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Genetical and bio-chemical analysis of glaucousness in wheat (*Triticum aestivum* L.)

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Abstract

The present investigation was carried out to study the inheritance of waxiness (glaucousness) in selected wheat genotype. Glaucousness is the visual expression of waxiness on stem and leaves. Sufficient diversity exists for this trait. Waxiness in Pusa T3336 was determined through genetic analysis as well as Gas Liquid Chromatography (GLC). The F_1 hybrids viz., Pusa T3336 x DL629 (non-waxy) and Pusa T3336 x DL605 (non-waxy) appeared non-waxy or non-glaucous indicating dominance of non-waxiness over waxiness. The visual expression data of individual plants in F_2 population derived from both the crosses segregated in 1 waxy : 3 non-waxy ratio. The genetic analysis carried out on field data revealed that waxiness in Pusa T3336 is recessive and controlled monogenically. The GLC was carried out for Pusa T3336 x DL629 only to differentiate waxy and non-waxy plants. The F_1 showed the presence of only C-homologue C_{32}, which was not scorable visually. The F_2 individuals showed quantitative differences in C-compounds which corresponds to the visual scores of individuals in F_2 population under field conditions. The trend indicated similar mode of distribution of wax and the individuals followed 1 waxy: 3 non-waxy segregation patterns. The GLC data also indicated that the production of wax on stem and leaves of Pusa T3336 is controlled by a single recessive gene. The GLC analysis revealed the presence of eight carbon compounds (C-homologues) in waxy parent Pusa T3336 and the same were absent in non-waxy parent DL629. However F_1 hybrid (Pusa T3336 x DL629) showed the presence of C-homologue C_{32}.

Key words: Wheat, glaucousness, non-glaucousness, inheritance, carbon compound, gas liquid chromatography

Introduction

Genetic markers are valuable tools for basic studies and manipulating the plants through selection of desirable traits for improvement with respect to yield and other traits. Literature reveals that only a few morphological, cytological and biochemical markers linked to the traits of interest are available in wheat. Many of the wheat varieties have wax (glaucousness) formation on the surface of leaf and stem. The presence of wax on cuticle of leaf and stem in many crops is an important trait for fundamental studies. The trait, glaucousness is the visual expression of waxiness on stems and leaves. The expression of glaucouness depends on the arrangement of wax deposits rather than the amount of wax [1]. The inert layers of epicuticular wax interposed between plants and their environment are involved in all processes, both physical and physiological, occurring within the primary surface tissues. It has been reported that impervious epicuticular components effectively reduce water loss due to transpiration, contribute to the control of gaseous exchange, impose and influence retention and redistribution of foliar-applied chemicals. The deposition of epicuticular wax on plant surfaces appears to be a drought resistance characteristic in many species including wheat [1, 2]. Also, the glaucous lines outyielded non-glaucous line in irrigated and rainfed conditions [1]. The waxy components effectively reduce water loss due to transpiration and show possible genetic relationship between waxy/waxless genes in wheat and barley altering ear glaucousness to give increased water repellency and reduction of in ear sprouting of wheat [3]. This external layer also provides a micro-habitat for a variety of parasitic and saprophytic organisms and acts as a barrier to fungal pathogens [4], e,g., waxing in Pusa T3336 of *Triticum aestivum* has been reported to have association with leaf blight resistance [5].

Genetic studies indicate that non-glaucousness can be either recessive or dominant. Recessive forms of non-glaucousness are apparently mutants of the genes that produce the wax-like deposits. Dominant non-glaucous phenotypes (as assessed visually) appear

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to be due to mutations that affect the molecular structure and reflectance of the wax like substances [6]. The granuale bound starch synthesis (waxy) proteins responsible for amylase synthesis are encoded by three genes located on chromosome arms 7AS (Wx-A1), 4AL (Wx-B1) and 7DS (Wx-D1) of bread wheat [7]. This wax seldom occurs as an even film over the cuticle but usually develops as discrete structures whose form is characteristic of the species and even variety and has been used as taxonomic character [8].

The gene involved in wax production and the "inhibitors" are duplicated in chromosomes 2B and 2D. Earlier work indicated that the wax producing genes and the inhibitors were allelic [6, 9], therefore recessive allele and dominant allele may result in non-glaucousness. Orthologous loci do occur in barley (*gs*1*, gs*6*, gs*8), rye (*wa*1) and maize (*g*2) [10]. A gene for spike glaucousness, *Ws* was mapped distally on the short arm of chromosome 1B in *T. durum* cv. Langdon/*T. dicoccoides* acc. Hermon H52. The wax condition in wheat has been found to be monogenically controlled but when Thatcher (waxy) was crossed with Kalyansona (non-waxy), F_1 produced waxy plants and F_2 segregated in 9 waxy: 7 non-waxy plants [11]. Non-glaucousness is controlled by single dominant gene in *T. durum* [12]. Non-glaucousness gene in line 3672 of *T. aestivum* was controlled by a single dominant gene, *IW* [13] and mapped on distal region of chromosome 2DS. The confirmation of the inhibitor of waxy gene was also done that $W2^I$ is located on distal end of chromosome 2DS in *T. tauschii* [14]. Epistatic inhibitor gene $W1^I$ of glaucousness was located on chromosome 2BS in variety Cornell Selection [15], while $W3^I$ was on 1BL [16].

Since there is sufficient genetic variability available in wheat and its progenitors and closely related species, therefore, the generation of new knowledge on genetics of glaucousness will always remain an area of research for further use. The variation in presence of wax on leaf surface is scattered and is characterized by the presence of different homologues of Carbon atoms ranging from C20-C44 [17]. An investigation was therefore, carried out to find out the genetics of presence of C-atom in waxy and non-waxy genotypes of *T. aestivum* through Gas Liquid Chromatography.

Material and methods

The present study on genetics and bio-chemical analysis of glaucousness (waxiness) were conducted during the *rabi* seasons of 2005-06 and 2006-07 in the net house of Division of Genetics, IARI, New Delhi. The materials consisted of a waxy genotype Pusa T3336 (DARF Kite/ Lok Bharti) and non-waxy (non-glaucous having bottle green bloom) derivatives from synthetic hexaploid wheat, DL605 and DL629 (Fig. 1). Two crosses, *viz*., Pusa T3336 x DL605 and Pusa T3336 x DL629 were made. The F_1 hybrids were raised in off-season nursery at Wellington, Tamil Nadu. F_2 populations were grown in net house at New Delhi. Recommended cultural practices were followed to raise F_1 and F_2 generations. The presence and absence of waxiness on leaves and leaf sheaths of individual plants was recorded visually and the plants with presence (waxy bloom) and absence of wax (bottle green foliage) were classified according to the expression of non-waxy and waxiness. The data were subjected to χ^2 (Chi-Square) test for testing goodness of fit.

Qualitative and quantitative analysis of wax

Differential amount of carbon compounds present in contrasting parents were estimated by using Gas Liquid Chromatography (GLC). Wax from leaves and leaf sheath of internode below the peduncle (10g) was extracted with hexane (100ml). The hexane was separated and dried over anhydrous sodium sulphate to remove moisture. The hexane extract was concentrated on a rotary evaporator to dryness. During analysis by GLC, 1 ml of hexane was added to each of these dried samples. The above samples were analyzed by GLC equipped with Hewlett Packard 5890A with Flame Ionization Detector (FID) and fitted with HP-I (0.53 mm id x 10 m) column. The operating conditions were as follows:

Column Temperature	150 to 270°C @ 10°C/min.
Injector Temperature	250°C
Detector Temperature	250°C
N_2 as carrier gas	20 ml/min.
H_2 and O_2 ratio ml/min	30:120

Samples were analyzed by injecting 3µl in the injector part. For identification of nature of hydrocarbon in waxy and non waxy parents, F_1 and segregating F_2 generation the Gas Chromatography – Mass Spectroscopy (GC–MS) was performed by using same extract as in GLC on HRGC–MEGA 2 series gas chromatograph coupled with a FISIONS TRIO 1000 ion trap mass spectrometer and connected to a Panasonic K x P1150 multimode printer and on a HP 5890 G C

coupled with HP 5790 mass selective detector (MSD). The ionization potential was equipped with OV – 101 capillary column (30 m x 0·25 mm i. d., film thickness 1 –0·15mm). The column temperature was programmed from 70°C to 270°C at a rate of 10°C per minute. Helium was used as a carrier gas with flow rate of 2 ml per min. For quantitative analysis, the solution of known concentration of each hydrocarbon was injected and based on the area of the amount present in unknown sample was calculated as per the following equation.

$$\text{Amount of C-atom homologue} = \frac{\text{Area of sample x concentration of standard}}{\text{Area of standard}}$$

Results and discussion

Investigations were carried out to determine the inheritance pattern of waxiness and its correlation with the qualitative and quantitative estimation by using Gas Liquid Chromatography (GLC). Expression of wax in stock Pusa T3336 starts appearing before the boot leaf stage. The deposition of wax is higher on leaf sheath than on the leaf surface. Both presence and the absence of wax is clearly observable trait. In both the crosses, Pusa T 3336 x DL605 and Pusa T3336 x DL629 the F_1 plants did not show the presence of wax visually but appeared bottle green in colour (non-glaucous). The absence of wax in both the F_1 hybrids indicated that

Fig. 1. Parents, Pusa T3336 with wax (left) and DL605 without wax (right) and their F_1 hybrid with non-waxy appearance (middle) on leaf sheath respectively

Table 1. Segregation for the presence of waxiness in F_2 generation

Cross/parent	Generation	Segregation for waxiness (observed values)		Expected ratio	χ^2 value	P value
		waxy	non-waxy			
Pusa T3336	P_1	15	0			
DL605	P_2	0	12			
Pusa T3336 x DL605	F_1	0	10			
	F_2	40	94	1 : 3	1.68	0.20-0.10
DL629	P_3	0	17			
Pusa T3336 x		—				
DL 629	F_1	0	10			
	F_2	32	97	1 : 3	0.003	0.98-0.95
	F_2	32	97	3 : 13	3.10	0.10-0.20
Pusa T3336 x		—				
DL 629*	F_2	21	47	1 : 3	1.25	0.40-0.30

*Plants based on chromatographs peaks were classified into two groups

non-glaucousness is dominant over waxiness. The F_2 populations derived from the cross, Pusa T3336 x DL605 segregated into a ratio of one waxy and three non waxy plants (1:3) with χ^2 value of 1.681 (P value 0.20-0.10), however, the F_2 population derived from the cross Pusa T3336 x DL629 fitted well in expected ratios of 1 waxy: 3 non-waxy as well as 3 waxy : 13 non-waxy with χ^2 value of 0.003 (P value 0.98-0.95) and 3.10 (P value 0.10-0.05), respectively (Table 1). The expression of glaucousness depends on the arrangement of wax deposits rather than the amount of wax. This phenotype was originally thought to be controlled by a series of wax-producing genes and an independent series of inhibitors [6] but later work [Stucky, J and Driscoll, C.J., cited in 18] indicated that the wax-producing genes and the inhibitors were allelic. Hence recessive alleles and dominant alleles may result in non-glaucousness.

Qualitative and quantitative analysis of wax and its pattern in parents, F_1 and F_2

Waxy and non-waxy plants differ in the carbon compounds they contain. Waxy and non-waxy sample of parents and F_1 analysed by GLC-MS showed the presence of C_{24}, C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{32}, and C_{40}, in waxy parent while these were absent in non-waxy parent. However, the F_1 showed presence of only C_{32}, with the value of retention time 19.471, which was not scorable visually (Table 2). The both amount and the nature of carbon compound varied in contrasting parents, F_1 and F_2 plants (Figs. 2 & 3). Variation in the estimation of carbon-compounds has been recorded earlier, where the quantity of each carbon-atom differed significantly. In waxy parent, C_{32} had intermediate value of 509 ppm, with a range of 15 ppm (C_{24}) to 755.10 ppm (C_{44}) [3]. Gas Liquid Chromatography was carried out to differentiate waxy and non waxy plants. Both the parents showed different peak for GLC which indicates their quantitative differences in C-compounds. GLC graph of F_1 (non-waxy) was close to non-waxy parent DL629 which corresponds to the dominance of non glaucousness. GLC was done in randomly selected 68 plants in F_2 generation of Pusa T3336 X DL629 cross. The chromatographs based on peaks were classified into two groups. Out of 68 plants 47 did not show the presence of any wax while 21 had visually scorable amount of wax. The trend indicated similar pattern in distribution of wax as observed in the study of mode of inheritance. When the data were subjected to χ^2 test it fitted well into expected ratio of 1:3 (waxy: non waxy) with χ^2 value of 1.254 at P value 0.40-0.30. It can therefore be presumed that other carbon-atom could not get detected because of the presence of inhibitors located in each of three genomes.

Table 2. Presence of hydrocarbons (C-homologue) present in parents and F_1 Pusa T3336 x DL629

S.No.	Retention time	C-homo-logue	Present in parents		F_1
			waxy	Non waxy	
1	11.863	C24	Present	-	-
2	12.634	C25	Present	-	-
3	13.281	C27	Present	-	-
4	13.448	C28	Present	-	-
5	16.400	C29	Present	-	-
6	17.717	C30	Present	-	-
7	19.471	C32	Present	-	Present
8	23.610	C40	Present	-	-

Waxiness (glaucousness) is the visual expression of deposition of wax on leaves and stems. Wheat plants may be either waxy (glaucous), non waxy (non glaucous, green or bottle green) or with low wax. However, the expression of waxiness depends on the arrangement of wax deposits rather than the amount of wax [1]. Many of the wheat varieties have waxy bloom but with variable degree of wax deposits. The waxiness is a scorable trait and such morphological traits have been reported to be associated with disease resistance [3]. Epicuticular wax on leaf and leaf sheath create microhabitat for a variety of parasitic and sporophytic organism and acts as a barrier to fungal pathogens [17]. Mutation at waxy locus is presumed to be frequent resulting into number of alleles. Electrophoretic analysis of waxy wheat protein permits to identify new variants at the waxy locus [18].

Understanding of genetics in wheat has been advanced through concentration on suitable experimentation systems. Clearly visible morphologically observable traits play a significant role for a well designed breeding programme. It is important, therefore to know inheritance pattern, identification of genes and linkage association between various traits of importance. In the crosses between fully waxy (glaucous) and bottle green (non waxy or non-glaucous) parents, the genetic analysis reveals that non waxiness is dominant over waxiness and is controlled by a single gene. However, involvement of two dominant complimentary genes and dosage effect of genes for waxiness have also been reported [9]. Sufficient genetic variability for waxiness exists in wheat and its related species. Orthologous loci in barley, rye and maize do also occur [8]. Single

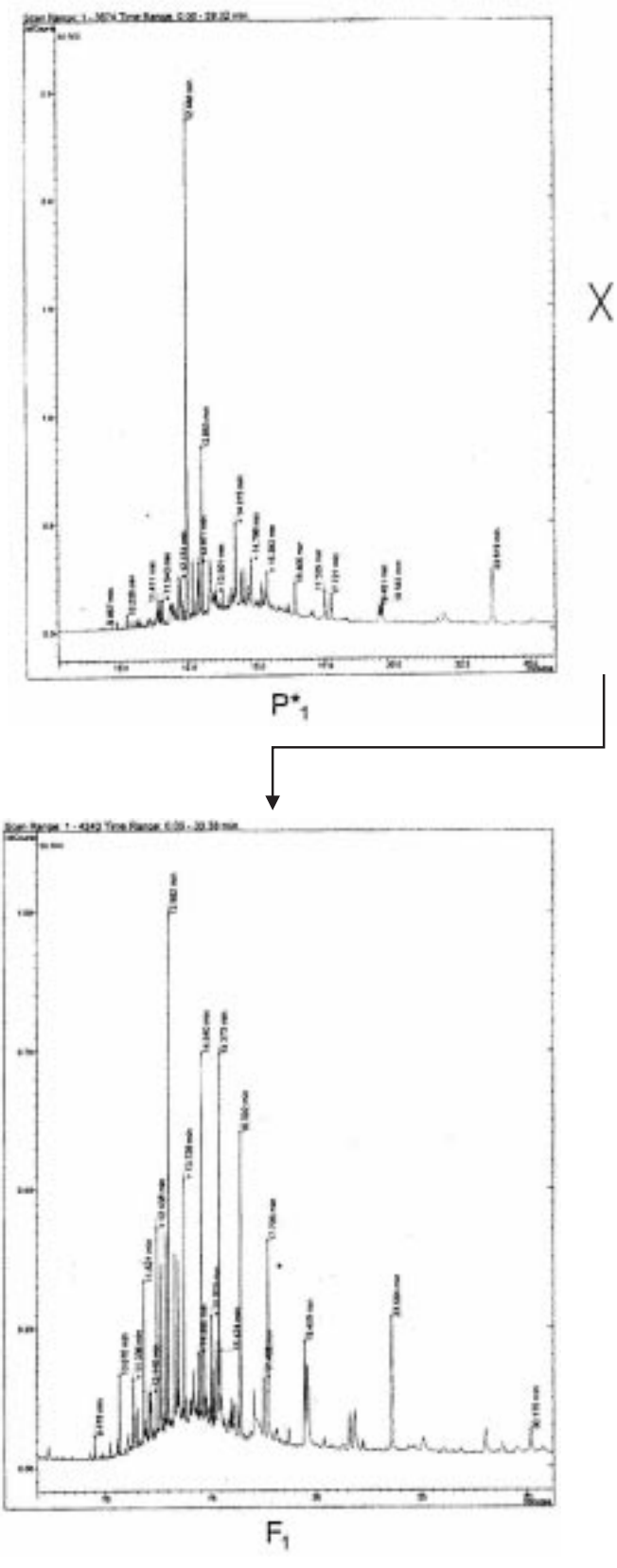


Fig. 2. GC-MS representation of presence of C-compounds in parents and F_1 hybrid. P^*_1: Waxy parent; P^*_2: Non-waxy parent

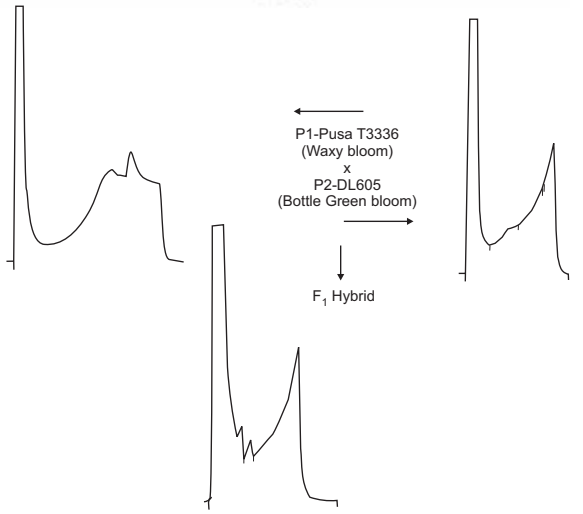
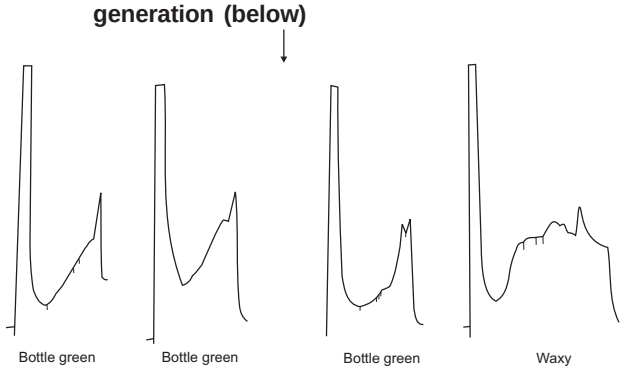


Fig. 3. Segregation pattern by gas liquid chromatographs in parents, F_1 and F_2 generation (below)



F_2 individuals indicating segregation

dominant gene governing the waxiness in *Triticum durum* has also been reported [19]. The earlier reports reveal that the presence of awnedness, pubescence and flag leaf waxiness in wheat may be contributing traits for drought tolerance [21]. Therefore, identification of gene for waxiness may serve as a useful marker in breeding. Further, the findings emanated from the study would help in understanding the genetic bases of individual carbon compound and its role in plant breeding.

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