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GLAUCOUS WHEAT MUTANTS. I. AGRONOMIC PERFORMANCE AND EPICUTICULAR WAX CONTENT

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ABSTRACT

This study was conducted to characterize five new induced glaucous wheat mutant lines in terms of wax load content and different agronomic traits and to estimate the variability in these desirable traits and compare them with their parent. Significant differences were observed among the six wheat genotypes for epicuticular wax (EW) content and agronomic traits. The mutant line GWM4 produced the highest quantity of EW, while the lowest amount was produced by the parent Sids1. The mutant GWM1 is characterized by a reduced plant height (82.3 cm). GWM2 is early flowering mutant (60.0 days) and has heavy 1000-grain weight (60.5 gm). GWM3 is characterized by a high number of spikes per plant, a high number of spikelets per spike and high grain yield per plant. GWM4 has also high number of spikelets per spike and a high number of grains per spike. GWM5 is charcterized by a high number of spikes per plant and high grain yield per plant. The phenotypic coefficient of variation (PCV) estimates were slightly higher than the genotypic coefficient of variation (GCV) estimates for all traits under study. The highest estimates of PCV and GCV were shown by number of spikes per plant and number of grains per spike. However, grain yield per plant, heading date and wax content exhibited moderate PCV and GCV estimates.

Key words: *Epicuticular wax, Glaucous, Mutation, Genetic Variation, Wheat, Triticum aestivum.*

INTRODUCTION

The success in developing improved varieties of crop plants through conventional breeding programs depends on the existence of genetic variation on which selection can act. Mutagenic agents, such as radiation and certain chemicals, are utilized to induce mutations and to generate variations from which desired mutants could be selected. During the past seventy years, more than 2,252 mutant varieties have been officially released (Maluszynski *et al* 2000). Many induced mutants were released directly as new varieties; others were used as parents to derive new varieties. Mutation induction with radiation was the most frequently used method to develop direct mutant varieties (89%). Gamma rays were employed to develop 64% of the radiation-induced mutant varieties, followed by X-rays (22%) (Ahloowalia *et al* 2004). Several classes of mutants have been induced in wheat (*Triticum aestivum* L.) i.e. reduced height, awnedness, increased yield, stem and leaf rust resistance, amber grain color, earliness, closed canopy phenotype and increased grain protein (Konzak 1987).

Glaucousness is the waxy covering which imparts a dull-white or bluish-green cast commonly referred to as bloom (Johnson *et al* 1983). The genetic variation in epicuticular wax (glaucousness) is of potential importance in breeding for drought tolerance. Variation in epicuticular wax in wheat has been previously reported (Johnson *et al* 1983), but only in a limited range of genotypes (Nizam Uddin and Marshal 1988). The waxy cuticle surface on wheat plants could be modified by induced mutation and these mutants are easily detected (Konzak 1981 and 1984). Johnson *et al* (1983) reported that glaucousness in wheat is controlled by a multiple allelic system of two dominant inhibitors on chromosomes 2B and 2D (Jensen and Driscoll 1962 and Stuckey 1972). They added that, a considerable scope exists for modifying the degree of glaucousness in wheat.

The present study was conducted to characterize new five induced glaucous wheat mutant lines that were derived via gamma irradiation in terms of wax load content and different agronomic traits and estimate the variability in these traits to help plant breeder in increasing the efficiency of selection for these desirable traits.

MATERIALS AND METHODS

Experimental plant materials and design

Five glaucous mutant lines of bread wheat i.e. GWM1, GWM2, GWM3, GWM4, GWM5 in the 5th mutated generation (M5) and their original parent, Sids1, were used as a genetic material in the present study. The five glaucous wheat mutant lines under study have different morphological and agronomic characters and they were selected in the second mutated generation (M2) from a glaucous wheat mutant which resulted from irradiation of Sids1 cultivar with 30 Krad (Kilo Rad) of gamma rays in the first mutated generation (M1) in the winter season, 2001/2002. In M3 and M4 generations, mutants were grown in spike-to-row progenies. Irradiation treatment was achieved by a Co-60 (Cobalt-60) Gamma Irradiation Unit, Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Egypt. The five glaucous mutant lines are characterized by a heavy epicuticular wax layer covering the stems, leaves and spikes, however, Sids1 is a non-glaucous spring wheat cultivar adapted under Egyptian agricultural conditions. The cultivar pedigree of Sids1 is as follows: HD2172 / Pavon "s" // 1158.571 Maya74 "s" SD 46-4sd-2sd-1sd-0sd.

In 2005/2006 season, seeds of the six wheat genotypes were grown at Inshas Experimental Farm of Plant Research Dept., Nuclear Research Center, Atomic Energy Authority, Egypt on the 15th of November, 2005. Irrigation was performed every 15 days in a sandy loam soil containing a sand, silt, and clay fractions of 63.75, 31.25, and 5.00%, respectively. Thirty one Kg of P_2O_5 fertilizer per faddan was incorporated. N and K_2O fertilizers were applied to the experiment at rates of 50 and 8 Kg per faddan, respectively.

Individual seeds were planted in 2-meter rows. Each row included 20 plants spaced 10 cm apart. Rows were spaced 30 cm apart in blocks (each block contains 30 rows). A randomized complete block design (RCBD) with three replications was used. Within each replicate, the experimental plot consisted of five rows for each genotype. Data were recorded on days to heading, plant height (cm), number of spikes per plant, number of spikelets per spike, number of grains per spike, 1000-grain weight (g) and grain yield per plant (g). Ten guarded plants from each replication were used for data recording.

Wax quantification

Wax quantification was performed according to the procedure used by (Nizam Uddin and Marshal 1988). Five samples each consisting of one flag leaf from the primary culms per plot were collected at anthesis to estimate epicuticular wax per unit leaf area. The colorimetric method of Ebercon *et al* (1977) was used for wax analyses. This method is based on the colour change produced by the reaction of wax with acidic $K_2Cr_2O_7$. After measuring the leaf area with an area meter (AM 100), each sample was cut into pieces, immersed and stirred for 15 seconds in 20-30 ml redistilled chloroform depending on the sample size. The extracts were filtered and evaporated to dryness in a water bath at 70°C. Five ml of acidic $K_2Cr_2O_7$ reagent was added to each sample and the samples were heated for 30 minutes in the water bath at 100°C. The reagent was prepared according to the procedure described by Ebercon *et al* (1977). After cooling, 12 ml of deionized water was added to each sample and several minutes were allowed for colour development. The optical density of the samples was then measured at 590 nm. The amount of wax was expressed as mg/dm^2 (both surfaces). The reagent was prepared by mixing 40 ml deionized water with 20 g powdered potassium dichromate. The resulting slurry was mixed vigorously with 1 liter concentrated sulfuric acid and heated (below boiling) until a clear solution was obtained. Standard wax solution was prepared from wax collected from leaf sheaths and blades of test plants (glaucous wheat mutants). Waxes were dissolved in redistilled chloroform and 15 ml aliquots containing a range of concentrations were prepared. The resulting standard curve was linear (r =1) throughout the concentrations used (Fig. 1).

Data analysis

Analysis of variance and least significant difference (LSD) were performed on the data for the six genotypes. The variance components (phenotypic, genotypic and environmental variance), phenotypic (PCV), genotypic (GCV) and environmental (ECV) coefficient of variation were calculated for the studied characters as described by Burton and De-Vane (1953) and Johnson *et al* (1955).

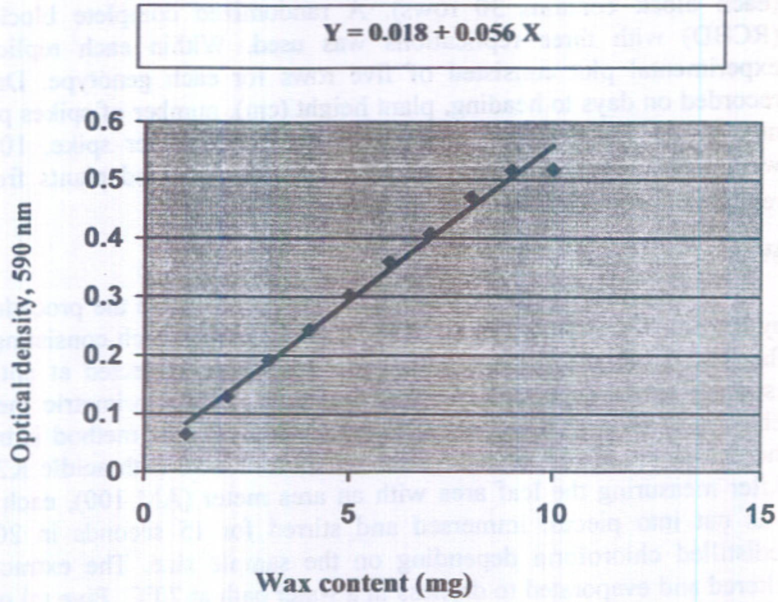

Fig. 1. Standard curve for glaucous wheat leaf wax.

RESULTS AND DISCUSSION

The selected glaucous wheat mutant lines under study are charcterized by a heavy epicuticular wax layer covering their stems, leaves and spikes as compared to their parent (Fig. 2). The quantity of this epicuticular wax layer a well as other studied traits varied significantly among the mutant lines and their parent (Table 1).

Table 1. Mean squares for wax content and 7 agronomic traits of five glaucous wheat mutant lines and their parent.

S. V.	d.f.	Wax content	Days to heading	Plant height (cm)	Spikes/ plant	Spikelets / spike	Grains/ spike	1000-grain weight	Grain yield/ plant
Blocks	2	0.02	57.7	7.1	3.4	1.5	71.5	40.5	14.1
Genotypes	5	0.94**	827.5**	324.3**	85.7**	41.2**	3505.6**	119.9*	435.2**
Error	10	0.07	54.6	11.2	2.9	3.4	324.7	26.5	68.5

*, ** Indicates significance at 0.05 and 0.01 levels of probability, respectively.

The mean performance of the five derived glaucous mutant lines and their parent for epicuticular wax content and agronomic traits is shown in Table (2).

Table 2. Mean performance for wax content (mg/dm^2) and 7 agronomic traits of five glaucous wheat mutant lines and their parent.

Trait	Genotypes						Average	$LSD_{(0.05)}$
	Sids1	GWM1	GWM2	GWM3	GWM4	GWM5		
Wax content (mg/dm^2)	2.62	3.78	3.42	3.63	4.30	3.27	3.50	0.48
Days to heading	89.0	77.0	60.0	107.8	93.0	75.0	83.64	13.44
Plant height (cm)	109.2	82.3	90.0	100.0	101.6	107.5	98.44	6.08
Spikes /plant	17.6	13.0	9.7	20.3	7.3	19.2	14.53	3.09
Spikelets/ spike	23.0	23.3	23.0	32.0	27.3	23.0	25.28	3.36
Grains /spike	75.6	99.7	74.6	105.4	163.4	76.3	99.16	32.78
1000-grain weight (g)	47.6	50.1	60.5	44.4	56.6	57.4	52.76	9.36
Grain yield/plant (g)	63.1	63.4	54.0	83.4	66.8	84.0	69.1	15.06

GWM: Glaucous wheat mutant.

Epicuticular wax (EW) content

Epicuticular wax (EW) content varied from 2.62 to 4.30 mg per dm^2 with an average of 3.50 mg per dm^2. The mutant line GWM4 is characterized by a high quantity of EW followed by GWM1, GWM3, GWM2 and GWM5. The lowest amount of EW was produced by the parent Sids1. The EW loads increased markedly by 44.27, 30.53, 38.55, 64.12 and 24.81% for the mutant lines GWM1, GWM2, GWM3, GWM4 and GWM5, respectively than their parent. The mean amount of EW per unit of leaf area found across the five glaucous mutant lines was considerably greater than that previously reported for wheat (Johnson *et al* 1983 and Nizam Uddin and Marshall 1988), rice (O'Toole *et al* 1979 and O'Toole and Cruz 1983), sorghum (Ebercon *et al* 1977 and Jordan *et al* 1983) and barley (Wettstein-Knowles, 1970).

Agronomic characters

The mutant lines differ in agronomic traits under study. These differences are significant as shown in Table (1). Each of the developed mutant lines is characterized by a significant increase or decrease in at least one agronomic character as compared to other mutants and their parent. GWM1 is characterized by a reduced plant height (82.3 cm). GWM2 is early flowering mutant (60.0 days) and has heavy 1000-grain weight (60.5 gm). GWM3 is characterized by a high number of spikes per plant, a high number of spikelets per spike and high grain yield per plant. GWM4 has a high number of spikelets per spike and a high number of grains per spike. GWM5 is charcterized by a high number of spikes per plant and high grain yield per plant. On the other hand, GWM3 flowers late (107.8 days). GWM1, GWM2 and GWM4 have reduced number of spikes per plant. GWM3 has the lowest 1000-grain weight. GWM2 is the lowest in grain yield per plant.

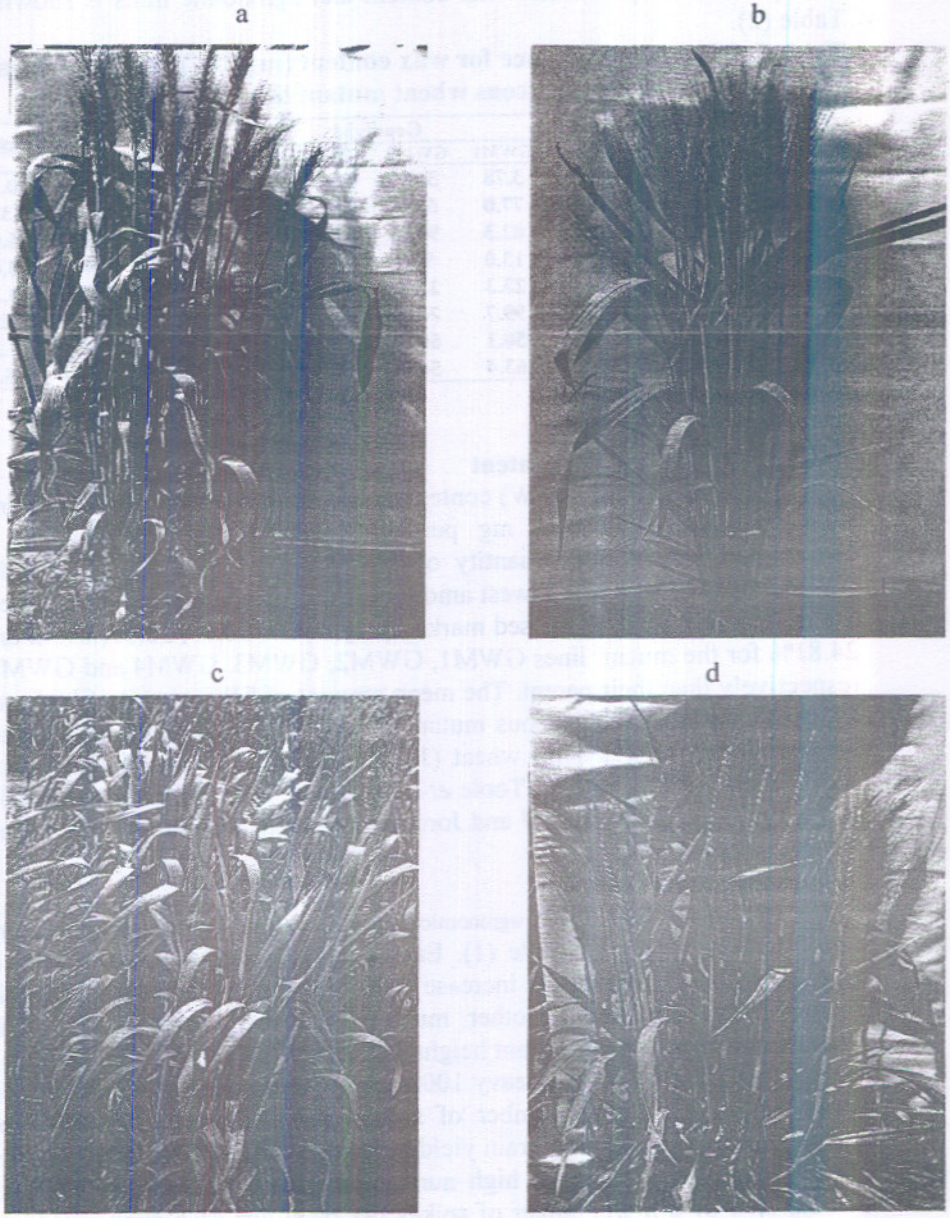

Fig. 2. a: Non-glaucous plant of Sids1 cultivar of wheat. b: Glaucous mutant line1, c: Glaucous mutant line4. and d: Glaucous mutant line5.

Variability

Variability present among the five glaucous wheat mutant lines and their parent was assessed for different traits using estimates of variance components, phenotypic (PCV), genotypic (GCV), and environmental (ECV) coefficient of variation (Table 3).

The PCV estimates were slightly higher than the GCV estimates for all traits under study. The GCV estimates for all traits were higher than that of ECV. This means that the genetic variation constitutes the major portion of phenotypic variation. The highest estimates of PCV and GCV were shown by number of spikes per plant and number of grains per spike. However, grain yield per plant, heading date and wax content exhibited moderate PCV and GCV estimates. The lowest estimates were shown by plant height and 1000-grain weight.

Table 3. Phenotypic (PCV), genotypic (GCV), and environmental (ECV) coefficients of variation, components of variance for wax content and 7 agronomic traits over five glaucous wheat mutant lines and their parent.

Trait	PCV	GCV	ECV	$\delta^2 ph$*	$\delta^2 g$*	$\delta^2 e/r$*
Wax content	15.91	15.38	4.04	0.31	0.29	0.02
Plant height (cm)	10.56	10.38	1.96	108.13	104.40	3.73
Days to heading	19.86	19.19	5.10	275.84	257.64	18.20
Spikes /plant	36.78	36.15	6.78	28.56	27.59	0.97
Spikelets /spike	14.67	14.05	4.22	13.75	12.61	1.14
Grains /spike	34.47	32.84	10.49	1168.53	1061.29	108.53
1000-grain weight	11.98	10.57	5.63	39.96	31.13	8.83
Grain yield/plant	21.32	19.36	8.93	145.08	122.25	22.83

$\delta^2 ph$, $\delta^2 g$ & $\delta^2 e/r$ are phenotypic, genetic, and error variances, respectively.

Variation observed among glaucous mutant lines and their non-glaucous parent in M5 generation indicates that positive genetic changes (mutations) occurred in the genetic factors (allelic forms) controlling these traits (epicuticular wax content and agronomic traits) in Sids1 cultivar after irradiation treatment and subjecting to homozygosity in the next segregating generations.

The increase in PCV and GCV estimates in this study are consistent with the finding of Scossiroli (1977), who stated that biometrical analyses of variation have shown that the increase in phenotypic variation in generations following irradiation, particularly in self-pollinated plants, is due mainly to an increase in homozygosity and this in the genetic variance, and it may be accounted for by the effects of mutations on the genetic factors influencing quantitative characters. From a plant-breeding point of view, this is an important effect because larger genetic variation means the possibility of larger responses to selection and higher chances for improvement. The existance of genetic variability among glaucous wheat mutant lines and their parent refers to the usefulness of these genetic materials to wheat breeders and for exploitation in selection or hybridization programs of bread wheat. Needless to report that the early GWM mutant if proved to be also early maturing will be a breakthrough genotype for wheat improvement.

Additional information is needed concerning determination of mode of inheritance, number of genes controlling the glaucousness trait induced in the present mutant lines, expression of glaucous trait, chromosome location of the genes, and role of wax content in drought tolerance.

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طفرات قمح شمعية. 1. الاداء المحصولي والمحتوى من الشمع السطحى

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يساعد تقدير محتوى الشمع على سطح الأوراق والصفات المحصولية وقيمة التباين الوراثى لهذه الصفات فى سلالات شمعية طفرية من قمح الخبز فى الاستفادة من تلك السلالات فى برامج التربية. شملت هذه الدراسة خمسة سلالات شمعية طفرية من قمح الخبز والأب الذى نشأت منه (الصنف سدس1) بهدف توصيفها فيما يتعلق بمحتواها من الشمع على سطح الأوراق وثمان صفات محصولية وزراعية مختلفة وكذلك تقدير التباينات الوراثية لهذه الصفات المرغوبة. لوحظ وجود فروق معنوية بين التراكيب الوراثية المدروسة فى محتواها من الشمع على سطح الأوراق وفى الصفات المحصولية والزراعية تحت الدراسة. تميزت السلالة الشمعية الطفرية رقم4 GWM4 بأعلى محتوى من الشمع على سطح الورقة بينما كان الأب سدس1 هو الأقل فى هذه الصفة. تميزت السلالة رقم1 GWM1 بطولها المنخفض (82.3 سم). تميزت السلالة رقم2 GWM2 بالتزهير المبكر (عند60 يوم) وارتفاع وزن ال 1000 حبة. تميزت السلالة رقم3 GWM3 بأكبرعدد سنابل وأكبرعدد سنيبلات ومحصول نبات عالى. تميزت أيضا السلالة رقم4 GWM4 بعدد سنيبلات وعدد حبوب فى السنبلة مرتفع. تميزت السلالة رقم5 GWM5 بعدد مرتفع من سنابل النبات ومحصول نبات عال. ارتفعت تقديرات معاملات الاختلاف المظهرية قليلا عن تقديرات معاملات الاختلاف الوراثية. أظهرت صفات عدد سنابل النبات وعدد الحبوب فى السنبلة أعلى تقديرات للتباين الوراثى بينما أظهر محصول النبات وعدد الأيام حتى التزهير ومحتوى الشمع على الأوراق تقديرات متوسطة من التباين الوراثى.

مجلد المؤتمر الخامس لتربيه النبات – الجيزه٢٧ مايو ٢٠٠٧

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