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PHENOL REACTION ON SEEDS AND GLUMES IN WHEAT SPECIES AT DIFFERENT LEVELS OF PLOIDY

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ABSTRACT

One hundred and thirty two accessions of diploid, tetraploid and hexaploid wheat and its related species were investigated for phenol reaction on seeds and glumes. Diploid species having A genome produced black or dark brown colour on seeds while those belonging to D, S, S^1, M, M^t, C, U and V genomes gave no phenol reaction. Varied reactions were observed among 4x species containing AABB and UUMM genomes while the species having AAGG genomes showed no reaction. All hexaploid wheats gave positive reaction except one accession each of *Triticum aestivum* ssp. *macha* (AABBDD) and *T. zhukovskyi* (AAAAGG). Variable reaction to phenol at tetraploid and hexaploid level are likely due to allelic variation. The coloration on glumes was different from that of seeds. The glumes of all the einkorn wheats except *T. urartu* gave variable degree of coloration. As in the case of seeds, the glumes of other diploid species except A genome species gave no reaction. The glumes of tetraploid species gave negative reaction, except a few accessions of *T. turgidum* ssp. *dicoccoides* and *carthlicum*, where variation in coloration was observed. Most of the hexaploid wheats reacted to phenol. The extent of colour development on seeds differs from that on glumes, suggesting that the factor(s) governing phenol reaction of seeds and glumes are different and independent of each other. The gene(s) controlling phenol reaction of seeds and glumes in 4x (AABB) and 6x (AABBDD/AAAAGG) species are contributed by A genome. However, the source of gene(s) controlling phenol reaction in species having UUMM genome is not known.

Key words: Wheat species, *Triticum*, *Aegilops*, phenol test, seed, glumes.

The phenol test of seeds and glumes is one of the criteria for ascertaining the genetic purity of a variety and has been successfully used in wheat [1–3] and rice [4]. Miczynski [5] studied the inheritance of phenol reaction in wheat seeds and reported that this character is controlled by one or two genes, while Joshi and Banerjee [6] suggested that it is governed by a single gene having multiple alleles. Bhowal et al. [7] suggested that the A genome contributed the gene controlling phenol reaction in hexaploid wheats. The present study reports the phenol reaction of seeds and outer glumes in 132 accessions of diploid (2x), tetraploid (4x) and hexaploid (6x) species of wheat and its relatives having different genomes and their combinations.

MATERIALS AND METHODS

One hundred and thirty two accessions of wheat species belonging to 2x, 4x and 6x groups having different genomes and genomic combination were used in this study. Seeds obtained from different sources were grown under uniform cultural practices at the IARI, Delhi. Species late in maturity were given extra light to induce flowering and maturity. The seeds and outer glumes (excluding lemma and palea, subsequently referred to as glume) were taken from the same ear of each entry. Ten seeds and the same number of glumes from each entry were wrapped with absorbant paper and soaked in water for 16 h at $20 \pm 1^{o}C$. Later the seeds and glumes were unwrapped, blotted dry and placed on Whatman No. 1 filter paper in covered Petri dishes with freshly prepared 1% aqueous solution of phenol at $25 \pm 1^{o}C$. After 4 h, the degree of darkening of seeds was scored as black, dark brown, brown, light brown or as no colour reaction (negative). The coloration of glumes were recorded on the outer surface, the marked localization of colour at either base or tip (beak) and the proportion of area covered was also scored. The test was repeated to confirm the results.

A classification of the genus *Triticum* and *Aegilops* has been used following Mackey [8] and Kihara and Tanaka [9], respectively. *Triticum boeoticum* ssp. *aegilopoides* and ssp. *thoudar* (two ecotypes) are used as described by Miller [10], and *T. sinskajae* is separated from the major einkorn group *T. monococcum* by Dorofeev and Korovina [11].

RESULTS

PHENOL REACTION ON SEED

The accessions of diploid wheats containing A genome, such as *T. monococcum* ssp. *boeoticum* and *T. boeoticum* ssp. *thoudar* showed black colour, while *T. urartu*, *T. boeoticum* ssp. *aegilopoides* and *T. sinskajae* produced dark brown colour (Table 1). Unlike the diploid species having A genome, the 2x wheats possessing D, S, S^1, M, M^t(T), C, U and V genomes gave negative reaction to phenol.

Among the tetraploid wheats possessing AABB genomes, black, dark brown, brown and light brown colour and negative reactions were recorded. Seven accessions of *T. turgidum* ssp. *dicoccoides* produced black colour, one light brown, and the remaining four gave negative reaction. Among the accessions of *T. turgidum* ssp. *durum*, six gave negative and two black reactions, while each of the two varieties of ssp. *dicoccum* showed dark brown and brown reactions. Out of the several accessions tested, one accession each of *T. turgidum* ssp. *turgidum* conv. *turgidum*, ssp. *turgidum* conv. *polonicum*, conv. *turanicum* and ssp. *pyramidale* produced light brown colour, and the remaining accessions did not react. The accessions of *T. turgidum* ssp. *carthlicum* showed all patterns of colour and none gave negative reaction.

Tetraploid wheats with AAGG genome *T. timopheevi* ssp. *timopheevi* and its mutant *T. militinae* showed no reaction. The other 4x wheats having CD, US, UC and DM genomes also did not react to phenol. However, species with UM genomes gave dark brown, brown as well as negative reaction.

A variety of reaction pattern were observed among the different accessions of 6x wheats. Of the two accessions of *T. aestivum* ssp. *macha*, one gave light brown and the other was negative. The only strain of *T. zhukovskyi* (AAAAGG) tested was also negative.

PHENOL REACTION ON OUTER GLUMES

The results of phenol reaction on the outer glumes are also presented in Table 1. One accession of *T. monococcum* ssp. *monococcum* produced black colour on the upper 1/3 part of the glume, while in the remaining five only the tip of the glume was black. The upper 1/3 part of the glumes of *T. boeoticum* ssp. *aegilopoides* and *T. sinskajae* turned black, while only the veins of the glumes were coloured in *T. boeoticum* ssp. *thoudar*. Among the accessions of *T. monococcum* ssp. *boeoticum*, the upper 3/4 portion of the glumes reacted to produce black colour and the lower 1/4 portion did not react. The glumes of *T. urartu* also did not produce any colour.

Table 1. Phenol colour reaction in 2x, 4x and 6x species of wheat

Species	Genome	Phenol reaction of seed	Phenol reaction of glume
1	2	3	4
Diploids (2n=2x=14):			
Triticum monococcum L. ssp. *monococcum*, ssp. *monococcum* vars. *nigraflavescens*, *vulgare* (3)	AA	Black	Only tip black
ssp. *monococcum* G 1372		Black	Upper 1/3 part black
ssp. *boeoticum* (Boiss.) Mk. G 1848, 4, G 2170 & G 2508 (4)	AA	Black	Upper 3/4 part dark brown
T. urartu Tum. G 1953 & PBI (2)	AA	Dark brown	Ne
T. boeoticum ssp. *aegilopoides* (Link.)		Dark brown	Upper 1/3 part black
T. sinskajae A. Filat and Kurk.	AA	Dark brown	Upper 1/3 part black
T. boeoticum ssp. *thoudar* Reut.		Black	Veins black
Aegilops speltoides Tausch, accs. A,D and K (3)	SS	Ne	Ne

(Contd.)

Table 1. *(contd.)*

1	2	3	4
Ae. longissima Schweinf & Muschl.,	S^1S^1	Ne	Ne
Ae. sharonensis Eig., acc. TH 02	SS	Ne	Ne
Ae. squarrosa L.,	DD	Ne	Ne
Ae. caudata L.,	CC	Ne	Ne
Ae. comosa Sibth & Sm., accs. A & G(2)	MM	Ne	Ne
Ae. mutica Boiss., accs. A & K(2)	M^tM^t	Ne	Ne
Ae. umbellulata Zhuk., accs. K 4033 K5901, PI 341797 and A (4)	UU	Ne	Ne
Haynaldia villosa	VV	Ne	Ne
Tetraploids (2n=4x=28):			
T. turgidum L. ssp. *dicoccoides* Korn., G 1377	AABB	Brown	Ne
ssp. *dicoccoides* vars. *fulvovillosum*, *spontaneonigram* and *aaronsohnii* (3)		Ne	Ne
ssp. *dicoccoides* accs. 19, PI 272582, G, VIR 26118 and V 1063 (5)		Dark brown	Ne
ssp. *dicoccoides* accs. TTD-15, and G 3034 (2)		Dark brown	Light brown
ssp. *dicoccoides* TTD-13		Ne	Light brown
ssp. *dicoccoides* G		Dark brown	Ne
ssp. *dicoccum* Schrank. NP 200	AABB	Brown	Ne
ssp. *dicoccum* 17		Dark brown	Ne
ssp. *turgidum* conv. *turgidum* accs. 2, 47 PI 352450, EC 119490, 16 and *lucitanicum* (6)	AABB	Ne	Ne
ssp. *turgidum* Ex-7		Black	Ne
ssp. *turgidum* Ex-17		Light brown	Ne
ssp. *turgidum* conv. *turanicum* (Jakubz.) Mk.			
accs. 11, SWAN 215, *orientale insigne* (3)		Ne	Ne
conv. *turanicum* G 678		Light brown	Ne
ssp. *turgidum* conv. *polonicum* (L.) Mk.	AABB		
accs. F 233, 12, G 586, EC 119482, EC 119483, EC 119484 (6)		Ne	Ne
conv. *polonicum* F 234		Light brown	Ne
conv. *polonicum nigrobarbatum*, TTP 01, non-loose glume (3)		Ne	Ne
ssp. *pyramidale* Perc. accs. TTP 02, PI 115812, Jordan Botanique, *recognitum*, EC 119488 (5)		Ne	Ne
ssp. *pyramidale* F-217		Brown	Ne
ssp. *carthlicum* Neveski (Mk) accs. WS 1786, *fuliginosum* Zhuk. (2)	AABB	Dark brown	Ne

(Contd.)

Table 1. (*contd.*)

1	2	3	4
ssp. *carthlicum stramineum* Zhuk.		Light brown	Light brown
ssp. *carthlicum rubiginosum* Zhuk.		Black	Ne
ssp. *carthlicum rubiginosum* US, 11556 (Glb) (2)		Black	Black beak & veins of upper 1/2 part black
ssp. *carthlicum* TCH 01		Brown	Ne
ssp. *carthlicum*-558		Dark brown	—
ssp. *carthlicum* conv. *persicum* Vav. accs. 53-2A and 53-2B (2)		Light brown	Ne
ssp. *abyssinicum* Vav. G 587		+	Ne
ssp. *aethiopicum* Jakubz.		Ne	Ne
ssp. *turgidum* conv. *durum* (Desf.) Mk. accs. PBW 34, MACS 9, Malvika, Bijaga Red, HD 4530, Raj.911, Bijaga Yellow, A-9-30-1 (8)		Ne	Ne
T. timopheevi ssp. *timopheevi* Zhuk., accs. 27, TIM 01, var. *typica*, G 921, K 28541, K 38553, K 47798 (7)	AAGG	Ne	Ne
T. militinae Zhuk. and Migusch.	AAGG	Ne	Ne
Aegilops cylindrica Host.	CCDD	Ne	Ne
Ae. crassa Boiss. accs.A & G (2)	DDMM	Ne	Ne
Ae. triuncialis L., acc. X	UUCC	Ne	Ne
Ae. columnaris Zhuk., acc. A	UUMM	Dark brown	Ne
Ae. triaristata Willd., acc. F	UUMM	Brown	Ne
Ae. biuncialis Vis., acc. A	UUMM	Brown	Ne
Ae. ovata L., accs. A & K (2)	UUMM	Brown	Ne
Ae. variabilis Eig., accs. TKK 02, A, G (3)	UUSS	Ne	Ne
Ae. kotschyi Boiss., acc. TKK 03	UUSS	Ne	Ne
Hexaploids (2n=6x=42)			
T. aestivum L., acc. HW 741	AABBDD	Black	Ne
Sujata		Light brown	Black
ssp. *compactum* (Host.) Mk. var. *icterinum*		Black	Light brown
ssp. *macha* (Dek. & Men.) Mk. accs. 5,		Ne	Ne
ssp. *macha* acc. PBI		Light brown	Light brown
ssp. *amplissifolium* L.		Brown	Ne
ssp. *spelta* L., G 526		Dark brown	Ne
ssp. *spelta*-4		Light brown	Dark brown
ssp. *spelta*-11		Light brown	Light brown
ssp. *spelta* var. *duhamelianum, album* (2)		Dark brown	Light brown

(*Contd.*)

Table 1. (*contd.*)

1	2	3	4
ssp. *spelta*-13		Brown	Ne
ssp. *spelta*, grey		Brown	Only tip black
ssp. *sphaerococcum* (Perc.) Mk. *rubiginosa*, 2(2)		Dark brown	Light brown
ssp. *sphaerococcum* Jordan Botanique		Light brown	Brown
ssp. *sphaerococcum* TAP 03		Brown	Brown
ssp. *vavilovi* (Vill.) Mk. G 529		Dark brown	Dark brown
ssp. *vavilovi* PBI		Black	Brown
T. zhukovskyi Men. & Er.,	AAAAGG	Ne	Ne
Ae. triaristata Willd.	UUMMUnUn	Brown	Ne

Ne—negative; - glume originally black; + grain originally purple coloured; numbers in parentheses are the number of accessions tested; accs.—accession.

As in the case of grains, the glumes of the other diploid wheats containing, D, S, S^1, C, M, M^t, U and V genomes did not react to phenol. The glumes of different accessions of *T. turgidum* ssp. *turgidum* conv. *turgidum* conv. *durum*, ssp. *turgidum* conv. *polonicum*, ssp. *turgidum* conv. *turanicum*, ssp. *aethiopicum*, ssp. *abyssinicum*, ssp. *persicum* and ssp. *dicoccum* showed negative reaction. Variation for phenol colour was observed in the glumes of only two subspecies having AABB genomes. The accessions of *T. turgidum* ssp. *dicoccoides* and ssp. *carthlicum* showed three types of colour reaction: complete glume light brown, black beak, black beak and veins. Three accessions of ssp. *dicoccoides* exhibited light brown colour and the remaining nine accessions were negative. The glumes of *T. timopheevi* and *T. militinae* showed negative reaction. The other tetraploid wheats having CD, US, UC, UM and DM genomes also produced negative reaction except *Aegilops triaristata* (UUMM), which showed brown colour.

The 6x species with AABBDD genomes exhibited different phenol reactions (Table 1), while *T. zhukovskyi* (AAAAGG) was negative.

DISCUSSION

The results for phenol reaction on seed of different *Triticum* species revealed that the factor(s) controlling this character are carried by the A genome, as most of the einkorn wheats tested produced black or dark brown colour. The diploid species of *Aegilops* and *Haynaldia villosa* did not react to phenol, indicating the absence of genes for this trait. However, the 4x species *Ae. columnaris*, *Ae. triaristata* and *Ae. ovata*, all containing UUMM genomes, produced dark brown or brown colour, indicating the presence of phenol reaction gene(s) in either of the genome of the tetraploid *Aegilops*. The negative reaction observed in diploid progenitors *Ae. comosa* (M) and *Ae. umbellulata* (U) may be due to the fact that a very few accessions (2 and 4, respectively) of these species have been tested. The other possibility is that these 4x species showing phenol reaction acquired the character through introgression in the course of evolution.

It is of interest to note that the accessions of *T. turgidum* ssp. *carthlicum*, ssp. *dicoccum* and ssp. *dicoccoides* showed a range of colour reaction (black, dark brown, brown and light brown). It is likely that the gene(s) controlling black and dark brown colour reactions have been contributed by einkorn wheats while those governing brown and light brown colour evolved at the tetraploid level. Thus, the wide range of phenol reactions exhibited by 4x wheats with AABB genomes indicate the existence of allelic variation at the tetraploid level. The negative phenol reaction amongst some tetraploid species could be due to mutations in the A genome. The absence of positive phenol reaction in *T. timopheevi* is presumably due to the loss of phenol reaction gene(s) from A genome or presence of inhibitory factor(s) in G genome; which is partially related to B/S genome.

The positive phenol reaction in 6x wheats, except one strain each of *T. aestivum* ssp. *macha* and *T. zhukovskyi*, supports the observations recorded earlier by Singhal and Prakash [3] in 78 cultivars of hexaploid wheat. The negative reaction in ssp. *macha* may be due to loss of the character during evolution, while in *T. zhukovskyi* the G genome may carry some inhibitory factor to suppress the expression of colour like one of its progenitor *T. timopheevi*. Bhowal et al. [7] also reported negative colour reaction in *T. zhukovskyi*.

The colour development on the glumes was different from that of seeds as has been reported earlier [3, 12]. In none of the wild einkorn and einkorn wheats complete glume coloration was observed but different portions of the glume developed varying degree of colour, indicating the presence of different alleles. The diploid wheats, except the einkorn group, produced negative phenol reaction, suggesting that the gene(s) governing this trait are present on A genome. Out of the 70 4x wheats involving A genome only six, three each of *T. turgidum* ssp. *dicoccoides* and ssp. *carthlicum*, gave positive reaction, indicating that the gene(s) controlling this trait are not widely distributed among 4x wheats. It is interesting to note that none of the A genome progenitors expressed colour on the full glume, while a few accessions of 4x and majority of 6x wheats produced colour on full glume. Possibly, the B genome carries some enhancer(s) which activate the enzymatic reaction to produce more colour.

The results indicate that the gene(s) controlling phenol reaction were contributed to hexaploid wheats by einkorn and emmer wheats. The pattern of phenol reaction observed in 4x (AABB) and 6x (AABBDD) wheats substantiates the evidence of polyphyletic origin of wheat. Since in many 6x wheats a complete glume produced colour like that of *T. turgidum* ssp. *dicoccoides* varieties TTD 13, TTD 15 and G 3034, and ssp. *carthlicum* var. *stramineum*, and in *T. aestivum* ssp. *spelta* (grey) only the beak was black as in *T. monococcum* var. *rubiginosum*.

A perusal of Table 1 reveals that the degree of colour development in seeds differs frequently from that on glumes. For example, the seeds of *T. urartu* (2x) and *T. turgidum* ssp. *carthlicum* var. *rubiginosum* (4x) were dark brown and black, respectively, while the glumes of same species produced negative reaction. In contrast, the seeds of one accession of *T. turgidum* ssp. *turgidum* conv. *polonicum* showed no reaction, while the glumes of the same accession produced light brown colour. The results are in agreement with those [3, 12] suggesting that the gene(s) controlling phenol reactions of seeds and glumes are different and independent of each other.

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