# ANALYSIS AND EVALUATION OF THE EPICUTICULAR WAXE OF TWO BREED WHEAT CULTIVARS

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**ABSTRACT**

**The evaluation of the epicuticular waxes and the molecular arrangement within the crystals were studied on biological and non-biological surfaces from wax platelets of leaves blades of two wheat (*Triticum aestivum* L.) cultivars: Inia- 66 and Uruq where collected from these two cultivars and which extracted from the oil obtained from soxhlet device extraction. The results revealed that the concentration of wax extracted from cv. Uruq leaves have a higher concentration of wax than Inia-66 cultivar, which means that Uruq cultivar has the superiority in protection and water lose by transpiration. The results also revealed that the wheat leaves of cv. Inia-66 wax contain 8 compounds and the cv. Uruq wax was different in quality and quantity content (mixture 11 compounds) and the absence of the alkane and ester compounds group in cv.Inia-66. These differences between two cultivars may appear in biological and physical activity mechanisms in these cultivars.**

**Key words: Epicuticular Waxes, Bread Wheat, Extraction**



## تحليل وتقييم المادة الشمعية في أوراق صنفين من حنطة الخبز

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**الـمـستخلص**

**تم تحليل المادة الشمعية الموجودة على أوراق للصنفين إينيا–66 وأوروك من حنطة الخبز(*Triticum aestivum* L.) وترتيب الجزيئات في البلورات على المستوى الأحيائي وغير الأحيائي فيها وقد أوضحت النتائج بأن تركيز المادة الشمعية المستخلصة من أوراق الصنف أوروك أعلى من من تلك الموجودة على أوراق الصنف إينيا–66 مما يدل ذلك على أن الصنف أوروك يتفوق على الصنف إينيا–66 في حمايته للأوراق من البيئة والتقليل من كمية فقدانها للمياه عن طريق النتح، وبينت الدراسة أن المادة الشمعية في الصنف إينيا–66 يحتوي على 8 مركبات بينما يحتوي الصنف أوروك على 11 مركباً، كما وتفتقد المادة الشمعية في الصنف إينيا–66 مركبات الألكينات والايسترات حيث ينعكس هذا الإختلاف على الفعاليات الفيزيائية والاحيائية فيهما.**

**كلمات مفتاحية: الطبقة الشمعية، حنطة الخبز، استخلاص.**

## INTRODUCTION

Wax is a ubiquitous material, which exist on the surface of plant material and on animal skins (1), it is a fat-like material which contains numerous substances and usually has high melting points (2). The development of architecturally complex cell walls for biomechanical support and structural protection, which typify modern land plants, can be traced back to divergence and radiation within the Charophycean green algae, their immediate ancestors (3). Armed with a protective skin, together with a range of adaptive strategies for acquiring and conserving water, as well as for avoiding or tolerating water stress, embryophytes now thrive in a wide range of desiccating environments (4). The plant cuticle is an extracellular hydrophobic layer that covers the aerial epidermis of all land plants, providing protection against desiccation and external environmental stresses such as water and moisture, even against microorganisms. Wax composition can vary substantially with species, ontogeny and environmental growth conditions (5). In most cases, the majority of compounds comprising the cuticular wax are derived from very long chain fatty acids including alkanes, aldehydes, primary and secondary alcohols, ketones and esters (6). Epicuticular wax is referred to as glucousness has been associated with drought tolerance in several crop species. It is reported that glucousness decreased transportation and increased yield and water use efficiency (7). Variation in Epicuticular wax in wheat has reported, but only in limited range of genotypes. This paper investigates the Variability in Epicuticular wax content and its relationship among two bread wheat (*Triticum aestivum* L.) cultivars: Inia- 66 and Uruq.

## MATERIAL AND METHODS

***Plant Material:*** Two bread wheat Leaf blades were collected from Inia-66 and Uruq cultivars. They were dried at 60 °C for 24 hrs and 80 °C for 24 hrs. The Wheat Leaf was filtered through a 20-mesh sieve, and packed in polyethylene bags and stored at a dry place for further use and stored at ‑20 °C. All chemicals used in the study were procured from Merck and BDH Ltd companies.

***Wax Extraction:*** Wax was extracted from Wheat Leaf blades as reported by Phukan and Boruah (8).

### *Analytical Methodologies*

***Spectrophotometric Analysis:*** A known quantity of wax (0.1% w/v) was dissolved in hexane for spectroscopic measurement. The solvent was scanned across wavelength of 200 to 700 nm by using Helios αspectrophotometer (Thermo Electron Corporation, USA). The thermal characteristics of the wax sample were measured according to the modified method of Athukorala *et al.* (9), with a differential scanning calorimeter (DSC- 60; Shimadzu, Japan).

***Gas Chromatography:*** 50 µl of the crude wax (1mg/ml CH2Cl2) was evaporated at 40ºC to dryness at 40.The GC-FID detector analyses were carried out as mentioned by Athukoral *et al*, (9) with a GC (Shimadzu Instruments, Japan) gas chromatograph equipped with an FID detector (Helium carrier gas).

***Mass Spectroscopy Analysis. GC-GCMS:*** The crude wax (4mg) was methylated as mentioned by Athukorala *et al.* (9). Crude wax was trimethyl-silylated with 200 µl of bis (trimethylsilyl) trifluoroacetamide and 100 µL toluene at 75◦C for 30 min. The reaction mixture was cooled and concentrated under nitrogen flush and redissolved in hexane. The analysis was conducted with GC-MS QP 2010 Ultra (Shimadzu, Japan) as mentioned by Deswarte *et al.* (10).

## *RESULT AND DISCUSSION*

***Wheat Wax Yield:*** The quantity of wax was found to be 4.95% (w/w) of dried wheat leaves blades of cv. Uruq. This percent was higher than cv. Inia-66 leaves (0.25 to 0.50%). This results agreed with the finding of Phukan and Boruah (8), that huge quantity of wheat wax extract gets filtered through Uruq leaf blades having higher concentration of wax than Inia-66, whereas Wheat Leaves blades are produced by shredding surface of wheat before crushing, thus affecting the total yield. The major component of wax in edible oil is wax esters, which consist of long chain alkyl esters and steryl esters. They are classified as a polar lipid species, whose polarities are lower than

triglycerides. Their low polarities and longer chain lengths contribute in crystallization. Not all wax esters are crystallizable, and some wax esters still remain in the sample.

***UV-Vis.* Analysis:**

The *UV-Vis*. of fatty acids, conjugated dienes, and hydroperoxides formed as a result of lipid oxidation absorb UV light at about 232nm and conjugated trienes at about 270 nm (10). In the UV range (100–400 nm) wheat leaves blades wax showed the peaks near 230 and 270nm indicating the presence of conjugated dienes and trienes (Fig.1).

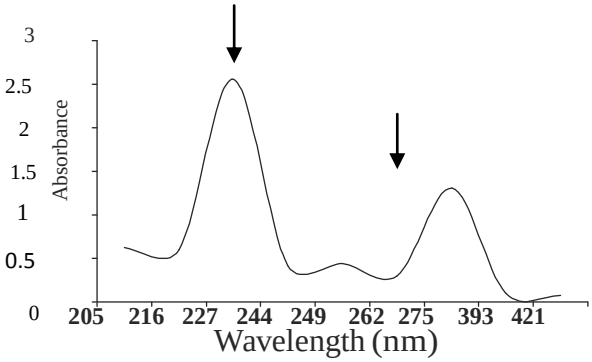


**Fig. 1: UV-Visible spectra of crude wax.**

***GC and GC-MS analysis results:***

Fig. (2) and Table (1): show the GC analysis results of wheat leaves of cv. Inia-66 wax contain a mixture of test components ranging (8 compounds) through different retention times in 1:5 ml/ml dilution percent.

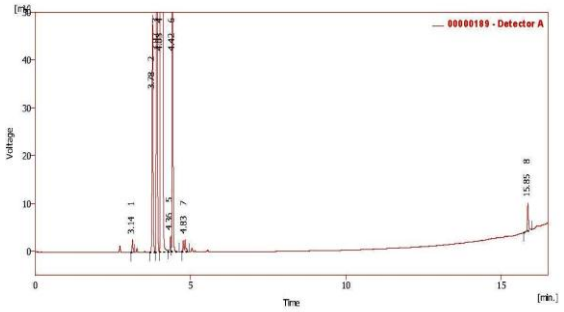


**Fig. 2: The elution of wheat leaves blades cv. Inia 66, a mixture of test components in retention times-RT ranging from 3.137 to 15.853**

**Table 1 . GC Analysis results of wheat leaves blade cv. Inia-66**

| wheat leaves blades (Inia- 66) | | |
|---|---|---|
| Area | RT. | No. |
| 2.670 | 3.137 | 1 |
| 49.633 | 3.780 | 2 |
| 142.127 | 3.920 | 3 |
| 990.665 | 4.033 | 4 |
| 3.292 | 4.360 | 5 |
| 68.393 | 4.423 | 6 |
| 2.574 | 4.827 | 7 |
| 5.900 | 15.853 | 8 |
| 4918.865 | | TOTAL |

Fig. (3) and Table (2): show the GC. Analysis results of wheat leaves cv. Uruq wax contain a mixture of test components ranging (11 compounds) through different retention times in 1:40 ml/ml dilution percent.

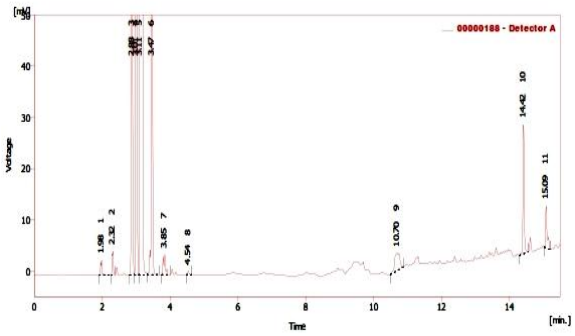


**Fig. (3): The elution of wheat leaves blade cv. Uruq, a mixture of test components in retention times ranging from 1.983 to 15.087**

**Table 2. GC analysis Results of wheat leaves blade cv. Uruq.**

| wheat leaves blades (Uruq) | | |
|---|---|---|
| No | Reten. Time | Area |
| 1 | 1.983 | 7.886 |
| 2 | 2.317 | 11.712 |
| 3 | 2.880 | 193.806 |
| 4 | 3.007 | 518.903 |
| 5 | 3.113 | 4583.976 |
| 6 | 3.467 | 247.018 |
| 7 | 3.847 | 17.750 |
| 8 | 4.540 | 1.725 |
| 9 | 10.700 | 35.384 |
| 10 | 14.420 | 64.273 |
| 11 | 15.087 | 26.021 |
| TOTAL | | 1433.722 |

Fig. (1 and 2): show as originally performed on the 10 m x 0.32 mm column. Resulting in analysis times that are almost half of the original analysis. The shorter analysis results in a higher FID detector signals for each eluting component due to decreased band broadening effects in the capillary column. Thus it provides an improved signal-to-noise ratio and better sensitivity. The maximum temperature of the VF-1ms column was normally set at 350 °C. However, the highly temperature stable VF-1ms liquid phase can be routinely pushed to 380 °C without any loss of column performance. A slightly higher column bleed level at 380 °C can be observed under these conditions. In addition, show the comparative chromatograms of a wheat leaf blades wax sample. The analysis on the 10 m x 0.15 mm VF-1ms GC column (Fig.1and2) provided a high-resolution separation in a significantly shorter analysis time and with improved sensitivity. Identified components

included normal alkanes with saturated free fatty acids. This initial composition may change with aging and depend on the environment, and other conditions. The ($C_3$) to Phthalic acid, cyclobutyl tetradecyl ($C_{26}$) contributing 4.51% of total extract, mass spectra of some of the major fatty acids are shown in table (3). 2,3,3-Trimethyl-4-nonene, the major fatty acid in the Uruq leaves sample has antimicrobial effect, indicating that plants must be producing it as defense mechanism against microbial attack. Apart from these major components aldehyde and alcohol, pentadecanol and isotridecyl alcohol were found to present its potential nutritional significance of the two samples. They were recently recognized after policosanol, which is a mixture of long chain saturated alcohols. Wax esters containing alkyl esters and steryl esters generate fatty acids, fatty alcohols, and sterols after hydrolysis or saponification. Phytosterols are known to improve blood lipid profile. Fatty alcohols like policosanol inhibit cholesterol synthesis in the liver. When absorbed into the body, wax esters are decomposed into those substances, which means that wax esters in wax or oil may have medicinal benefits. However, the exact mechanisms have not been fully studied and there have been only a few studies on the separation or purification processes for those substances from wax, which could be utilized in biological tests.

## CONCLUSION

This study revealed that the amount of crude wax from dried wheat leaves blades of cv. Uruq is higher than cv. Inia-66 and contain of many classes of compounds like alkane, ester, and different in concentration of other classes (alcohol, fatty acids) that refers to the different in major component of wax, and leads to differences in many beneficial effects that can be utilized for medicinal purpose. For example alkans have its application as insecticide. Fatty acids playing significant role in human nutrition were found to be present in the wax. Apart from medicinal, it can be used as food preservativers by coating edible fruits and vegetables.

**Table 3. comparison results of GCMS analysis of the leave blades of the two cultivars**

| Class of compos. | Uruq | Retention time | Area | Inia- 66 | Area |
|---|---|---|---|---|---|
| | Name of compounds | | | Name of compounds | |
| Alkane | 2-Isopropyloxetane | 26.95 | 24245 | — | |
| | 2,2-Dimethylbutane | | | | |
| | 1-Iododecane | | | | |
| | 1-Iodotetradecane | | | | |
| | 3-Hexanone | | | | |
| Fatty acid | Dimethylacetic acid | 25.925 | 15772 | — | |
| | 5-Methylhexanenitrile | | | | |
| | 2,3,3-Trimethyl-4-nonene. | | | | |
| | Cyclopentanol, nitrate | | | | |
| | Borane, ethylisopropylmethyl | | | | |
| | Phthalic acid, 4-cyanophenyl nonyl ester. | | | | |
| | Phthalic acid, 4-bromophenyl octyl ester | | | | |
| | Phthalic acid, cyclobutyl tetradecyl | | | | |
| Ester | Nitrous acid, isobutyl ester. | 20.433 | 23054 | Nitrous acid, butyl ester. | 17798 |
| | 3-methylbutyl ester | | | Isopentyl alcohol, nitrite. | |
| | Nitrous acid, n-butyl ester | | | Di-n propoxymethane | |
| | Butanoic acid, 2-hydroxy-2-methyl-, methyl ester. | | | | |
| Aldehyde | 6-Ethyl-2-methyldecane | 28.617 | 109879 | 2-Isopropyloxetane | 22045 |
| | 1-Iodoundecane | | | 2,2,5-Trimethyl-3,4-hexanedione | |
| | 1-Iodo-2-methylundecane | | | 2,2-Dimethylbutane | |
| | 3,7-Dimethylnonane | | | 2-propenyl ester. | |
| | Sulfurous acid, 2-ethylhexyl isohexyl ester | | | | |
| Alcohol | 5-Methylhexanenitrile | 22.225 | 28944 | Borane, ethylisopropylmethyl. | 19734 |
| | 2,3,3-Trimethyl-4-nonene | | | 5-Methylhexanenitrile | |
| | Cyclopenta-nol, nitrate | | | 2-Iodododecanoic acid | |
| | 1-Allyl-cyclohexane-1,2-diol | | | Oxalic acid, cyclobutyl hexyl ester | |
| | Borane, ethylisopropylmethyl | | | (4E)-2,3,3-Trimethyl-4-nonene | |

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