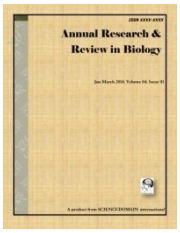

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Evaluation of Chemical Compositions of Tobacco (*Nicotiana tabacum* L) Genotypes Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MTM and NAT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MTM and NAT managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of this research was to determine the chemical and fatty acids composition of different genotypes of tobacco seeds.
Place and Duration of Study: Ten tobacco genotypes grown in Sulaimani-Iraq were investigated in this study. The tobacco seeds were provided by Agricultural station of Bazian, Ministry of Agriculture. The investigations were carried out on air-dried seeds during 2013 in University of Sulaimani, Faculty of Agricultural Sciences.
Methodology: Plants were sampled at different locations of Sulaimani to determine the chemical and fatty acid composition. The seed chemical and fatty acids compositions were determined by Soxhlet, Kjeldahl, Column chromatography, TLC and Gas chromatography methods.
Results: There were significant differences ($p< 0.05$) among genotypes for all studied chemical composition. Protein, ash, fiber and oil contents ranged from 20.861 to 23.872%, from 2.067 to 3.467%, from 13.66 to 19.33% and from 24.56 to 41.933% respectively. The results obtained from phospholipid, sterols and tocopherol showed significant difference among all genotypes. The content of phospholipids, sterols and tocopherols in the oils was 0.453-1.167%, 0.2-0.373% and 0.005-0.007% respectively. The results showed significant

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difference ($p < 0.05$) among genotypes for all saturated and unsaturated fatty acid. Palmitic saturated fatty acid (21.33 to 25.667%) and oleic unsaturated fatty acids (17.00 to 26.667%) were predominant in the oils.
Conclusion: The results indicated that all genotypes contained large amount of lipid, protein and fiber. Our data showed that the amount of unsaturated fatty acids is higher than the amount of saturated fatty acids in tobacco seeds. The knowledge of the present studies on different genotypes of tobacco seeds could be important to its appropriate industrial use and for improvement in the nutritional value.

Keywords: Tobacco seed; chemical compositions; fatty acid; genetic variation.

1. INTRODUCTION

Tobacco (*Nicotiana tabacum* L) is an economically important crop, widely cultivated all over the world, especially in China. Nicotiana belongs to family Solanaceae and has been divided into three subgenera (*Rustica, Tabacum and Petunioides*) containing more than 64 recognised species [1,2]. Only two species, *Nicotiana tabacum* L. and *Nicotiana rustica* L. have been widely cultivated. Tobacco breeding aims to develop varieties with wide adaptability, higher yield potential and suitable chemical constituents for cigarette industry. To explore the genetic potential and select suitable parents, it is necessary to study genetic diversity of tobacco germplasm resources.

The chemical characterization of *Nicotiana tabacum* seeds has been found important to look at alternative products of the crop i.e. oil and meal and find some uses of these products. Tobacco is known as an economic crop. There are many tobacco varieties and landraces and the number of new varieties in the world steadily increases. Tobacco seeds are rather different than grain or grain-legume seeds. They are very small (1g contains 10 000 to 18 000 seeds), with a high lipid content of 37-45%. The protein content of tobacco seed is 23-28 % and most of them are globulins [3]. In evaluation of four tobacco varieties by capillary electrophoresis of alcohol-soluble proteins was found that each variety possess a unique protein profile [3]. Tobacco seed as a secondary product of tobacco leaf production contains oil in a wide range of 36- 41% of the seed weight depending on a number of factors including the variety, growing conditions of tobacco and plantation area [4,5]. The major fatty acids in seed triacylglycerols are linoleic acid (C18:2; 60%-80%), oleic acid (C18:1; 10%-20%), and palmitic acid (C16:0; 10%-20%) [6,7,8].

The high content of linoleic acid in tobacco seed oil is very important for the oleo-chemicals production [9]. Linoleic acid can be used in formulation of protective coatings, plastics, as surfactant, dispersants, biolubricant, cosmetic and a variety of synthetic also it can be used in the preparations of other long chain compounds [10]. The knowledge of lipid composition of the seeds has taxonomic significance for plant classification and is useful for preserving seed purity for tobacco manufacturing industry. The aim of this study was to determine differences in chemical and fatty acids composition among ten genotypes of tobacco since this is the main quality trait currently important in breeding programs.

2. MATERIALS AND METHODS

2.1 Plant Material

The tobacco seeds were provided by Agricultural station of Bazian, Ministry of Agriculture. All genotypes were constructed by Dr. Mohammad Tofiq Mohammad. The investigations were carried out on air-dried seeds.

2.2 Oil Extraction

Oil was extracted by Soxhlet using n-hexane (Merck) for 2 hours. The temperature was brought to 69ºC. The solvent was distilled off at 80ºC. The oil content was calculated from the mass of oil and the mass of seeds [11].

2.3 Protein Content

The nitrogen content was determined by the Kjeldahl method and it was converted to protein content by using the conversion factor 6.25 [12].

2.4 Fatty Acid Composition

The fatty acid composition of triacylglycerols was identified by capillary gas chromatography of their methyl esters. The esterification was carried out by the Metcalfe and Wang technique [13]. Fatty acids composition of tobacco seeds was determined as their methyl esters prepared by boron-trifluoride methanol complex. A GCD PYE Unicam gas chromatograph equipped with flame ionization detector was used to determine the fatty acids methyl esters. Nitrogen carriers gas was used at a flow rate of 30 mL min^{-1}. Fatty acids were separated on a 1.8 mx 2 mm i.d. glass column packed with 6% BDS (Butanediol succinate polyesters) on solid support Anakorm ABS (100/120) mesh. Analysis was carried out at isothermal column temperature 190ºC, injector and detector temperature for all analysis were 230ºC. The peak was identified by comparison with standard fatty acids methyl esters.

2.5 Phospholipid Content

Lipids were extracted from the seeds by Folch procedure [14]. Polar lipids were divided from unpolar lipids by column chromatography [15]. Polar lipid was dividing from unipolar ones by column chromatography. The phospholipid constituent were separated by two-directional thin layer chromatograph on silica gel 60 G (Merck), impregnated with 1g per 100g $(NH_4)_2SO_4$ water solution. The first direction was carried out in chloroform (Merck): methanol (Merck): ammonia (Merck) 65:25:5 v/v/v and second in chloroform (Merck): methanol (Merck): ammonia (Merck): acetic acid: water 50:20:10:5 v/v/v/v. The spots of the separated individual phospholipids were identified by spraying with specific reagents. In addition, R_f and standard spots were used for definitive identification. The quantitative evaluation was carried out spectrophometrically at 700 nm.

2.6 Sterol Content

The free and esterified sterols were separated from the other oil constituents by preparative TLC on Silica gel 60 G “Merck” and mobile phase n-hexane: diethyl ether 1:1 v/v. The

esterified sterols were saponified with ethanolic KOH, extracted and purified by TLC [7]. The quantitative evaluation and individual composition were determined by gas chromatography, using HP 5890 A unit with FID, 25 m capillary column impregnated with OV-17 under the following conditions: column temperature 260-300ºC with a change 6ºC/min, detector temperature 320ºC, injector temperature 300ºC, gas-carrier-nitrogen. Identification was confirmed by retention time comparison of the individual constituents with those of authentic samples. Betuline was used as internal standard for quantitative evaluation of total sterols.

2.7 Tocopherol Content

In order to determine the tocopherol content, samples of tobacco oil were saponified using 60% KOH. After saponification the unsaponifiable substances were extracted three times using peroxide free diethyl ether. Then the ether was distilled off and the residue was dissolved in n-hexane. The quantification were carried out by HPLC [16]. “Merck-Hitachi” unit fitted with column Nucleosil Si 50-5250 x 4 mm and fluorescent detector “Merck-Hitachi” F 1000 was used. The operating conditions were as follows: excitation 295 nm, emission 330 nm, mobile phase n-hexane: dioxan 94:4 v/v, rate of mobile phase 1 cm^3/min. the peaks were identified using authentic individual tocopherols.

2.8 Statistical Analyses

All chemical and physical determinations were conducted in triplicate. Statistical differences among cultivars were estimated from ANOVA test at the 5 % level (P=0.05) of significance for all parameters evaluated. Whenever ANOVA indicated significant difference, a pairwise comparison of mean by Least Significant Difference test (LSD) was carried out using SPSS version 21.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Seeds

Statistically significant differences were found among genotypes for protein contents according to test LSD. Protein content ranged from 20.861 to 23.872% in all genotypes. The highest value of protein content was recorded by genotype H12 (23.872%) while the lowest value was found in HBALA (20.861%) Fig. 1. Abbas et al. [9] measured the protein content in three varieties of tobacco. The authors showed that the protein content varied from 19-21%. Fig. 2 showed significant difference among genotypes for ash content. The ash content varied from 2.067 to 3.467%. The maximum ash content was registered by genotype H3 (3.467%) while the minimum ash content was showed by genotype H5 (2.067%). Abbas et al. [9] obtained the same ash content in Jati and Virigina tobacco varieties. Ash content can be regarded as a general measure of quality and often is a useful criterion in identifying the authenticity of a food. High ash content suggests the presence of an inorganic adulterant. The result of fiber content were represented in Fig. 3 confirmed that the differences among genotypes were highly significant. Maximum fiber content recorded by HBALA genotype (19.33%). H14 tobacco genotype gave minimum fiber content (13.66%). Data of oil content Fig. 4 showed significant differences among genotypes. The highest values of oil content produced by H5 genotype was 41.933%, whereas the lowest oil content values exhibited by H14 genotype (24.56%). Zlatanov et al. [7] estimated the oil content in seeds tobacco species. They found that the oil content varied from 38 to 49%. The oil content in our genotypes is higher than the value of 29.82% that reported by Abbas et al. [9]. Fig. 5 shows

the percentage of phospholipid in different seed genotypes. However, the content of phospholipid from different seeds genotype was found to be significantly different. Significant greater amount of phospholipid was recorded in the genotype (1.167%) H15 than those contained in other genotypes. Abbas et al. [9] measured the phospholipid content in three varieties of tobacco. These authors obtained 5-6% of phospholipid in different genotype seeds. The sterols content varied from 0.2 to 0.373%. High significant difference was observed among tobacco genotypes. The highest sterols content was observed in genotype H15 (0.373%), while the lowest sterols content was found in genotype H14 (0.2%) Fig. 6 Zlatanov et al. [7] found high content of sterols in different species of tobacco which ranged from 0.2 to 0.8%. High significant difference was found among tobacco genotypes for tocopherol content Fig. 7. The highest tocopherol content was recorded by genotype H5 (0.0067%), while the lowest tocopherol content was found in genotype H14 (0.0046%). Zlatanov et al. [7] found high content of tocopherol in different species of tobacco which ranged from 0.0002 to 0.02%.

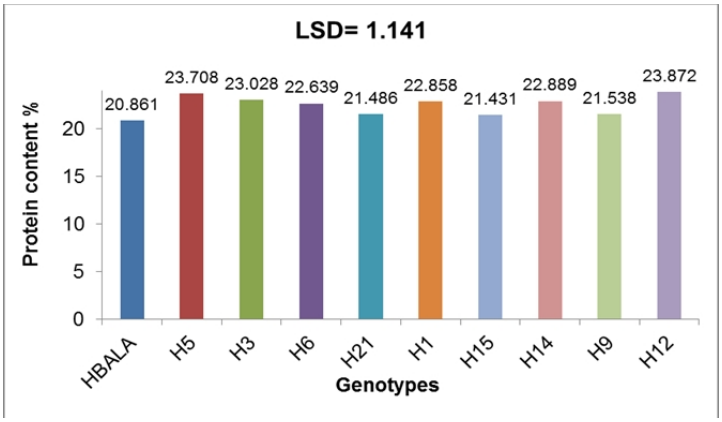

Fig. 1. Seed protein content (% dry weight) in different genotypes of tobacco

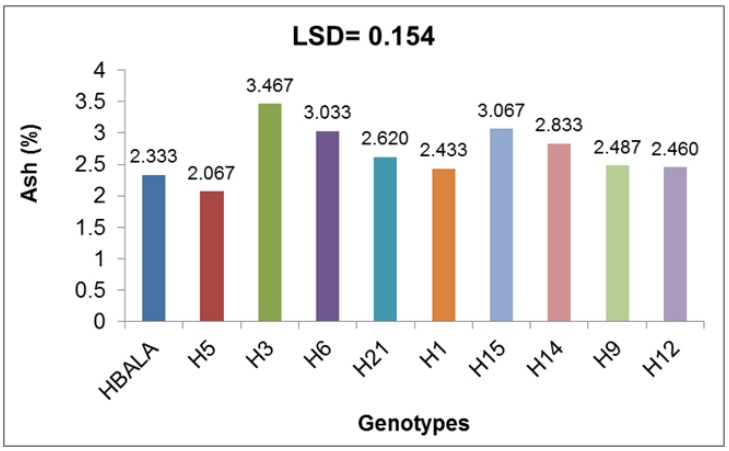

Fig. 2. Seed ash content (% dry weight) in different genotypes of tobacco

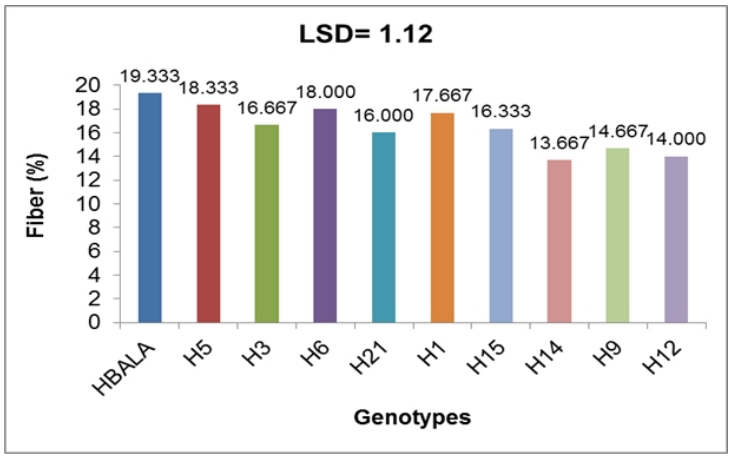

Fig. 3. Seed fiber content (% dry weight) in different genotypes of tobacco

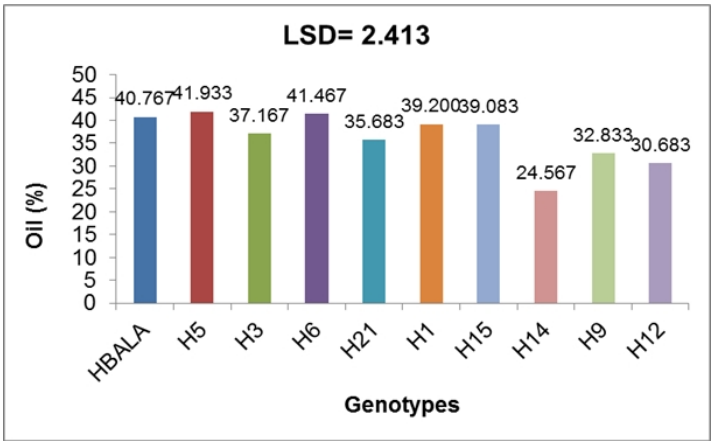

Fig. 4. Seed oil content (% dry weight) in different genotypes of tobacco

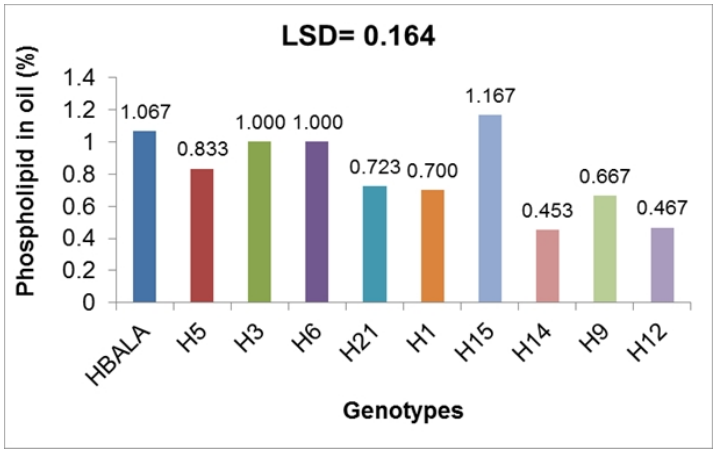

Fig. 5. Seed phospholipid content (% dry weight) in different genotypes of tobacco

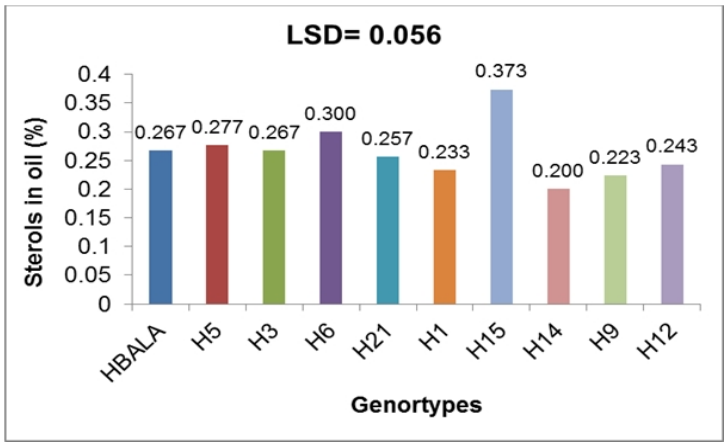

Fig. 6. Seed Sterols content (% dry weight) in different genotypes of tobacco

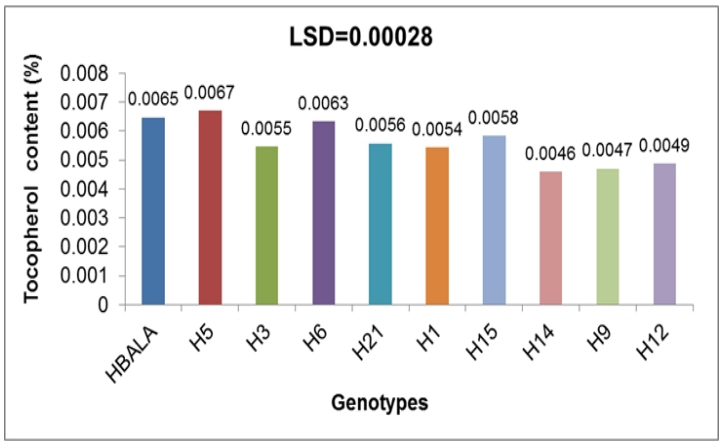

Fig. 7. Seed tocopherols content (% dry weight) in different genotypes of tobacco

3.2 Fatty Acid Composition

Table 1 shows the saturated fatty acid composition of the glyceride oil. Statistically significant differences were found among genotypes for saturated fatty acid composition. Palmitic fatty acid was found to be the predominant component in the oil. The relative percentage of major fatty acids ranged from 0.567 to 1.233% for lauric acid (C12:0), 1.267 to 1.7% for miristic (C14:0), 21.33 to 25.667% for palmitic acid (C16:0) and 4 to 8 for stearic acid (C18:0). Genotype H 14 presented the lowest percentage for all studied saturated fatty acid. High concentration of all studied saturated fatty acid was observed by genotype H15. This result was agreed with the result of Abbas et al. [9]. This result dose completely agree with some reported works [17,18]. The difference in our results could be explained by variation in soil and climatic conditions that could alter fatty acid content in oil.

Statistically significant differences were found among genotypes for unsaturated fatty acid composition Table 2. Table 2 shows that tobacco seeds oil contains unsaturated fatty acid ranging from C16 to C18. The predominant fatty acid was oleic acid (C18:01). Significant difference was detected in all unsaturated fatty acid. The content of plamiticoleic (C16:01),

oleic (C18:01), linoleic (18:02) and linolenoic acid (18:03) was ranged from 10.333 to 14.667%, 17.00 to 26.667%, 4.2 to 9.233% and 0.3 to 0.583% respectively. The minimum concentration of plamitoleic, oleic, linoleic and linolenoic acid was recorded by genotype H14, while the maximum concentration of plamitoleic, oleic, linoleic and linolenoic acid was registered by genotypes H6 and H5. The content of linoleic acid is lower than that reported (45-47%) by Gofur et al. [4] and Koivai et al. [19]. The high amount of unsaturated fatty acid in seed could increase to autoxidation and polymerization, resulting in cross-linked and tough films upon exposure to air. Although tobacco seed oil is non-edible oil, it can be utilized for drug and Cosmetics industry. Cosmetics are care substances used to enhance the appearance or odor of the human body. The addition of tobacco seed oil methyl esters to the diesel fuel reduce CO and SO_2 emission [20]. On the other hand, high concentration of saturated fatty acid especially palmitic acid lead to preparing of the fatty acid methyl esters with comparatively high freezing point and high viscosity.

Table 1. Saturated fatty acid (%) composition of oil extracted from different genotypes of tobacco

Genotypes	Lauric acid (C12:0)	Miristic acid (C14:00)	Palmitic acid (C16:00)	Stearic acid (C18:00)
H1	0.853	1.450	24.333	5.900
H12	0.710	1.433	23.333	5.000
H14	0.567	1.167	20.000	4.000
H15	0.800	1.633	25.667	7.000
H21	0.633	1.267	21.333	5.167
H3	0.700	1.233	22.000	5.000
H5	1.033	1.700	23.000	8.000
H6	1.233	1.550	24.667	7.000
H9	0.677	1.300	22.000	5.333
HBALA	0.867	1.600	23.000	6.000
LSD	0.077	0.095	1.03	0.546

Table 2. Unsaturated fatty acid (%) composition of oil extracted from different genotypes of tobacco

Genotypes	Plamiticoleic acid (C16:01)	Oleic acid (C18:01)	Linoleic acid (C18:02)	Linolenoic acid (C18:03)
H1	12.333	24.000	6.267	0.400
H12	12.333	21.000	7.333	0.467
H14	10.333	17.667	4.200	0.300
H15	14.000	22.000	7.267	0.550
H21	11.667	22.000	7.233	0.500
H3	11.000	24.000	6.000	0.367
H5	14.000	26.667	9.000	0.533
H6	14.667	26.000	9.233	0.583
H9	11.667	20.333	6.333	0.500
HBALA	11.667	25.000	7.000	0.367
LSD	0.982	0.538	0.667	0.11

4. CONCLUSION

The phytochemical characteristics can be helpful to identify the quality of oil and oil products for possible industrial or commercial uses. It has been observed that all genotypes contain high percentage of unsaturated fatty acids as compared to saturated fatty acids ranging from C16 to C18, which is the charcheristics of vegetable oils. From the quality of view, tobacco seed oil classified as oleic and linoleic acid, can be used in paint industies and cosmetic as potiential raw materials. The findings also imply that tobacco seeds may, therefore, be used as a potentially attractive source of lipid, protein and fiber. Protein also can be utilized as a nutritive complement. Further study is needed to understand how stage of maturity, growing region and harvesting conditions influence characteristics of oil and nutritive values of the seeds of tobacco.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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