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LABORATORY METHODS FOR THE RECOGNITION OF SEEDS OF SOME WHEAT (*Triticum aestivum* L.) VARIETIES

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ABSTRACT

Attempts were made to find methods for distinguishing seeds of four wheat varieties (Sisd 12, Sids 13, Misr 1 and Misr 2) during 2009/2010 season. Methods such as phenolic acid, ammoncial silver nitrate and ninhydrin treatments, reputed in other crops to stain different seed cultivars with different colours were undertaken. Reaction to phenolic acid and some reagents, reputed to deal with tyrosinase enzyme were tested on grain. Other trials based on chromatography techniques were undertaken. It was found that the quickest method which produced distinct differences in colour was when seeds were set on seed test paper moistened with water and exposed to phenol vapour for different concentrations and different hours, and the colour of spots were then recorded. In another treatment seed sample was placed on seed testing paper moistened with water and then exposed to ammonical silver nitrate reagent. The spots formed by Misr 1 and sids 12 seeds became yellow and seeds of Sids 13 and Misr 2 became orange yellow. Moreover, the ninhydrin test gave the best violet spots by seed of Sids 13 and Misr 2 and light violet spots by seeds of Misr 1, whereas gave the negative reaction by seeds of Sids 12. Qualitative measure standards should be further investigated based on the present findings. It could be concluded that this tests are used for discrimination among genotypes of cereal crops such as wheat, barley and rice.

INTRODUCTION

The breeding program of a superior new variety takes great efforts and tremendous period of time. The quantity of breeder seed class produced does not usually exceed several kilograms. This quantity of the breeder seed class has to be increased through successive multiplication stages in order to produce the required quantity of seed which cover the designed area for production on a country level. The successful seed program must be designed to assure the maintenance of certain standard of purity throughout the multiplication stages.

The intensive research work for the breeding of new varieties have almost entirely used up the wild parents materials. In addition the other available parents material were involved in most of the new varieties progeny. In other words most of the new varieties chose some similar parental composition The superiority of the newly released variety depends on the maintenance of its genetic purity and avoid mechanical admixture occurrence. Furthermore, it is imperative to apply measures to keep the entity of the varieties throughout the multiplication process. This could be only achieved by recognizing the varieties with specific characteristics. With regard to the necessity to maintain varietal purity, specific characteristics must be recognized for various stages of growth. However, there are several methods for varietal purity testing. One of the laboratory methods is the chemical staining reaction of the seed variety to certain reagents.

It has been reported by different works that placing seed on moistened filter paper with water left certain colour on the paper. These papers including the spots are considered as chromatogram. Thus when exposed to reagents such as silver nitrate, ninhydrin or phenol tests produced variation either in colour or its intensity.

By definition, each cultivar of a cultivated species should differ from other cultivars in one or more characteristics. Thus, if cultivars are distinct, there should be corresponding chemical differences. To date chemistry has been little used in characterizing plant cultivars. However, the application of chemical methods, such as chromatographic, electrophoretic, serological and finger print techniques to plant taxonomy at the species level and above is becoming of increasing importance. The problem is how to elicit these differences which are often minute and quantitative rather than qualitative in nature, making their isolation and identification difficult, especially in the case of individual seeds which are the basic material for varietal purity test.

Fortunately, it was noticed during routine germination tests that a lemon yellow spot is produced under each individual seed of Misr 2 and Sids 13 on the filter paper. Taking these spots tentatively as finger print, it is decided therefore, to find out whether the seeds of both cultivars would produced different spots or react differently to chemical reagents. In addition, other methods such as ammonial silver nitrate (Block *et al.*, 1958), Payne (1993 a,b) and ninhydrin test (Alexander and Block 1960) and phenol test (Jushi and Banerjee 1970, Maguire *et al*, 1975, Niemyski and Grazelak 1975 and Gandy 1996) which were reported in other crops to color the seeds themselves were tried.

This study was carried out on each cultivar individually as a primary step in order to produce distinct differences but not to identify the chemical compounds.

MATERIALS AND METHODS

This study was carried out on wheat varieties (Sids 12, Sids 13, Misr 1 and Misr 2) during 2009/2010 season with the aim to finding out a variable method for the recognition of the cultivars. Therefore, several methods were undertaken to test these varieties. The following schedules were carried out on four samples. Each sample represented one cultivar from a given locality. Each treatment was carried out on 200 seeds of each sample divided into eight subsamples (25 seeds each).

Phenol test (carbolic acid): Four random replicates each of 25 seeds each, were placed on germination paper lining Petri dish large enough to accommodate each replicate. Phenol solutions at concentration of 0.2, 0.3, 0.4, 0.5 and 1% were used as paper moistened media. These tests were carried out at room temperature. Change in grain colour were recorded instantly at the first four hours and, then after 24 hours, according to Banerjee and Chandra, 1974.

Phenol vapour: Twenty five seeds of each subsamples of the four samples were set on one 9 cm circle of whatman filter paper, which was placed in a petri dish and moistened with distilled water as usually done for the germination test. The lids of the dishes were taken off and the bottom halves with the seeds were placed under glass bell-jars which were saturated with phenol vapour for 24, 48 or 72 hr. The phenol vapour was introduced by placing a wide shallow glass container full of phenol solution under the bell-jar during the time of the incubation period. The concentrations of phenols were 0.5, 1, 2 and 3%. All treatments to phenol vapour exposures were carried out simultaneously. Eight dishes, each with 25 seeds placed on filter paper moistened with distilled water were left covered with their lids for eight hours without exposing them to phenol vapour as control. These controls were carried out currently with exposure tests.

Chemical reagents: Seeds of each subsample were set in Petri dishes lined with filter paper and moisted with distilled water. Ten seeds of each sample were kept at 10°C and exposed to ammonical silver nitrate (0.1N silver nitrate, 5N ammonium hydroxide, 1:1 v/v), and other ten seeds of each sample were exposed to 0.3% ninhydrin in ethanol.

At the end of each incubation period the seeds were removed and the filter papers subjected to standard chemical reaction. For the sake of simplicity the filter paper and spots will be referred to from now on as the chromatogram (Gaber, 1978 as well as Gaber and Rammah 1979).

RESULTS

Effect of the liquid phenol reaction on the wheat varieties.

The colour reaction of the wheat varieties to phenol was investigated. The results presented in Table 1 revealed that by using the concentrations of 0.2, 0.3, 0.4, 0.5 and 1%, the grains of Sids 13, Misr 1 and Misr 2 took light brown colour which intensity was deepened after 1 hr. in all concentrations. This attitude was achieved at all the concentrations of phenol, after the elapse of one hour which developed to dark brown in the second hour and finally became almost black after three hours.

Table 1: The phenol reaction of wheat grain varieties at certain concentration and several intervals.

Varieties	Conc. / Time	0.2%	0.3%	0.4%	0.5%	1%
Sids 12	1 hour	-	-	-	-	-
	2 hours	-	-	-	+	+
	3hours	-	-	+	++	++
Sids 13	1 hour	+	+	++	++	++
	2 hours	++	++	+++	+++	+++
	3hours	++	++	++++	++++	++++
Misr 1	1 hour	+	+	++	++	++
	2 hours	++	++	++	++	+++
	3hours	++	++	+++	+++	++++
Misr 2	1 hour	+	+	++	++	++
	2 hours	++	++	++	++	+++
	3hours	++	++++	++++	++++	++++

*(-) indicated absence of colour , + = light brown ; ++ = brown ; +++ = dark brown ; ++++ = black

On the other hand, the grains of Sids 12 did not take coloration with the lower concentration of phenol after 1 and 2 hr. and also no coloration was achieved with the higher concentration of phenol up to three hours period of time.

Nevertheless, faint brown colour began to appear after the elapse of three hours on the grain of Sids 12 treated with the higher concentration (0.5 and 1%) of phenol.

Effect of the phenol vapour reaction of the wheat varieties.

Varieties seed began to germinate after 10 to 14 hrs under room temperature when water only was used as the germination media. On the other hand, when seeds were exposed to phenol vapour, germination did not take place during the test period. Results in Table 2 showed that phenol vapour from a 1% solution was a good producing clear spots on the paper on the varieties Sids 13 and Misr 2 after 48 hr. but gave the light spots on the varieties Misr 1 and Sids 12.

On the other hands, the phenol vapour from 2% concentration at 24 hr. gave good spots with Sids 13 and Misr 2 varieties, but gave the light spots with Sids 12 and Mise 1 varieties. Also, the phenol vapour from 0.5% at 72 hr. and 3% at 6 hrs. gave the clear spots for Sids 13 and Misr 2 varieties but gives the low spots for Misr 1 and Sids 12 varieties.

All the germination and phenol vapour treatments were consequently carried out under the ambient conditions.

Table 2: Effect of phenol vapour of wheat grain varieties.

Conc. / Varieties	0.5% / 72 hr	1% / 48 hr.	2% 24 hr.	3% / 6 hrs.
Sids 12	+	+	+	+
Sids 13	+++	+++	+++	+++
Misr 1	++	++	++	++
Misr 2	+++	+++	+++	+++

+ = light brown ; ++ = brown ; +++ = dark brown

Effect of different reagents on wheat varieties:

Chromatograms which were dipped in ammoncial silver nitrate (0.1N silver nitrate, 5N ammonium hydroxide 1:1 v/v) and 0.3 % ninhydrin in 95% ethanol, they gave ambiguous results the treatment of the grain of wheat varieties with the reagents as ammonical silver nitrate and ninhydrin indicated that the Sids 13 and Misr 2 varieties reacted with the ammonical silver nitrate reagent but the Sids 12 and Misr 1 varieties gave the light reaction with the same reagent after 24 hr. On the other hand, the sids 13 and Misr 2 varieties gave the pest violet colour with ninhydrin reagent and the Misr 1 variety gave the light violet colour with the same reagent but Sids 12 variety gave the negative reaction with the ninhydrin reagent as shown in Table 3.

Table 3: Effect of chemical reagents on the coloration of wheat grain varieties.

Reagents / Varieties	Ammonical silver nitrate	Ninhydrin
Sids 12	+	-
Sids 13	++	++
Misr 1	+	+
Misr 2	++	++

*(-) Indicated the absence colour *(+) Indicated the presence colour

DISCUSSION

Certain qualitative differences between some varieties, noted when extracts from bulked seeds or bulked plant material were chromatographed, could not be demonstrated when extracts from individual seeds or plants were used (McKee ,1973) as well as (Crisp and Wrigley,_1974).In view of this and because individual seeds are the basic material for seed testing, a chromatographic approach might not be a panacea for all problem of cultivar purity test. Nevertheless, the method tentatively called the finger print method to thought be the most suitable for characterizing the cultivars of wheat varieties under test (Mahoney and Ramsay, 1992).

The result showed that the spots on the chromatogram react positively with ninhydrin with Sids 13, Misr 1 and Misr 2, this indicated that these varieties contained certain amino acids which react with ninhydrin at this concentrations but Sids 12 variety gave negative react which indicate that this concentration under test of ninhydrin was not competent to react directly with the amino acids.

Since the discovery of the classical phenol test by Pieper (1922) it has been used to assist in distinguishing seed cultivars. The reaction of phenol takes place and the stain produced in the testa. This reaction is primarily controlled by the enzyme tyrosinase (Salih , 2009), (Menezes and Belle, 1995) and Csala (1972). In addition Salih (2009) stated that the existence of enzyme tyrosinase is a good index of drought resistance. This might help the plant breeder in the course of selection in his programs. .

Nevertheless, the spots seemed to contain oxidisable chemical compounds. Ammonical silver nitrate is reputed to be a convenient reagent for detecting easily oxidisable compounds such as many complex phenols, flavonoids and anthocyanins (Block *et al.*, 1958). The oidation reaction in the spots produced by ammonical silver nitrate differed with time. The spots of Sids 13 and Misr 2 varieties became brown earlier than the spots of Sids 12 and Misr 1, thought they both became the same colour after a period of time. Moreover, the Sids 12 variety gave negative spots with ninhydrin reagent than another varieties.

In view of the results obtained with ammonical silver nitrate and ninhydrin reagent, it is advisable to either investigate other treatments that deals specifically with individual amino acid reaction and individual types of phenols in order to be able to select more accurate methods of varietal recognition.

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طرق معملية لتمييز حبوب بعض أصناف القمح

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أجريت هذه الطرق لتمييز حبوب أربعة أصناف جديدة من القمح وهى (سدس١٢، سدس ١٣، مصر ١، مصر ٢) و ذلك خلال موسم ٢٠٠٩/٢٠١٠ وكانت الطرق المستخدمة هي المعاملة بالفينول، نترات الفضة الامونية، جوهر كشاف النهيدرين والذى يعود اختلاف اللون لحبوب المحاصيل المختلفة إلى اختلاف عملية الصبغ.

أوضحت النتائج إن أسرع طريقة هي التي تظهر اختلافات واضحة في لون الحبة المختبرة عن طريق وضع الحبوب على ورقة ترشيح مرطبة بالماء ثم تعريضها إلى بخار الفينول بتركيزات مختلفة ولمدد مختلفة ويسجل لون البقعة الناتجة. بعض الحبوب الاخرى تعرض الى جوهر كشاف نترات الفضة الامونية وقد لوحظ أن البقع المتكونة لحبوب الصنف مصر ١، الصنف سدس ١٢ كانت البقع المتكونه صفراء اللون بينما البقع المتكونة لحبوب الصنف سدس ١٣، الصنف مصر٢ كانت برتقالية مصفرة، اما بالنسبة لطريقة استخدام جوهر كشاف النهيدرين فقد سجل الصنف سدس ١٣ والصنف مصر ٢ افضل بقعة بنفسجية وسجل الصنف مصر ١ بقعة بنفسجية خفيفة بينما أعطى نتيجة سلبية لحبوب الصنف سدس ١٢.توصي هذه الدراسة أن هذه الطرق تستخدم فى تمييز اصناف محاصيل الحبوب مثل القمح والشعير والارز. وهي الطرق الأسرع و الأدق لتمييز أصناف القمح تحت الدراسة.

قام بتحكيم البحث

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