SOME FACTORS AFFECTING THE VALUE OF PHENOL TEST USED IN WHEAT (*TRITICUM AESTIVUM* L.) IDENTIFICATION

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Abstract

The use of phenol test for wheat varietal identification should be taken with some caution where differences in grain colouration might not always give reliable comparison. The objective of the present study were to determine the effect of nitrogen and phosphorous fertilizers supplied to the mother plants, seed weight, temperature and duration of grain staining on phenol testing results. Two field experiments were conducted at El-Gemmiza experimental stations during 2001/2002 and 2002/2003 season. Each experiment included five wheat varieties namely Sakha 94, Gemmiza 7, Gemmiza 9, Gemmiza 10 and Giza 170 and three nitrogen and phosphorous fertilizer treatments. Grains harvested from each treatment were undergone to phenol test according to the International Rules for Seed Testing. The results indicated that wheat varieties were responded differently at varying levels of nitrogen and low level of nitrogen (40 kg N/Fed) was associated with relatively light colors, while high levels of nitrogen (92 kg/Fed) and phosphorous (30 kg P_2O_5 /Fed) levels with dark colors. Grain weight categories might also react differently to phenol test where large grain stained darker than small ones. Furthermore, grains produced in 2002/2003 season gave lighter colouration than those grains produced in 2001/2002 season. Low staining temperature (17-18° C) caused slow grain coloration while high temperature (26-27° C) caused over staining of grains. There was a gradual change in grain color with time and it would be useful to investigate colouration of grains at hour intervals to maximize the benefit of phenol test to distinguish between wheat varieties.

INTRODUCTION

According to the procedures outlined by the International Rules for Seed Testing (ISTA,1999), wheat varieties can be identified by visual observation of morphological characteristics in the field and grain reaction to phenol test in the laboratory. The phenol test has been used as a routine measure for purity analysis for a long time in several countries such as Sweden ; Germany ; U.K and U.S.A. The advantage of phenol test is that it serves as an easy, quick and reliable test. Biochemical studies on phenol color reaction have been shown that it involves the

enzymic oxidation of phenol through diphenols to quinones and finally to dark brown melanins. This enzyme system has variously been called monophenoloxidase or tyrosinase (Zeven, 1972). The enzyme tyrosinase uses phenol as a substrate and the seed coat of various wheat cultivars is the site of biochemical reaction giving different colors (Steen *et al.*,1986; Jensen and Legaspi 1979).This reaction appears to be inherited and controlled by a small number of genes. (Wrigley and shepherd, 1974 ; Olsen,1975). Moreover, the test has been reported to be indicative of genotype and independent of growth conditions provided grain ripens less than about 30% moisture (Wrigley and Mc Intosh,1975). In this connection, phenol color reaction has been proposed as a test for identification of wheat varieties (ISTA Rules,1999; Payne,1993 and AOSA,1991). However, several researchers have pointed out the effect of crop environment on test results. Clancy *et al.*, 1982 stated that no work has been found on phenol testing of immature seed.Steen, *et al.*,1986 recommended further studies on the date of harvest and crop location on the phenol reaction. This study was undertaken to invesigate the effect of different levels of nitrogen fertilizers supplied to the mother plant, seed weight which is related to a large extent to seed maturity, temperature and time of grain staining on phenol testing results.

MATERIALS AND METHODS

Two field experiments were carried out during two successive seasons 2001/2002 and 2002/2003, at El-Gemmiza Agricultural Research Station, Gharbia Governorate . In each season, A randomized complete block design was arranged in split plot design with four replications. The wheat varieties were allocated in the main plots and the fertilizer treatments were in the sub plots. The sub- plot area was $10.5m^2$ (3 x 3.5m). Five seed samples of five Egyptian wheat varieties namely Sakha 94, Gemmiza 9, Gemmiza 7, Gemmiza 10 and Giza 170 were grown at three different levels of nitrogen (ammonium nitrate, 33.5% nitrogen) and three different levels of phosphorous (mono calcium phosphate 15.5% P_2O_5) as follows:

N_1= 46 kg N/Fed ; N_2=69 Kg N/fed and N_3= 90 Kg/fed.

P_0= no phosphours ; P_1= 15 Kg P_2O_5 /fed and P_2= 30 kg P_2O_5 /fed

Seeds were sown in rows and the distance between rows was 30 cm by hand and ordinary agricultural practices were applied during the growing season. After harvesting the crop , the seeds were separated by hand from the shaff and phenol test was carried out on material harvested from the middle rows of each plot using

the AOSA (1991) protocol. Four replications of fifty seeds from each treatment were taken at random from the bulck of threshed ears and then placed over two layers of filter paper (Whatman 15 cm diameter) previously soaked in 5 ml of distilled water in petri dishes. The dishes were covered , allowing the seeds with the ventral crease downwards, to soak for 18 hours at 22-23C°. The seeds were removed from the distilled water and deposited on two new layers of filter paper in petri dishes and 5 ml of 1% v/v freshly made phenol solution at 4.8 pH was added . The dishes were covered and incubated at 22-23 C° for 4 hours after that the seeds were classified into five colour groups (- = negative; + = light brown ; ++ = brown ; +++ = dark brown and ++++ = black) according to the procedures outlined by (Saavedra and Laverack 1996). In addition , seed sample of each variety from the recommended treatment (N_2P_2) were taken at random and divided into three sub-samples. Four replications of 50 grains of each sub sample were undergone to three different examination to study the effect of staining time (1 to 6 hours), seed weight (large, small and ungraded) and staining temperature (16-17 ; 22-23 and 26-27°C) on grain colouration . The weight of 100 seeds was determined according to the procedures outlined by (ISTA rules 1999). other phenol testing procedures were followed as mentioned before.

RESULTS AND DISCUSSION

No significant variation was found either between seasons or between replications. Furthermore, not all grains stained similarly at a given time, but there were a few seeds less than 5% did react to phenol solution as the remaining grains (95%) which are considered in the results reported here. A visual assessment of grain colour after staining is shown in Table 1. The results showed different reaction between varieties as to the same nitrogen and phosphorous treatment. In Sakha 94 grains show no colour at low nitrogen level (N_1), whereas they showed brown colour at P_0N_2, P_0N_3, P_1N_3 levels; dark brown at P_1N_2, P_2N_2, P_2N_3.. In Gemmiza 7, grains stained dark brown at N_1 and N_3 and black at N_2 , irrespective of phosphorous levels. In Gemmiza 9, grains stained brown at N_1 nitrogen level and dark brown at N_2 and N3 nitrogen levels regardless of phosphorous treatment. In Gemmiza 10, grains stained dark brown at N_1 nitrogen level and black at N_2 and N_3 levels. In Giza 170, grains stained light brown at N_1 level and dark brown at N_2 , N_3 levels . Generally, the results show that wheat varieties behaved differently at varying levels of nitrogen and low

level of nitrogen (N_1) was associated with relatively light colours while high nitrogen and phosphorous level , with dark colour. This means that wheat varieties differed in their response to phenol solution , but there was a trend towards darker colour at N_2 level in all varieties, one exception with variety Gemmiza 7, where grains stained black at N_2 and brown at N_3 level . These results indicated that nitrogen and phosphorous treatments of wheat growing environment may affect phenol reaction of grains and the use of phenol test in routine seed and variety testing should be taken with some caution. In other words, a reliable comparison between wheat varieties require information on nitrogen and phosphorous status of mother plant environment.

Table 1. Visual assessment of phenol reaction of wheat grains from plants grown under different phosphorus and nitrogen levels.

Variety	P_0			P_1			P_2		
	N_1	N_2	N_3	N_1	N_2	N_3	N_1	N_2	N_3
Sakha 94	-	++	++	-	+++	++	-	+++	+++
Gemmiza 7	+++	++++	+++	+++	++++	+++	+++	++++	+++
Gemmiza 9	++	+++	+++	++	+++	+++	++	+++	+++
Gemmiza 10	+++	++++	++++	+++	++++	++++	+++	++++	++++
Giza 170	+	+++	+++	+	+++	+++	+	+++	+++

* Key - = negative colour ; += light brown ; ++=brown; +++= dark brown; ++++ black .

The results in table 2 show that grains of the varieties sakha 94 and Giza 170 reacted the same to phenol solution regardless of grain weight , but large grains of the Gemmiza varieties gave darker color than small and ungraded grains . This means that grain weight might react differently to phenol solution.In some cases, it is to be noted that grains produced from 2001/2002 season gave lighter coloration than those of the same varieties and weight category which have been produced in 2002/2003, (Gemmiza 10 and Giza 170). This might be explained by seasonal variation of the environment surrounded the mother plants.

Table 2. Effect of grain weight on reaction to phenol solution (1%) during 2001/2002 and 2002/2003 seasons.

Variety	100 seed weight/season					
	Large		Small		Ungraded	
	1/2002	2/2003	1/2002	2/2003	1/2002	2/2003
Sakha 94	5.94	5.98	2.19	1.99	4.30	4.43
	+	+	+	+	+	+
Gemmiza 7	6.44	6.37	3.46	3.60	5.27	5.36
	+++	+++	++	++	++	++
Gemmiza 9	6.11	6.16	2.93	2.98	4.70	4.88
	+++	+++	++	++	++	++
Gimmeza 10	5.79	5.42	2.89	2.95	4.61	4.59
	++	++	+	+	++	+
Giza 170	5.59	5.49	2.95	2.88	4.56	4.52
	++++	+++	++++	+++	++++	+++

* Key - = negative colour ; += light brown ; ++=brown; +++= dark brown; ++++ black

Table 3 shows the reaction of wheat varieties to phenol solution at hour intervals. Grains of the variety Sakha 94 did not give any reaction to phenol after staining for 2 hours and they gave light brown color after 3 till 6 hours . Furthermore, the variety Sakha 94 can be easily distinguished from other varieties at any time of staining with phenol solution. Similarly, grains of the variety Giza 170 gave brown color after an hour and they changed to dark brown and balck after 2 and 3-6 hours, respectively and therefore it can be distinguished from other varieties. On the other hand , phenol testing failed to distinguish between the varieties Gemmiza 7, Gemmiza 9 and Gemmiza 10 and this might be due to limited diversity of genetic background of these varieties. Generally, the results in Table (3) indicated that there was a gradual change of grain wheat with time and this should be considered if phenol test is included many varieties.

Table 3. Reaction of various wheat varieties to phenol solution (1%) at different time.

Variety	Staining time (hours)					
	1	2	3	4	5	6
Sakha 94	-	-	+	+	+	+
Gemmiza 7	+	++	+++	+++	++++	++++
Gemmiza 9	+	++	+++	+++	++++	++++
Gemmiza 10	+	++	+++	+++	++++	++++
Giza 170	++	++	++++	++++	++++	++++

* Key - = negative colour ; += light brown ; ++=brown; +++= dark brown; ++++ black.

Table (4) shows the effect of staining temperature on grain colouration. Low temperature of 17-18° C caused slow grain colouration , while high temperature caused overstaing of grains so that color differences between varieties at 17-18° C and 22-23° C disappeared at 26-27° C. Color differences between low temperature of 17-18° C and medium temperature of 22-23° C might be due to variation in enzyme activity at these temperatures.

Table 4. Effect of staining temperature on grain colouration of five wheat varieties.

Variety	Staining temperature		
	17-18°C	22-23°C	26-27°C
Sakha 94	++	+++	++++
Gemmiza 7	+++	++++	++++
Gemmiza 9	++	+++	++++
Gemmiza 10	+++	++++	++++
Giza 170	+++	+++	++++

*Key - = negative colour ; += light brown ; ++=brown; +++= dark brown; ++++ black.

From the previous results the use of phenol test as a routine test for varietal identification should be taken with some caution where differences between varieties may not always give reliable comparisons. Taking into account the environment of mother plant, seed weight, staining time and temperature would be helpful to obtain accurate evaluation when using phenol testing.

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بعض العوامل المؤثرة علي دقة اختبار الفينول المستخدم في تمييز أصناف القمح

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قسم بحوث تكنولوجيا البذور ، معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية ، جيزة ، مصر

توصي القواعد الدولية لفحص البذور بإجراء اختبار التميز Distinctness تحت ظروف الحقل واختبار الفينول تحت ظروف المعمل للتمييز بين أصناف القمح . ويتميز الاختبار الثاني عن الاختبار الأول بسرعة وسهولة الأجراء وانخفاض تكاليفه. وبالرغم من أن نتائج اختبار الفينول من حيث تلوين بذور صنف ما ترتبط بتركيبه الوراثي ، ألا أن دقة نتائج هذا الاختبار ربما تتأثر ببعض العوامل البيئية مثل معدلات التسميد النيتروجيني التي تضاف خلال موسم النمو ، وكذلك بعض الظروف المعملية أثناء إجراء الاختبار مثل زمن ودرجة الحرارة التي تتعرض لها البذور أثناء النقع بمحلول الفينول ، بالإضافة إلى وزن البذرة الذي يعبر إلى حد كبير عن درجة النضج . لذا أجريت تجربتان حقليتان بمحطة البحوث الزراعية بالجميزة موسمي ٢٠٠١/٢٠٠٢ ، ٢٠٠٢/٢٠٠٣ واشتملت كل تجربة علي زراعة خمسة أصناف من القمح المستنبطة حديثا هي سخا ٩٤ ، جميزة ٧ ، جميزة ٩ ، جميزة ١٠ ، جيزة ١٧٠ وثلاث معاملات سمادية للنيتروجين هي (٤٦ ، ٦٩، ٩٢ كجم نيتروجين/فدان) ، ثلاث معاملات سمادية من الفوسفور (٠ ، ١٥ ، ٣٠ كجم فوسفور/فدان) . بعد حصاد المحصول وفصل الحبوب من السنابل أخذت عينات ممثلة لكل صنف وخضعت للتقييم المعملي . أوضحت النتائج المتحصل عليها اختلاف استجابة أصناف القمح التى شملتها الدراسة لمحلول الفينول ، وبناء عليه أمكن تمييز الصنفين سخا ٩٤ ، جيزة ١٧٠ عن باقي الأصناف ، في حين تعذر تمييز الأصناف الأخرى عن بعضها بوضوح . كما أن تلوين الحبوب نتيجة التعرض لمحلول الفينول تأثر بمعدلات التسميد خاصة النيتروجيني منها والتي تضاف إثناء موسم نمو المحصول . كذلك أوضحت النتائج أهمية متابعة تلوين الحبوب علي فواصل زمنية (ساعة) بدلا من تسجيل النتائج بعد ٤ ساعات كما توصي بذلك القواعد الدولية لفحص البذور . كما دلت النتائج علي وجود تأثيرا لوزن البذور علي درجة تلوينها بمحلول الفينول. لذا توصي الدراسة باستبعاد البذور صغيرة الوزن بقدر الإمكان من العينات التي تخضع للاختبار . كذلك أشارت النتائج إلى أن درجة الحرارة المنخفضة تودي إلى بطء تلوين الحبوب بالفينول ، في حين تودي درجة الحرارة المرتفعة إلى تلوين سريع مما يصعب معه أحيانا تمييز الأصناف كما هو الحال عند درجة حرارة ٢٦/ ٢٧ م.