly the crop is grown in *Aljazeera* project in Central Sudan [2]. Gossypiumhirsutum species encompass 90% of cotton world production [3].

As Sudan is a party of many international conventions on biosafety such as Cartagena Protocol on Biosafety (CPB), and the Convention on Biological Diversity (CBD), Sudan has developed a National Biosafety Framework (NBF), which legalized the use of GM cotton seeds in Sudan [4]. Bt cotton variety named “Seeni 1” was commercially approved by the Sudanese Authority in 2012 [5]. Seini is a Chinese Bt-cotton genotype (*G. hirsutum*), it carries *Cry 1A* gene from *Bacillus thurengenesis* (Bt), this gene codes for a toxin against Lepidoptera larvae protecting the cotton crop against bollworms [5]. The area of genetically modified (Bt) cotton cultivation was increased by three folds between 2012 and 2013 (overall increases of 129–166%) indicating the positive experience of the farmer [2].

Genetically-modified cotton was introduced in 1996 [6], the global adoption rates for the cultivated GM cotton in 2013 reached 70%. In season 2000/2001, transgenic cotton represents 12% of the total transgenic crop-area in the world [7], particularly in developing countries [8, 9]. Martha *et al*. (2018) [10] reported that GM technology had reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%.

GM foods were legalized in some countries and restricted in others [11, 12], regulated by the EU. In Europe (2014). Some countries now have developed their own GM cotton hybrids (Bt), such as China and India [13]. India has adopted the transgenic cotton cultivation, having different Crystal genes (*cry genes*) isolated from soil bacterium *Bacillus thuringiensis* (Bt) [13]. In India, commercial planting was approved since the 2002/2003 season. By 2014, 96% of cotton in the US were genetically modified varieties [14].

As many benefits were gained by these modifications, the benefits and the risk of GM technology adoption and their effect on health remain controversial and a matter of debate**.**

Although the availability of genetic variation affects the outcome of breeding programs of farmers and farming companies [15], farmers select varieties with high seed oil content according to phenotypic information [16,17], Cottonseeds can be described using phenotypic characteristics, i.e. using characteristics resulting from the transgenic processes, these characteristics may be either morphological or physiological. Many structural compounds such as proteins, polysaccharides, and minerals were expected to vary between the organic and the GM seeds [1]. However, the information concerning the genetic basis of cottonseed contents (such as oil and protein) is still lacking [18]. The genetic association with seeds varieties must be considered when looking for value-added seed properties.

Cotton seeds may be longer and finer, or shorter and thicker according to the species and variety [3]. The similarity of phenotypic characters of cottonseeds varieties (natural & GM) makes it difficult to differentiate between them [19].

However, methods like physiochemical markers often can be easier than DNA molecular markers technology for the identification of the GM seeds from the practical point of view, because they are easier and cheaper [19]. The complexity of the genetic analysis made it necessary to look for an easier and applicable investigating methods to differentiate the GM from the natural cottonseeds [20].

As cotton is a main income source for farmers, they suffer from the high costs of fertilizers and pesticides, that is why they welcomed the growing of genetically modified cotton, which reduces the high expenses.
The information reported in this research work may help in optimizing management practices for best utilization of these types of cottonseeds as renewable natural resources. To that end, the main target of this study was to investigate the presence of morphological or physiological differences between natural cottonseeds used by Sudanese cotton breeders, and some genetically modified cottonseeds imported from some other countries. Although the study uses local seeds (from Sudan) with seeds imported mainly from China, the results of the study and its idea can be applied globally.

Materials and Methods

For the procurement of samples, the natural cottonseeds (non-transgenic) were acquired from the local market in Omdurman city in Sudan, while the GM cottonseeds (transgenic) were acquired from Shambat Research Centre, and confirmed by the Sudan Ministry of Agriculture, and experts of Shambat Research Center. About 500g of seeds of each type had been brought for testing. Besides the Chinese transgenic seeds (CN1), additional transgenic seeds from India and Brazil had been tested for morphological comparing purposes. The investigations had been carried in the Sudan Customs laboratory – Khartoum**.** Standard AOAC (2005) [21] methods were used for physiochemical analysis**.** Three different samples were analyzed in duplicate.
Seeds were processed in a gravitational table to become uniform in terms of density, seeds of different sizes were compared **.**The solvent extraction method was used with soxhlet reflux apparatus according to Akpan *et al*. (2006) [22].
For morphological investigations, apparent features had been noticed by the naked eye and were reported. Dimensions of both seed types were measured, photos of the two types had been documented and reported. Weight of 1000 Seeds (W1000S), (also called mass of thousand seeds, MTS), was determined following the methodology recommended by the ISTA (ISTA, 2013) [23].
The parameters studied in proximate analysis include percentage of moisture, crude protein, and ash content, were determined following standard methods of AOAC (2005) [21]. Total carbohydrate content was calculated by difference. For the crude protein content determination, ammonia nitrogen (NH_3-N) concentration in the incubation medium was determined after centrifugation using Kjeldahl method and expressed as mg/100 ml of incubation medium [24]. The fiber values were expressed exclusive of ash as per Goering and Van Soest (1970) [25]. For the determination of lipid content, the reflection done by hexane as by Okonkwo & Okafor (2016) [24]. Calcium and phosphorus in both types of cottonseeds were analyzed as per Talapatra *et al*. (1940) [26].

Results and Discussion

Results highlight the similarities, and the differences of some morphological features (seeds from China, India, and Brazil vs seeds from Sudan), and physiological characteristics of transgenic cottonseeds (seeds from China vs seeds from Sudan) (*Gossypiumhirsutum* L.), and non-transgenic cottonseeds (organic seeds) (seeds from Sudan).

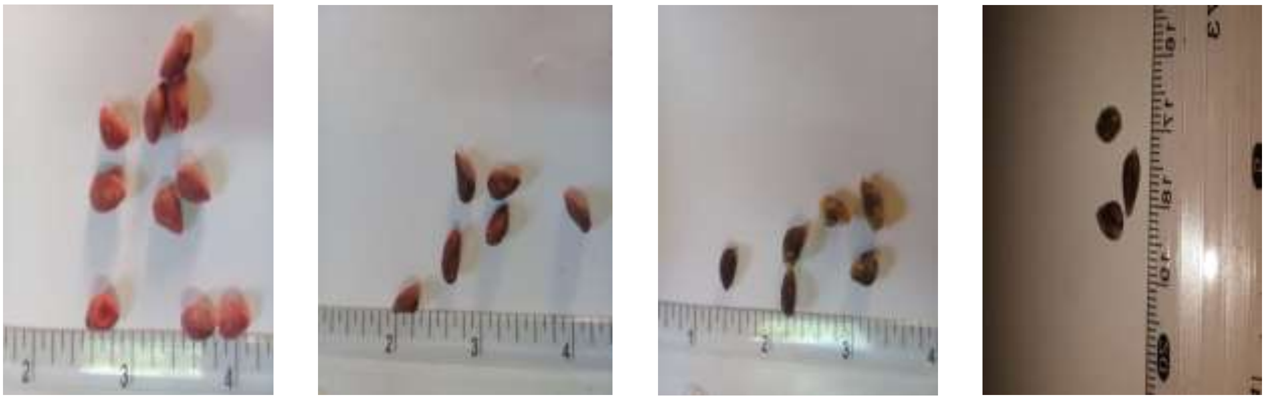
Morphological features

The sample bulk contained seeds with different lengths and thickness. The non- transgenic (Sudanese) seeds were densely covered with white or rusty cotton layer before treatment**.** Both the natural cottonseeds (Non-transgenic), and transgenic ones were apparently oblong, and ovoid in shape (egg-shaped), both types were very hard "stony" and non-swelling. The GM (transgenic) cottonseeds had approximately similar size and hardness as that of Non-transgenic seeds, they were, in general, about 1 Cm long, and half Cm wide {Table 1}. Our results were not far from the 9.37 mm length, and 5.5 mm width of some native species (Gossypium spp.) cottonseeds from Mexico, which was reported by Pérez-Mendoza *et al*. (2019) [27].

Table 1: Ranges of lengths and widths of some transgenic, and non-transgenic cottonseeds

Type	Length range	Width range
Inidian (transgenic)	0.8 – 1 cm	0.3 – 0.5 cm
Brazilian (transgenic)	0.7 – 1 cm	0.3 – 0.4 cm
Chinese (transgenic)	0.9 - 1 cm	0.4 – 0.5 cm
Sudanese (non-transgenic)	0.7 – 0.8 cm	0.4 cm – 4.5 cm

When the hairs removed from the non- transgenic (Sudanese) seeds, they had dark or dark brown natural color, but the transgenic seeds had different colors, seems as artificial coloring made to distinguish transgenic seeds from Non-transgenic ones.
Krakhmalev (1991) [28] (by roentgenograms) reported high microhardness of almost all constituent structural layers of cottonseeds coat. Cottonseed hardness is clearly evident, and maybe related to the chemical heterogeneity in the structural elements of the germs and seed peel [29]. Probably, the presence of inhibiting substances in cotton seeds give rise to the seeds become stony [30].
Some studies assumed that the increased concentration of various chemical elements in structural formations of the seeds results in, for example, an abnormal jump of microhardness in the zone of a so-called light-line [29]. The structural features of the stony cottonseeds (*Gossypiumhirsutum* L.) were studied deeply by Krakhmalev (1991) [28]. True dry matter degradability (TDMD) of Bt and non-Bt cottonseeds observations reported by Bertrand *et al*. (2005) [31] indicated the hardness and rigidity of both cottonseeds varieties.
The masses of thousand seeds (MTS) were similar for all tested varieties of the cottonseeds (range: 130-120g), it was lower than MTS for some beans types reported by Zdzisław Kaliniewicz (2019) [32], and slightly higher than MTS of sunflower seeds which reported by Marta Krajewska (2016) [33]. However, the conventional measurement of the variation in the characteristics of the seed has been difficult, usually with biases and systematic errors, and more time-consuming [34].
Figure 1. Shows a comparison between the shape and color of the natural non- transgenic cottonseeds (from Sudanese local markets), and the transgenic cottonseeds (from China). While Figure 2. Shows comparisons between two other transgenic cottonseeds types (from India and Brazil) used in Sudan.



A-1 A-2 B-1 B-2

Fig 1: Comparison between the shape and the color of the natural non- transgenic cottonseeds (from Sudanese local markets), and the transgenic cottonseeds (from China).
A-1: Transgenic cottonseeds from China (before washing with water)
A-2: Transgenic cottonseeds from China (after washing with water)
B-1 & B-2: non- transgenic cottonseeds (natural seeds from Sudan)

A -1 A -2 B-1

Figure 2. Comparison between two other transgenic cottonseeds types (from India and Brazil) used in Sudan.
A-1: Transgenic cottonseeds from India (red - colored)
A-2: Transgenic cottonseeds from India (black - colored)
B-1: Transgenic cottonseeds from Brazil

Physiochemical analysis

The results of the present study revealed that most of the physiochemical characteristics of the natural organic cottonseeds (Non-transgenic) were almost similar to those of the cottonseeds brought from Research Centers (GM seeds) (transgenic), with some variations in the moisture and ash contents. {Table 2.}

Table 2: Comparison of the proximate compositions and mineral content of Transgenic and Non-transgenic cottonseeds (*gossypiumhirsutum*)

Parameter	Natural Non-transgenic cottonseeds	Transgenic cottonseeds
Moisture	10.87 ± 0.10	7.90 ± 1.10
Ash	3.55 ± 0.06	5.19± 1.06
Crude protein	26.87 ± 0.03	27.0± 0.90
Lipid	25.88 ± 0.65	25.00 ±1.05
Total carbohydrate	27.00 ± 0.40	26.70 ± 1.00
Calcium	0.26	0.25
Phosphorus	0.40	0.42

Each data is mean of three replicates ± standard deviation (SD)

The similarity in physiochemical characteristics of Sudanese and Chinese cottonseeds (regardless of their transgenic situation) was confirmed by the similarity of their constituents reported in this study. However, the variations in the moisture and ash contents between (Non-transgenic) and GM (transgenic) seeds may be due to different circumstances such as storage periods and the maturity stages of each seed type, regardless of their transgenic situation. However, our results of moisture content are not far from Krakhmalev (1991) [28] results which was 8-10% moisture of mature natural cottonseeds. The storage temperature may affect seeds moisture range, seed size, volume, and 1000 seed weight [35], furthermore, resistance to fracture decreased with an increase in moisture content [36].

Our results of the similarity of the crude protein content of the natural cottonseeds and the transgenic cottonseeds; Calcium level; and Phosphorus level, confirmed the results reported by Mohanta (2011) [37] who report 26.7% and 27.0% of crude protein in the Bt cottonseeds and Non-Bt cottonseed, respectively; and were in accordance with Okonkwo and Okafor (2016) [24] who reported 27.27±0.03% of crude protein, while mean protein content of 38.0% were observed among some genotypes of cottonseeds [38].

Results of the total carbohydrate content of both transgenic, and non-transgenic seeds were lower than the 30.49%+0.4 carbohydrate content of the natural (non-transgenic) seeds reported by Okonkwo and Okafor (2016) [24].

Our results of the Lipid content were closer to the results of Okonkwo and Okafor (2016) [24] studies which reported 27.83%±0.35 natural cottonseeds oil content, and they were lower than results reported by Yan (2010) [39] of 31.33% oil content of natural cottonseeds. Jana Orphal (2005) [38] found average crude oil 31.0%, and total fat contents varied from 23.11 to 37.70%.

Our results of the similarity of physiochemical composition between transgenic and Non-transgenic whole cottonseeds were corresponding with results of Mohanta *et al*. (2011) [37] studies, and within the range described by Mujahid *et al*. (2000) [40] for some South Asian cottonseeds. Our results about comparing Bt and non-Bt cottonseeds were very closed to the findings obtained by Kumar and Singhal (2004) [41], and Bertrand *et al*. (2005) [31]. We found similar Calcium and Phosphorus content in both types of whole cottonseeds, the same results were obtained by Mohanta *et al*. (2011) [37] who found similar minerals' contents in both types of cottonseeds (Bt & non-Bt), and they found that both types of cottonseeds – either contains *Cry* protein or not -, were having similar degradability for dry matter and organic matter. However, the studies of Bertrand *et al*. (2005) [31], and Kumar and Singhal (2004) [41] had found only minor variations in the chemical composition between the transgenic cottonseeds and their near-isogenic counterparts, indicating a similar feeding values of both varieties. Our results may confirm the fact that the genetic modification does not affect the morphological features of cottonseeds.

The pronounced differences may also be detected to check varietal identity and provides a great advantage to seed users, since it could be done by a cheap and simple technique, and it is possible to have test results without planting a posteriori control seeds, in addition to the analysis of other phenotypic characteristics.

Moreover, if performed under standardized conditions, phenotype will reflect the genotype interactions. Nevertheless, to make it possible to distinguish varieties with accuracy, this technique requires several markers, shape, weight, and rigidity, that are sufficiently uniform for these characteristics. Today, this technique is used for some crops, such as maize.

Further studies are needed to evaluate the applicability, rapidity and reliability of methods that enable differentiating natural (non-transgenic) cottonseeds from the GM (transgenic) seeds, such as seed protein electrophoresis techniques, and techniques of cotton seed radiography for determination of the seed hardness. However, these pronounced differences between the transgenic and non-transgenic seeds of the same genetic background should be verified in further pair-wise studies. Developing new simple analytical techniques may help farmers to differentiate the natural cottonseeds from the GM seeds.

Conclusion

The studied Cottonseeds (*gossypiumhir sutum*) showed almost similar phenotypic features and physiochemical properties within the two types of seeds, the natural (Non-transgenic) and the genetically modified (transgenic) seeds, suggesting a difficulty in the differentiation approach. The apparent colors of the seeds, and some differences in Ash and moisture levels remain the characteristics that enables the differentiation between the two cottonseeds types. The knowledge of the phenotype of seeds may allow the identification of the cotton seeds condition either genetically modified or natural seeds. Our results may confirm the fact that the genetic modification does not significantly affect the morphological features of cottonseeds. These results may help farmers for the best utilization of these types of cottonseeds as renewable natural resources.

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References

1. He ZM, Shankle H, Zhang TR, Way H, Tewolde, Uchimiya M. Mineral composition of cottonseed is affected by fertilization management practices. Agron. J. 2013; 105:341-350. doi:10.2134/agronj2012.0351.
2. Naglaa A Abdallah. The story behind Bt cotton: Where does Sudan stand?. Journal GM Crops & Food Biotechnology in Agriculture and the Food Chain. 2014; 5(4):241-243. Published online: 18 Dec 2014 https://doi.org/10.1080/21645698.2014.997119.
3. Ikitoo EC. Gossypiumhirsutum L. In: Brink M & Belay G, PROTA (Eds.). (Plant Resources of Tropical Africa / Ressourcesvégétales de l'Afriquetropicale), Wageningen, Netherlands, 2011.
4. El Wakeel AS. Agricultural biotechnology and biosafety regulations in Sudan: The case of Bt cotton. Agric-biotech-conf. 2014. Available from http://www. nasaconline.org.
5. Latif AH, Babiker EA. An interview and current status of agricultural biotechnology in Sudan. Southern and Eastern Africa Cotton Forum (SEACF) meeting. 2012. Available from https://www.icac.org/wp-content/Sudan _Report.
6. Brien RD, Jones LA, King CC, Wakelyn PJ, Wan PJ. Cottonseed Oil: chapter 5. In: Bailey's Industrial Oil and Fat Products, Sixth Edition, John Wiley & Sons, Inc, 2005.
7. ICAC. Cotton: Review of the world situation. Vol. 55, No. 5. International Cotton Advisory Committee, Washington DC, USA, 2002a.
8. Smale M, Zambrano P, and Cartel M. Bales and balance: A review of the methods used to assess the economic impact of Bt cotton on farmers in developing economies. Ag. Bio. Forum. 2006; 9(3):195-212.
9. Klümper W, Qaim M. A Meta-Analysis of the Impacts of Genetically Modified Crops. Plos One. 2014; 9(11):e111629. Doi: 10.1371/journal.
10. Martha Rocha-Munive G, Ario Soberón, Saúl Castañeda, Esteban Niaves, Enrique Scheinvar, *et al*. Evaluation of the Impact of Genetically Modified Cotton After 20 Years of Cultivation in Mexico. Front. Bioeng. Biotechnol, 2018; Reviewed from https://doi. org/10. 3389/fbioe.2018.00082.
11. Bashshur, Ramona, FDA and Regulation of GMOs. American Bar Association. (Retrieved February 2016).
12. Sifferlin, Alexandra, Over Half of E.U. Countries Are Opting Out of GMOs. Time, 2015. Available From (https://ec.europa.eu, 2018).
13. James C. Global status of commercialized biotech/GM crops. ISAAA. 2013; Brief No. 46. Ithaca, NY: ISAAA.
14. USDA ERS-Adoption of Genetically Engineered Crops in the U.S, 2018. Available From www.ers.usda.gov.
15. Silva So, Souza Junior MT, Alves EJ, Silveira jrs, Lima MB. Banana breeding program at Embrapa. Crop Breeding and Applied Biotechnology. 2001; 1(4):399-436.
16. Azhar FM, Ahmad M. Inheritance pattern of cotton seed oil in diverse germplasm of G. hirsutum L. Pak. J.

Biol. Sci. 2000; 3:1250-1252. http://dx.doi.org/10.3923 /pjbs.
17. Pahlavni MH, Miri AA, Kazemi G. Response of oil and protein content to seed size in cotton. Int J Agric. Biol. 2008; 10:643-647.
18. Jiwen Y, Shuxun Y, Shuli F, Meizhen S, Honghong Z, Xingli L, *et al*. Mapping quantitative trait loci for cottonseed oil, protein and gossypol content in a Gossypiumhirsutum × Gossypiumbarbadense backcross inbred line population. Euphytica. 2012; 187:191-201. http://dx.doi.org/10.1007/s10681-012-0630-3.
19. Krishnan HB, Sleper DA. Identification of tall fescue cultivars by sodium dodecyl sulfate polyacrylamide gel electrophoresis of seed proteins. Crop Science. 1997; 37:215-219.
20. Gilliland TJ, Coll R, CAlsyn E, De Loose M, Van Eijk MJT, Roldan-Ruiz I. Estimating genetic conformity between related ryegrass (Lolium) varieties. Molecular Breeding. 2000; 6:569-580.
21. AOAC. Official Methods of Analysis, 18th edition. Association of Analytical Communities, AOAC International, Arlington, VA, USA, 2005.
22. Akpan UG, Jimoh A, Mohammed AD. Extraction, characterization and modification of castor seed oil. Leonard Journal of sciences. 2006; 8:43-52.
23. ISTA (International Seed Testing Association). International rules for seed testing. Seed Sci. & Technol. Bassersdorf, Switzerland, 2013, 243.
24. Okonkwo SI, Okafor EC. Determination of the Proximate Composition, Physicochemical Analysis and Characterization of Fatty Acid on the Seed and Oil of Gossypium Hirsutum. International Journal of Chemistry. 2016; 8(3):57. ISSN 1916-9698 E-ISSN 1916-9701. Published by Canadian Center of Science and Education.
25. Goering HK, Van Soest PJ. Forage Fiber Analysis. (Apparatus, reagents, procedures, and some applications), Agricultural Handbook 379. United States Department of Agriculture, Washington, DC, USA, 1970.
26. Talapatra SK, Roy SC, Sen KC. The analysis of mineral constituents in biological materials: Estimation of phosphorus, chlorine, calcium, manganese, sodium and potassium in foodstuffs. Indian Journal of Veterinary Science and Animal Husbandry. 1940; 10:243-258.
27. Pérez-Mendoza C, Tovar-Gómez MR, García de los Santos G, Suárez-Espinosa J. Physical Characterization of Cottonseeds in Native Species (Gossypium spp.) From Mexico. Journal of Agricultural Science, 2019, 11(10). ISSN 1916-9752 E-ISSN 1916-9760.
28. Krakhmalev VA. Cotton seed radiography. Acad. Sci. of Uzbekistan. Preprint N. 40. Institute of Electronics. Tashkent, 1991, 24.
29. Krakhmalev VA, Sultanova M. Microhardness of Cotton Seeds. Fan. Tashkent, 1983, 103.
30. Baskin C, Quarterman E. Germination requirements for the seeds of Astrgalus Tennesseans. Bull. Torrey Bot. Club. 1969; 96:3.
31. Bertrand JA, Sudduth TQ, Condon A, Jenkins TC, Calhoun MC. Nutrient content of whole cottonseed. Journal of Dairy Science. 2005; 88:1470-1477.
32. Zdzisław Kaliniewicz, Piotr Markowski, Andrzej Anders, Krzysztof Jadwisieńczak, 2019. Physical Properties of Seeds of Eleven Fir Species. *Forests*. DOI: 10.3390/f10020142. https://www.researchgate.net /publication/331009967.
33. Marta Krajewska, Beata Ślaska-Grzywna, Dariusz Andrejko. Physical Properties Of Seeds Of The Selected Oil Plants. Agricultural Engineering. 2016; 20(1):69-77. ISNN 2083-1587; e-ISNN 2449-5999. DOI: 10.1515/agriceng-2016-0007. www.wir.pti r.org.
34. Gyulai G, Rovner I, Vinogradov S, Kerti B, Emödi A, Csákvárf E, Gyulai F. Digital seed morphometry of dioecious wild and crop plants development and usefulness of the seed diversity index. Seed Sci. & Technol. 2015; 43:492-506. https://doi.org/10.15258/ sst.2015.43.3.15.
35. Aviara NA, Gwandzang MI, Haque MA. Physical Properties of Guna Seeds. Journal of Agricultural Engineering Research. 1999; 73(2):105-111.
36. Khan Nadiya Jan, Paramjit S, Panesar, Sukhcharn Singh. Effect of moisture content on the physical and mechanical properties of quinoa seeds. Int. Agrophys. 2019; 33:41-48. Doi: 10.31545/intagr/104374.
37. Mohanta RK, Singhal KK, Ebrahimi SH, Rajput YS, Mohini M. Comparative nutritional evaluation of transgenic cottonseeds containing Cry1C protein for ruminant feeding. Livest. Res. Rural Dev, 2011, 23.
38. Jana Orphal. Comparative Analysis of the Economics of Bt and Non-Bt Cotton Production. Publication of the Pesticide Policy Project, Hannover, Special Issue Publication Series, 2005; 8:3.
39. Yan ZW. Composition of fatty acid and physiochemical properties of cotton seed oil. CNKI Journal. 2010; 227(5):10-13.
40. Mujahid A, Abdullah M, Barque AR, Gilani AH. Nutritional value of cottonseeds and its derived products: I. Physical fractionations and proximate composition. Asian-Australasian Journal of Animal Science. 2000; 13:348-355.
41. Kumar RS, Singhal KK. Chemical composition and nutritional evaluation of transgenic cottonseed for ruminants. Indian Journal of Animal Science. 2004; 74:868-871.