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# DETERMINATION OF GENETIC PURITY AS AFFECTED BY SEED SOURCES IN FOUR BREAD WHEAT VARIETIES

Mohamed E.S. Kammoura<sup>1\*</sup>, A.A.G. Ali<sup>2</sup>, H.A. Awaad<sup>2</sup> and M.I. Elemery<sup>1</sup>

1. Seed Technol. Res. Section, Field Crop Res. Inst. ARC, Giza, Egypt

2. Agron. Dept., Fac. Agric., Zagazig Univ., Egypt

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**ABSTRACT:** Seed categories and crop varieties play an important role in wheat production in Egypt, which effect on genetic purity of wheat genotypes. So, this investigation aimed to study the effect of seed sources and varieties on yield and its attributes. Two field experiments were conducted at the Experimental Farm of Giza Agricultural Research Center (ARC), Giza Governorate, Egypt, during two successive winter growing seasons of 2014/2015 and 2015/2016 on the optimum sowing date 15<sup>th</sup> of November. Four wheat varieties (Misr1, Gemmeiza 11, Giza 168 and Sids 12) and their seed categories (Basic, Certified and Farmer saved seeds) were used in this study. Yield and its attributes and off- type percentage were estimated in the field experiment, further more phenol color reaction and ISSR technology were carried out at Seed Technology Department Laboratory. The results indicated significant differences between wheat varieties and its seed categories for the most traits. Gemmeiza 11 variety gave the highest value in most studied traits *i.e.*, flag leaf chlorophyll content SPAD, flag leaf area, spike length, No of spikes/m<sup>2</sup>, No of grains/spike, 1000-grain weight and grain yield/fad., while the lowest value of those traits were recorded by Misr 1 variety. For the effect of seed categories on yield and yield attributes, basic seeds gave the greatest value for all characters, except for plant height where farmer seed recorded the highest value. Meanwhile insignificant effects were obtained from seed categories on flag leaf chlorophyll content SPAD. The interaction effects between varieties and seed categories indicated that Gemmeiza 11 and Sids 12 varieties with Basic and Certified seed categories gave the highest value of most traits, while Misr 1 Varity with farmer seed category gave the lowest value for yield traits. Insignificant effects were noticed between wheat varieties for the off- type % while seed categories showed highly significant effects where, Farmer saved seed recorded the highest value and Basic seed gave the lowest value for this trait. Wheat varieties showed different color reaction to phenol while seed categories took the same pattern in phenol color reaction for each variety. Eight ISSR primers were used for fingerprinting the four wheat varieties and their seed categories produced 97 band, 31 of them were polymorphic (68.04%) polymorphism. The highest level of polymorphism was observed in primer HB-12 which showed 95.00% polymorphism, while the lowest polymorphism was 28.57% in primer HB-09.

Key words: Wheat, seed category, phenol test, ISSR,

# **INTRODUCTION**

Wheat (*Triticum aestivum* L.) is the most important nutritional cereal crops in Egypt and all over the world. Increasing production per unit area appears to be one of the important factors for narrowing the gap between production and consumption. Egypt's area of wheat is estimated by about 3.4 million faddan with production of 8.9 million ton, while it imports 10 million tons of wheat (FAO STAT, 2020/ 21). Seed categories and varieties play an important role in wheat production in Egypt. Improved varieties and its seed quality are the key good agriculture progress. High degree of

<sup>\*</sup> Corresponding author: Tel. :+201225595291 E-mail address: kammoura@icloud.com

genetic variability was recorded between five wheat genotypes *i.e.*, Gemmeiza 7, Sakha 93, Giza 168, Gemmeiza 9 and Sids13 in flag leaf area, plant height, spike length, number of grains/spikes, 1000-grain weight and biological yield/plant. Not enough foundation (basic) seed could be produced to sowing all the areas allocated to production of registered seed (Attia et al., 2015). Therefore, the genetic composition of the variety initially developed by the breeders must be the same as marketed to the grower after several generations. It means that genetic purity of varieties must be found in every class of seed certification program which included breeder seed, basic seed, registered seed and certified seed (Amal, 2004). Khan et al. (2007) found that pre-basic category of Bakhtawar-92 obtained from CCRI, Pirsabak gave the maximum spikes/m<sup>2</sup> (347.3), grains/spike (52.30), 1000grain weight (52.75 g), grain yield (4931 kg ha-1), followed by basic and certified categories of the same cultivars and location. Grain yield and genetic purity based on number of off-types and seed fingerprint were significantly reduced from planting farmer-saved seeds compared with basic, registered and certified seeds. So, it is possible to utilize farmer-saved seed for one year of certified seed provided that the farmer takes out the off-types from the part of the field and keep it for next planting (El-Kalla et al., 2010). Muhammad et al. (2012) indicated that number of fertile tillers/ m<sup>2</sup> were significantly higher in certified seed source than the farmer's own seed that resulted in significant increase in grain yield. Other yield contributing parameters including number of spikelet/spikes, numbers of grains/spike and 1000 grain weight were at par in both seed sources. Therefore, healthy and pure seed source gave high seed germination and good crop stand which enabled the plants to withstand abiotic stress especially drought during the crop season. The average physical purity of seed obtained from the formal sector was 99.41% compared with seed obtained from the informal sector, e.g., neighbors/other farmers, traders / markets, or own saved seed. The formal sector seed had the highest analytical purity and lowest mean contamination of other crop seeds and noxious weeds where all samples met the standard for certified seed (Bishaw et al., 2012). Arif et al. (2015) suggested that there is the need for strengthening agricultural extension services in the study area in order to educate the farming community to ensure the use of recommended certified seed and modern agriculture technology for getting high yield of wheat crop.

Generally, there was virtually no difference about physiological quality between certified seed and farm-saved seed sector that is related to lower quality of certified seed. The certified produced seeds had the lower number of weed seed, species and genus before and after cleaning. The highest seed purity and 1000 seed weight was obtained from the certified seed production system. The need for cleaning the farm-saved seed samples before sowing is one of the important findings of this survey (Khazaei et al., 2016). Meanwhile, Duric et al. (2016) indicated that genotype PKB Lepoklasa and seed category pre-basic seed had the highest values for the largest number of yield traits, while the genotype BG Merkur and the certified seed of the first generation had the lowest values. Furthermore, Certified seed categories of the first generation and basic seed had similar reactions to the interaction between factors. The use of uncertified seed by farmers contributed to high disease incidence (Tenge et al., 2016). Adinew and Mohmmed (2019) showed that the interaction of seed source and variety had significant effect on all physical and physiological seed quality parameters. Alemu (2019) found that seed source influenced growth and yield during the growing season but the response depended on seed size and variety. The source of seed can also be important since location influences seed nutrient content. Number of tillers were significantly affected by main effect of variety, seed source and seed rate. Interaction effect of variety and seed source has highly significant effect on number of tillers per 1 m length.

Survey off-types in certified and farm-saved seed samples to compare varietal purity based on type of seed producer and IR wheat history was performed by **Gaines** *et al.* (2007). Ninetytwo samples of non-IR varieties were taken from certified and farm-saved seed growers. One certified sample and three farm-saved samples exceeded the 0.1% threshold for off-types in certified wheat seed. Laboratory testing including phenol test of the seed came the same conclusion. **Squires** *et al.* (2014) implied three primary sources for seeds of off-type colour *i.e.*, admixtures of genetically different varieties, true-to-type varieties and the last source of

variation was attributed to unknown environmental interactions in genetically pure samples. A variety of phenol reactions among the wheat species having different genomes were detected by (Niranjana et al., 2018). Pavlic et al. (2018) used phenol method according to UPOV (1994) guidelines for DUS testing on 40 different wheat genotypes. Grain color was determined according to the coloration grade, on a scale from 0 to 9. All genotypes showed certain degree of pericarp coloration. Coloration grades were from 1 (one genotype) to 9 (12 genotypes), 32.5% of genotypes were scored as medium low to very low coloration of grain.

ISSR have been successfully used to assess the genetic diversity of wheat varieties. The relationships estimated genetic by the polymorphism of ISSR markers revealed greater level of genetic variability in wheat accessions of wide adaptability and applicability (Singh and Jaiswal, 2016; Heidari et al., 2017; Mandoulakani et al., 2017). The present investigation aimed to evaluate seed degree (Basic-Certified and Farmer-saved seed) of four varieties under field and laboratory conditions and determine the importance of seed source and to assure the quality seed.

### MARTIALS AND METHODS

Two field experiments were conducted at the Experimental Farm of Giza Agricultural Research Center (ARC), Giza Governorate, Egypt, during the two successive winter growing seasons of 2014/2015 and 2015/2016 on the optimum sowing date 15<sup>th</sup> of November.

The field experiments were laid out in split plot design with four replications. The main plots were devoted to four wheat varieties *i.e.*, Misr1, Gemmeiza11, Giza168 and Sids12 and the sub plots devoted to three seed categories or classes i.e. (Basic, Certified and Farmer-Saved seed). Sup plot area was  $3.6 \text{ m}^2$  contain 6 rows, 3 m long; raw to raw was 20 cm. The soil was clay in texture. All recommended cultural practices were applied. Random sample of ten guarded plants were taken from each sup plot to estimate; morpho-physiological characters i.e., flag leaf chlorophyll content (SPAD) using chlorophyll meter (SPAD-502 Soil Plant Analysis Development (SPAD)Section Minolta Camera Co, Oska, Japan), flag leaf area cm<sup>2</sup>, plant height (cm) and spike length (cm). Yield and its components *i.e.*, number of spikes/ $m^2$ , number of grains/spike and 1000- grain weight (g) was estimated. Grain yield (Kg/fad) was determined from a center area (4 rows) of each sub plot.

### **Off Type Percentage**

was estimated by divided the number of offtype plants by the total number of plants of every seed category for the studied varieties following the standard of the Seed Testing and certification administration, Ministerial Decree, 368 (1998),

### **Phenol Color Reaction**

Four random replicates, each of 100 grains, were placed on germination paper lemming Petri dish large enough to accommodate each replicate. Phenol solution at 1% was used as watering media. Change in grains color was recorded at one, two, three and four hours, according to **Banerjee and Chandra (1974)**.

# Analysis of Inter Simple Sequence Repeats (ISSR)

The Genomic DNA was extracted using DNeasy Plant Mini Kit (QIAGEN) from young leaves. The PCR was performed using a set of eight primers which are presented in Table 2. The reaction was performed in 30-µl volume tubes according to Williams et al. (1990). The reaction mixture included 3 µl dNTPs, 3 µl MgCl<sub>2</sub>  $3\mu$ l Buffer (10 x), 2  $\mu$ l primer (10 pmol), 0.2µl Taq DNA polymerase (5U/µl), 2 µl Template DNA (25 ng) and 16.8  $\mu$ l H<sub>2</sub>O (D.W.). The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94° C. 1 min at 57° C, and 2 min at 72° C, and the reaction was finally stored at 72° C for 10 min.

Agarose (1.50 gm) was mixed with (100 ml)  $1 \times TBE$  buffer and boiled in the microwave. The ethidium bromide (5 µl) was added to the melted gel after the temperature became 55°C. DNA amplified product (15 µl) was loaded in each well. DNA ladder (100 bp) mix was used as standard DNA with molecular weights of 3000, 1500, 1000, 900, 800, 700,600, 500, 400, 300, 200 and 100 bp. The run was performed for about 30 min at 80 V in a mini-submarine gel Bio-Rad.

	8	-	
	Breeder	Basic	Certified
Off types allowable	0 %	0 %	1 %
Percentage %	0: 10.000	0: 10.000	1: 100

Table 1. Allowable off- types in different seed categories according to the Ministerial Decree No.368 of 1998 setting seed testing standards and procedures for certification

Table 2. List of ISSR primers and their nucleotide sequences used in the study

No.	Name	Sequence	No.	Name	Sequence
1	14A	5'-CTCTCTCTCTCTCTCTCTG-3`	5	HB-10	5'-GAGAGAGAGAGACC-3`
2	44B	5'-CTCTCTCTCTCTCTCTGC-3`	6	HB-12	5'-CACCACCACGC-3`
3	HB-8	5'-GAGAGAGAGAGAGAGG-3`	7	HB-13	5'-GAGGAGGAGC-3`
4	HB-9	5'-CACCACCACGC-3`	8	HB-15	5'-GTGGTGGTGGC-3`

# **Data Analysis**

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among varieties as revealed by dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among genotypes (**Yang and Quiros, 1993**).

All characters were subjected to analysis of variance (ANOVA) to test the significance of variance sources using XLSTAT2016.05. Combined analyses between the two seasons were conducted, and the least significant difference (LSD) values were calculated at the 5% and 1% probability levels (Steel and Tonle, 1997).

# **RESULTS AND DISCUSSION**

### **Morpho-Physiological Characters**

The statistical analysis of flag leaf chlorophyll content (SPAD), flag leaf area, plant height and spike length for wheat varieties during the two seasons and combined are listed in Tables 3 and 4.

### **Effect of varieties**

Statistical analysis of variance revealed highly significant differences between varieties for morpho-physiological characters. These results indicated the presence of adequate amount of genetic differences between wheat varieties for these traits. Sids 12 variety recorded the greatest value of flag leaf chlorophyll content (SPAD) followed by Gemmeiza 11 in both seasons and its combined (Table 2), while the lowest flag leaf chlorophyll content (SPAD) was recorded by Misr 1 then Giza 168. Whereas the latest " Giza 168" variety had the broadest flag leaf area (cm<sup>2</sup>) followed by Gemmeiza 11 and Sids 12 varieties in the two seasons and it's combined without any significant differences between them, while the narrower flag leaf area (cm<sup>2</sup>) was recorded by Misr1 (Table 2). Awaad et al. (2010) and Abaza et al. (2020) recorded significant difference between some wheat varieties in respect to flag leaf chlorophyll content. Attia et al. (2015) found significant differences in flag leaf area between five wheat varsities. The maximum flag leaf area was produced from Sakha 93 (24.00 cm<sup>2</sup>), but the lowest flag leaf area (14.68 cm<sup>2</sup>) was resulted from Sids 13.

Gemmeiza 11 appeared to produce taller plants and longer spikes in both seasons and its combined. Meanwhile, Sids 12 was the shortest plant height and Misr 1 behaved the same in spike length (cm) through the two seasons and their combined (Table 3) compared with the other evaluated wheat varsities. Abd El-Rahman and El-Saidy (2016) indicated significant differences between wheat genotypes in plant height, and Sids 12 was the shortest compared

with other genotypes. Attia *et al.* (2015) found significant differences between wheat genotypes Gemmeiza 11, Sakha 93, Giza 168, Gemmeiza 9 and Sids13 in spike length.

### Effect of seed categories

As shown in Table 3 results indicated insignificant differences between seed categories in both seasons and the combined for flag leaf chlorophyll content (SPAD). Meanwhile basic seeds category achieved the broadest value of flag leaf area (cm<sup>2</sup>) followed by certified seed category, while farmer seed category had the lowest one for flag leaf area  $(cm^2)$  in both season and their combined. Otherwise, Khazaei et al. (2016) reported that no differences about physiological quality between certified seed and farm-saved seed sector that was related to the lower quality of certified seed. Moreover, the highest plant height was observed in farmer seed categories, while the shortest one was recorded by basic and certified categories without any significant differences between them (Table 4).

The attained results in Table 4 displayed significant differences between seed categories in spike length in the 1<sup>st</sup> season and highly significant differences in the 2<sup>nd</sup> one and the combined. Basic seeds had the longest spike length in two seasons and it's combined without significant differences with certified seeds in the 1<sup>st</sup> season, while the farmer seeds category had the shortest spike length in the two seasons and their combined. **Shahwani** *et al.* (2014) stated that plant height and spike length were affected significantly by seed size.

### **Effect of interaction**

Regarding the effect of interaction between wheat varieties and seed categories on flag leaf chlorophyll content (SPAD), highly significant effects were noticed in the 1<sup>st</sup> season, while it was insignificant in the 2<sup>nd</sup> one and the combined analyses.

In the light of highly significant interaction effects between wheat varieties and seed categories in the 1<sup>st</sup> season on chlorophyll content, results given in Table 3-a indicated that Sids 12 variety with all wheat seed categories and Gemmeiza 11 variety with basic and certified seed categories gave the higher value of flag leaf chlorophyll content. On the other hand, the lower value of flag leaf chlorophyll content was obtained by Giza 168 with certified and farmer saved seed categories.

Highly significant interaction was found between wheat varieties and their seed categories for flag leaf area in the 1<sup>st</sup>, 2<sup>nd</sup> seasons and their combined. As given in Table 3-a during the 1<sup>st</sup> season, Gemmeiza 11 and Sids 12 varieties with basic seed category gave broadest flag leaf area  $(61.98 \text{ cm}^2)$  and  $(60.90 \text{ cm}^2)$ , respectively. Whereas, Misr1 with farmer seed category gave the narrower flag leaf area (47.85 cm<sup>2</sup>). In the 2<sup>nd</sup> season (Table 2-b), the higher value of flag leaf area (55.98  $\text{cm}^2$ ), (54.90  $\text{cm}^2$ ) and  $(54.53 \text{ cm}^2)$  were achieved by Gemmeiza 11, Sids 12 and Giza 168 varieties with basic seed category, respectively. On the other direction, the lowest value of flag leaf area (41.85 cm<sup>2</sup>) was recorded by Misr1 with farmer seed category. Based on the combined (Table 2b), the higher estimates of flag leaf area 58.98, 57.90 and 57.53  $\text{cm}^2$  were attained by Gemmeiza 11, Sids 12 and Giza 168 varieties with basic seed category, respectively. Whereas the lowest flag leaf area 44.85 cm<sup>2</sup> was registered by Misr 1 with farmer seed category.

Highly significant interaction between the two studied factors in two seasons and its combined were found on plant height. During the 1<sup>st</sup> season, as illustrated in Table 3-a, the tallest plant height 109.65 cm was achieved by Gemmeiza 11 with farmer seed category, while the shortest plant height (93.13 cm) was found by Sids 12 with farmer seed category. In the 2<sup>nd</sup> season (Table 4-a), the tallest plant height (125.97 cm) has been achieved by Gemmeiza 11 with farmer seeds. On the other hand, the shortest plant height (102.70 cm) was recorded by Sids 12 with farmer seed category. Based on combined analyses, results of Table 3-a revealed that the tallest plants (115.76 cm) were registered by Gemmeiza 11 with farmer seed category, whereas the shortest plants (96.76 cm) were registered by Sids 12 with same category.

Spike length display insignificant interaction in the two seasons and significant in the combined (Table 4-b). According to combined analysis (Table 4-b), the longest spike length 14.19 cm and 13.59 cm were achieved by Gemmeiza 11 and Sids 12 varieties with basic seed category. While, the shortest spike length (10.90 cm) was recorded by Misr 1 variety with farmer seed category, this result refers to the deterioration happened in seed propagation process.

Main effect and	Flag leaf chl	orophyll con	tent (SPAD)	F	Flag leaf area (cm <sup>2</sup> )			
interaction	2014-2015	2015-2016	Combined	2014-2015	2015-2016	Combined		
Varieties								
Misr 1	46.85 c	47.19 b	47.02 c	52.52 c	46.52 c	49.52 c		
Gemmeiza 11	50.41 b	50.11 a	50.26 b	57.58 ab	51.58 ab	54.58 ab		
Giza 168	46.44c	45.99 b	46.22 d	58.41 a	52.41 a	55.41 a		
Sids 12	53.02 a	51.78 a	52.40 a	57.01 b	51.01 b	54.01 b		
F- test	**	**	**	**	**	**		
Seed categories								
Basic seed	49.48	49.46	49.47	60.28 a	54.28 a	57.28a		
Certified seed	49.44	48.56	49.00	55.54 b	49.54 b	52.54b		
Farmer seed	48.61	48.29	48.45	53.32 c	47.32 c	50.32c		
F-test	NS	NS	NS	**	**	**		
Interaction	**	NS	NS	**	**	**		

Table 3. Mean performance of flag leaf chlorophyll content (SPAD) and flag leaf area (cm²) for<br/>some wheat varieties under three seed categories during two successive seasons (2014-<br/>2015 and 2015-2016) and their combined

NS and \*\* indicated insignificant and significant at 0.01 level of probability, respectively.

Table 3-a. Flag leaf chlorophyll content (SPAD) and flag leaf area (cm<sup>2</sup>) as affected by the interaction between wheat varieties and seed categories (1<sup>st</sup> season)

	Flag l	eaf chlorophyll (1 <sup>st</sup> seas	l content ( son)	Flag leaf area (cm <sup>2</sup> ) (1 <sup>st</sup> season)				
Varieties Seed categories	Misr1	Gemmeiza 11	Giza 168	Sids 12	Misr1	Gemmeiza	11 Giza 168	Sids 12
Basic seed	В	А	В	А	С	А	В	AB
	47.00 a	52.13 a	47.08 a	51.73 b	57.70 a	61.98 a	60.53 a	60.90 a
Certified seed	В	А	В	А	С	А	В	А
	46.55 a	52.00 a	46.13 a	53.10 a	52.00 b	58.03 b	55.20 b	56.93 b
Farmer seed	В	В	В	А	С	В	А	В
	47.00 a	47.10 b	46.13 a	54.23 a	47.85 c	52.73 c	59.50 a	53.20 c

Table 3-b.Flag leaf area (cm<sup>2</sup>) as affected by the interaction between wheat varieties and seed categories (2<sup>nd</sup> season and combined)

	Fla	Flag leaf area (cm <sup>2</sup> ) (2 <sup>nd</sup> season)				Flag leaf area (cm <sup>2</sup> ) (combined)			
Varsities Seed categories	Misr1	Gemmeiza 11	Giza 168	Sids 12	Misr1	Gemmeiza 11	Giza 168	Sids 12	
Basic	С	А	А	AB	С	А	В	В	
	51.70 a	55.98 a	54.53 a	54.90 a	54.70 a	58.98 a	57.53 a	57.90 a	
Certified	С	А	В	А	D	А	С	В	
	46.00 b	52.03 b	49.20 b	50.93 b	49.00 b	55.03 b	52.20 c	53.93 b	
Farmer Seeds	С	В	А	В	D	С	А	В	
	41.85 c	46.73 c	53.50 a	47.20 c	44.85 c	49.73 c	56.50 b	50.20 c	

Main effect and	Р	lant height (c	em)	SI	Spike length (cm)			
interaction	2014-2015	2015-2016	Combined	2014-2015	2015-2016	Combined		
Varieties								
Misr 1	102.88 a	111.62 b	107.25 b	11.17 d	11.16 c	11.16 c		
Gemmeiza 11	106.00 a	117.13 a	111.57 a	13.75 a	13.56 a	13.65 a		
Giza 168	98.38 c	107.23 c	102.81 c	12.29 c	12.14 b	12.22 b		
Sids 12	96.28 d	104.50 d	100.39 d	13.03 b	13.63 a	13.33 a		
F- test	**	**	**	**	**	**		
Seed categories								
Basic seed	100.37 b	109.27 b	104.82 b	12.62 ab	13.13 a	12.87 a		
Certified seed	99.49 b	108.66 b	104.08 b	12.75 a	12.51 b	12.63 b		
Farmer seed	102.81 a	112.42 a	107.61 a	12.31 b	12.23 b	12.27 c		
F- test	**	**	**	*	**	**		
Interaction	**	**	**	NS	NS	*		

Table 4. Mean performance of plant height(cm) and spike length (cm) for some wheat varieties under three seed categories during two successive seasons (2014-2015 and 2015-2016) and their combined

NS, \* and \*\* indicated insignificant, significant at 0.05 and 0.01 levels of probability, respectively.

Table 4-a. Plant height (cm) as affected by the interaction between wheat varieties and seed categories (1<sup>st</sup>, 2<sup>nd</sup> season and combined)

	Plant height (1 <sup>st</sup> season)					Plant height (2 <sup>nd</sup> season)			I	Plant height (combined)		
Varieties	Misr1	Gemmeiza 11	Giza 168	Sids 12	Misr1	Gemmeiza 11	Giza 168	Sids 12	Misr1	Gemmeiza 11	Giza 168	Sids 12
Seed categories												
Basic seed	А	А	В	В	А	А	В	В	А	А	С	В
	105.10 a	103.53 c	95.95 b	96.90 b	117.91 a	116.87 b	106.73 b	107.90 a	109.46 a	108.72 c	100.01 b	101.10 a
Certified seed	В	А	С	В	BC	А	С	В	В	А	С	В
	98.15b	104.83 b	96.15 b	98.83 b	108.42 b	118.69 b	196.34 b	109.07 a	102.54 b	110.21 b	100.24 b	103.31 a
Farmer Seed	В	А	С	D	В	А	С	D	В	А	С	D
	105.40 a	109.65 a	103.05 a	93.13 c	118.04 a	125.97 a	115.18 a	102.70 b	109.75 a	115.76 a	108.17 a	96.76 c

Table 4-b. Spike length (cm) as affected by the interaction between wheat varieties and seed categories (combined)

	Varieties	Misr1	Gemmeiza 11	Giza 168	Sids 12
Seed categories	5				
Basic seed		D	А	С	В
		11.33 a	14.19 a	12.32 a	13.59 a
Certified seed		D	А	С	В
		11.26 ab	13.88 b	12.09 b	13.30 b
Farmer seed		С	А	В	А
		10.90 b	12.90 c	12.18 ab	13.10 b

# Yield and its Components

The statistical analysis of variance for wheat grain yield and its components for wheat varsities, their seed categories and interaction between them are tabulated in Tables 5 and 6.

## Effect of varieties

The results show insignificant during the two seasons and significant differences between wheat varieties for No. of spikes/m<sup>2</sup> in the combined (Table 5). Giza 168 variety achieved the greatest No. of spikes/m<sup>2</sup> followed by Gemmeiza 11 and Misr 1 varieties without significant differences between them, while Sids 12 recorded the lowest No. of spikes/m<sup>2</sup> in the combined. Therefore, wheat varieties differed in genes controlling No. of spikes/m<sup>2</sup>.

Whereas, highly significant differences were detected between wheat varieties for No. of grains/spike during the two seasons and their combined. Giza 168 and Sids 12 varieties had greater No. of grains/spike without significant differences between them, whereas Misr 1 had the lower No. of grains/spike in the two seasons and their combined (Table 5).

As shown in Table 6, insignificant differences between wheat varieties were noticed in the 1<sup>st</sup> season, while, significant and highly significant differences were recorded between varieties in  $2^{nd}$  season and combined for 1000-grain weight. Sids 12 variety recorded the heaviest 1000grain weight (gm) followed by Gemmeiza 11 variety without any significant differences between them and then Misr 1, while Giza 168 variety gave the lightest 1000- grain weight (gm) in  $2^{nd}$  season and combined. These results matching with **Duric** *et al.* (2016) and Abd El-**Rahman and El-Saidy** (2016) who found significant differences between genotypes for this trait.

For grain yield/fad., Table 6 results revealed that highly significant effects between wheat varieties were noticed in the 1<sup>st</sup> season. Hereby, wheat varieties varied in genes controlling grain yield. Gemmeiza 11 and Sids 12 gave the greatest grain yield/fad., with values of 2063.32 and 2034.89 kg/fad., respectively. It is interest to note that Sids 12 showed superiority in grain yield, and each of the two components *i.e.*, No. of grains/spike and 1000-grain weight. While

Misr 1 and Giza 168 gave the lowest grain yield/ fad., valued 1899.49 and 1950.59, respectively. On the other hand, no significant effects between wheat varieties were recorded in the 2<sup>nd</sup> season and combined for this trait. Whereas, significant genetic variation was observed for wheat grain vield and its components by Khan et al. (2007) and Duressa and Ayana (2020) and Awaad (2021). Al-Otayk (2019) displayed significant variation for number of spikes/m<sup>2</sup>, kernels spike<sup>-1</sup>, 1000-kernel weight, grain yield and straw yield. The genotypes AC-3, AC-5 and BS-1 had higher grain yield and stable in performance across seasons. Abd El-Rahman and El-Saidy (2016) indicated that Giza 168 recorded the greatest No. of spikes/ $m^2$  among the studied wheat genotypes. Cvetkovic et al. (2016) reported genetic differences between genotypes for No. of grains/spike, and the greatest number of grains per spike was obtained by cultivar Etida (54.8) and lowest in Zvezdana (51.4).

### Effect of seed categories

As shown in Tables 5 and 6, the results showed significant or highly significant differences between seed categories through the two seasons and it's combined for No. of spikes /m<sup>2</sup>, 1000-grain weight (gm) and grain yield Kg/fad. Whereas, insignificant and highly significant differences were detected between seed categories through 1<sup>st</sup> season and both of the 2<sup>nd</sup> season and combined in No. of grains/spike, respectively.

Generally, the greatest No. of spikes/m<sup>2</sup> and No. of grains/spike, 1000- grain weight (gm) and grain yield kg/fad were achieved by basic seed category then certified seed category, while the farmer seed category had the lowest values in grain yield and its components in two season and its combined. Muhammed et al. (2012) indicated that number of fertile tillers/  $m^2$  were significantly higher in certified seed source than the farmer's own seed that resulted in significant increase in grain yield. Other yield contributing parameters including numbers of grains/ spike and 1000- grain weight were at par in both seed sources. It was concluded that pure seed source gave the best yield attributes. Whereas, Tyagi et al. (1985) found that farmer's seeds gave the lowest yield consistently. Duric et al. (2016) indicated that seed category pre-basic seed had

Table 5.	Mean performance of No. of spikes/m <sup>2</sup> and No. of grains/spike for some wheat varieties
	under three seed categories during two successive seasons (2014-2015 and 2015-2016)
	and their combined

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Main effect and	Ν	lo. of spikes/m	2	No	. of grains/sp	pike
interaction -	2014-2015	2015-2016	Combined	2014-2015	2015-2016	Combined
Varieties						
Misr 1	327.83	331.28	329.56 ab	48.27 c	43.73 b	46.00 b
Gemmeiza 11	365.00	338.51	351.75 a	52.58 b	44.13 b	48.35 b
Giza 168	345.50	374.78	360.14 a	54.10 ab	53.35 a	53.73 a
Sids 12	307.50	326.23	316.87 b	57.74 a	49.51 a	53.63 a
F- test	N. S	N. S	*	**	**	**
Seed categories						
<b>Basic seed</b>	362.00 a	371.67 a	366.83 a	54.24	52.42 a	53.33 a
Certified seed	327.63 b	344.66 b	336.14 b	54.12	47.87 b	50.99 b
Farmer seed	319.75 b	311.77 с	315.76 c	51.15	42.75 c	46.95 c
F – test	**	**	**	N.S	**	**
Interaction	*	N.S	N.S	NS	N.S	N.S

N.S, \* and \*\* indicated insignificant and significant at 0.05 and 0.01 levels of probability, respectively.

categor	categories (1 season)									
	Varieties	Misr1	Gemmeiza 11	Giza 168	Sids 12					
Seed categories										
Basic seed		В	А	В	С					
		358.00 a	434.50 a	342.50 a	313.00 a					
Certified seed		AB	AB	AB	В					
		331.00 ab	330.50 b	352.00 a	297.00 a					
Farmer seed		В	AB	А	А					
		294.50 b	330.00 b	342.00 a	312.50 a					

Table 5-a. No. of spikes/m<sup>2</sup> as affected by the interaction between wheat varieties and seed categories (1<sup>st</sup> season)

Table 6. Mean performance of 1000- grain weight and grain yield (kg/fad) for some wheat<br/>varieties under three seed categories during two successive seasons (2014-2015 and<br/>2015- 2016) and their combined

Main effect and	1000- graiı	n weight (g)		Grain yield	l (kg/fad)	
interaction	2014-2015	2015-2016	Combined	2014-2015	2015-2016	Combined
Varieties						
Misr 1	48.17	46.94 bc	47.55 b	1899.49 b	2701.40	2300.45
Gemmeiza 11	50.48	51.15 ab	50.81 ab	2063.32 a	2700.28	2381.80
Giza 168	45.24	43.96 c	44.60 b	1950.59 b	2903.42	2427.01
Sids 12	50.98	54.73 a	52.85 a	2034.89 a	3317.50	2676.20
F- test	N. S	*	**	**	N.S	N. S
Seed categories						
<b>Basic seed</b>	51.64 a	52.33 a	51.99 a	2172.95 a	3232.40 a	2702.68 a
Certified seed	48.38 b	50.25 a	49.32 a	2064.13 b	2967.70 b	2515.92 b
Farmer seed	46.12 b	44.99 b	45.56 b	1724.12 c	2516.85 c	2120.49 c
F – test	**	*	**	**	**	**
Interaction	N.S	N.S	N.S	*	N.S	N.S

NS, \* and \*\* indicated insignificant and significant at 0.05 and 0.01 levels of probability, respectively

	Varieties	Misr1	Gemmeiza 11	Giza 168	Sids 12
Seed categories					
Basic seed		В	А	В	А
		2046.10 a	2286.26 a	2091.33 a	2268.11 a
Certified seed		В	AB	В	А
		1989.43 a	2083.68 b	1995.73 a	2187.69 a
Farmer seed		BC	А	AB	С
		1662.93 b	1820.01 c	1764.70 b	1648.87 b

# Table 6-a. Grain yield kg/fad as affected by the interaction between wheat varieties and seed categories (1<sup>st</sup> season)

the largest number of yield traits. Akhter *et al.* (2015) indicated that farmers who had access to certified wheat seed were able to achieve higher crop yields, higher income, and lower poverty as compared to farmers having no access to certified wheat seed. Also, **Tenge** *et al.* (2016) reported that use of uncertified seed by farmers contributed to high disease incidence.

### **Effect of interaction**

As presented in Table 5-a, results indicated significant effects in the  $1^{st}$  season and insignificant in the  $2^{nd}$  one and combined between the two studied factors on No. of spikes/m<sup>2</sup>, while it was insignificant during two seasons and it's combined on No. of grains/ spike, 1000- grain weight as well as significant interaction effects were noted regarding grain yield (kg/ fad) in the  $1^{st}$  season, and insignificant interaction effects in the  $2^{nd}$  one and the combined.

Respecting the 1<sup>st</sup> season, results in Table 5-a revealed that the highest value of No. of spikes/m<sup>2</sup> (434.50) was achieved by Gemmeiza 11 with basic seed category, while the lowest value for No. of spikes/m<sup>2</sup> (294.50) was recorded by Misr 1 with farmer seed category.

Interaction effects between wheat varieties and their seed categories in the 1<sup>st</sup> season for the grain yield (Table 6-a), revealed that higher grain yield (kg/fad.) was obtained by Gemmeiza 11 and Sids 12 varieties with basic seed category. While the lower grain yield (kg/fad.) was recorded by Misr1 and Sids 12 varieties with farmer seed category. In this manner, Khan et al. (2007) found that pre-basic category of Bakhtawar-92 obtained from CCRI, Pirsabak gave the maximum spikes  $m^2$  (347.3), grains/ spike (52.30), 1000-grain weight (52.75 g), grain yield (4931 kg ha), followed by basic and certified categories of the same varieties and location. Grain vield and genetic purity based on number of off-types and seed fingerprint were significantly reduced from planting farmersaved seeds compared with basic, registered and certified seeds. In continuous, Adinew and Mohmmed (2019) recorded significant interaction between seed source and wheat variety on grain yield/ha. Otherwise, Duric et al. (2016) indicated insignificant effects of the interaction between seed categories and varieties on 1000grain weight, but significant interaction on No. of grains/spike.

### **Specific Characters**

### **Off- type parentage**

Off- type percentage as affected by wheat varieties and seed categories in addition the interaction between them during both seasons and their combined are presented in Table 7.

# **Effect of varieties**

Data presented in Table 7 indicated insignificant effects between wheat varieties for off-type trait during the both seasons and the combined.

### Effect of seed categories

Regarding the effect of seed categories on off- types percentage, the results in Table 7 indicated highly significant influenced of the off- types percentage by seed categories in both seasons. Farmer seed gave the maximum offtypes percentage (3.62 and 3.25) in both seasons. On contrast, basic seed category provided the minimum off-types percentage (1.68 and 1.56) in both seasons. These results reflect the good yield measurements recorded by the basic seed category during this study. Meanwhile, El-Kalla et al. (2010) indicated that grain yield and genetic purity based on number of off-types and seed fingerprint were significantly reduced from planting farmer-saved seeds compared with basic, registered and certified seeds. So, it is possible to utilize farmer-saved seed for one year of certified seed provided that the farmer takes out the off-types from the part of his field he keeps for next planting. Squires et al. (2014) implied three primary sources for seeds of offtype colour *i.e.*, admixtures of genetically different varieties, true-to-type varieties and the last source of variation was attributed to interactions unknown environmental in genetically pure samples.

### **Effect of interaction**

Insignificant interaction effects were found between wheat varieties and seed categories on off- types percentage as obvious in Table 7.

### Phenol color reaction test

Results of phenol test in Table 8 showed that phenol color reaction was diverse between varieties under different times. Misr 1 and Gemmeiza 11 varieties took the light brown color after the 1<sup>st</sup> hour and then the brown color

Main effect and interaction		Off- type percentage	2
	2014-2015	2015-2016	Combined
Varieties			
Misr 1	2.58	2.25	2.41
Gemmeiza 11	2.08	2.08	2.08
Giza 168	2.33	2.08	2.20
Sids 12	2.75	2.25	2.29
F- test	NS	NS	NS
Seed categories			
<b>Basic seed</b>	1.68	1.56	1.62
Certified seed	2.00	1.86	1.84
Farmer seed	3.62	3.25	3.28
F - test	**	**	**
Interaction	N.S	N.S	N.S

Table 7. Mean performance of off- type percentage for some wheat varieties under three seed<br/>categories during two successive seasons (2014-2015 and 2015-2016) and their<br/>combined

NS, \* and \*\* indicated insignificant and significant at 0.05 and 0.01 levels of probability, respectively

Table 8.	Effect	of	phenol	reaction	of	wheat	varieties	and	their	seed	categories	using	1%
	concen	trat	tion und	er one, tw	7 <b>0, t</b>	hree an	nd 4 hours						

Time		1 hour		2 hours			3 hours			4 hours		
Varsities	Basic seed	Certified seed	Farmer seed	Basic seed	Certified	Farmer seed	Basic seed	Certified seed	Farmer seed	Basic seed	Certified seed	Farmer seed
Giza 168	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Misr 1	+	+	+	++	++	++	+++	+++	+++	++++	++++	++++
Gemmeiza 11	+	+	+	++	++	++	+++	+++	+++	++++	++++	++++
Sids 12	-	-	-	-	-	-	-	-	-	+	+	+

- indicate absence color + indicate light brown ++ indicate brown +++ indicate dark brown ++++ indicate very dark brown

after the  $2^{nd}$  hour and then dark and very dark brown after the  $3^{rd}$  and  $4^{th}$  hours, respectively. While, Giza 168 variety took the brown color after the  $1^{st}$  hour and then the very dark brown color after the  $2^{nd}$  hour till the end of the test. On the other hand, Sids 12 didn't have any color with the phenol till the third hour and took the light brown color after the  $4^{th}$  hour. Seed categories took the same pattern in phenol color reaction for each variety. In this regard, wheat varieties showed different color reactions to phenol by **Eman (2011), Niranjana** *et al.* **(2018) and Pavlic** *et al.* **(2018)** 

### **ISSR marker technology**

ISSR markers were able to distinguish morphologically distinct and similar accessions and provide steady information on the genetic variability among wheat varieties that will help in breeding programs. In the current study eight primers of ISSR were selected to distinguish among wheat varsities. These primers produced multiple bands, which ranged between 2 polymorphic bands for primer HB-09 with 28.57% polymorphism to 19 bands for primer HB-12 which showed 95.00% polymorphism. The total number of bands was 97 of them 66 were polymorphic with 68.04% polymorphism as shown in Table 9 and Fig. 1. Primer 14A produced 11 bands with the fragment sizes ranging from 250 bp to 1290 bp, 8 of them were polymorphic (72.72% polymorphism). Primer 44B produced 14 bands with the fragment sizes ranging from 290 bp to 2270 bp, 8 of them were polymorphic (57.14% polymorphism). HB-8 formed 9 bands with the fragment sizes ranging from 270 bp to 1240 bp, 5 of them were polymorphic (55.55% polymorphism). Primer HB-10 generated 16 bands with the fragment sizes varied from 200 bp to 960 bp, 12 of them were polymorphic (75.00% polymorphism). Whereas, primer HB-12 produced 20 bands with the fragment sizes ranging from 220 bp to 1110 bp, 19 of them 95% were polymorphic which giving polymorphism. Primer HB-13 produced 8 bands with the fragment sizes ranging from 305 bp to 740 bp, 5 of them were polymorphic (62.50% polymorphism). Primer HB-15 showed 12 bands with the fragment sizes ranging from 195 bp to 1590 b, 7 of them were polymorphic (58.33% polymorphism). ISSRs have been

successfully used to assess the genetic diversity of wheat varieties. The genetic relationships estimated by the polymorphism of ISSR markers revealed greater level of genetic variability in wheat accessions of wide adaptability and applicability (Singh and Jaiswal, 2016; Heidari *et al.*, 2017; Mandoulakani *et al.*, 2017; Nazarzadeh *et al.*, 2020).

### **Cluster Analysis**

The dendrogram resulting from the UPGMA cluster analysis showed that the studied varieties could be divided into two main clusters (Fig. 2). The first cluster contained two sub-clusters. The first sub-cluster contained two varieties (Mr1Cer S. and Mr 1 Bas S.), the second sub cluster contained two varieties (Gm 11 Cer. S. and Mr 1 Far S. The second cluster was divided into two sub-clusters, the first sub-cluster included one variety (Gm 11 Far S.), the second sub cluster contained 8 varieties (Sd 12 Far S., Sd 12 Cer S., Gz 168 Cer S., Sd 12 Bas S., Gz 168 Far S, Gz 168 Bas S., Gm 11 Bas S. and Gm 11 Far S.). In this concern, molecular markers have been exploited in wheat breeding programs and detected higher percentage of polymorphisms among wheat varieties. Genetic diversity of wheat varieties has been detected based on ISSR molecular markers by Mandoulakani et al. (2017) and Varsha et al. (2018).

## **Genetic Diversity**

The similarity matrix revealed high percentage of similarity between the varieties. Results in Table 10 showed the genetic diversity coefficients among wheat varieties. The highest similarity was observed between the varieties Gz 168 Far S. and Sd 12 Bas S. with similarity coefficient value of 0.89, while the lowest similarity value was obtained by the varieties Mr 1 Cer S. and Gm 11 Far S. with similarity coefficient value of 0.66. Appearance of differential differences between the studied materials might be due to the effect of the genotypic effect and their interaction with different seed categories on the DNA. Similar results were reported by Singh and Jaiswal (2016), Heidari et al. (2017) and Varsha et al. (2018).

Table 9. Number of monomorphic, polymorphic bands and polymorphism percentage

produced by each ISSR primer of the tested wheat varieties

Primers	Size of bands (bp)	Total no. of bands	Monomorphic bands	Polymorphic bands	Polymorphism %		
14 A	250:1290	11	3	8	72.72		
44 B	290:2270	14	6	8	57.14		
HB- 10	450-1250	16	4	12	75.00		
HB- 12	220-1110	20	1	19	95.00		
HB-13	305-740	8	3	5	62.50		
HB -15	195-1590	12	5	7	58.33		
HB-08	270-1240	9	4	5	55.55		
HB-09	450-1250	7	5	2	28.57		
Total		97	31	66	68.04		



Fig. 1. ISSR pattern obtained by 8 primers of the tested wheat varieties

1210



Fig. 2. A dendrogram based UPGMA cluster analysis of ISSR showing the relationships between the different wheat varieties

0.95

1.00

0.90

Lane	Mr 1 Bas. S.	Mr 1 Cer. S.	Mr 1 Far. S.	Gm 11 Ba. S.	Gm 11 Cer. S.	Gm 11 Far. S.	Gz 168 Bas. S	Gz 168 Cer. S.	Gz 168 Far. S.	Sd 12 Bas. S.	Sd 12 Cer. S.	Sd 12 Far. S.
Mr 1 Bas. S.	1											
Mr 1 Cer. S.	0.86	1										
Mr 1 Far. S.	0.85	0.79	1									
Gm 11 Ba. S.	0.8	0.75	0.8	1								
Gm 11 Cer. S.	0.81	0.82	<mark>0.8</mark> 3	<mark>0.7</mark> 9	1							
Gm 11 Far. S.	0.73	0.66	0.82	0.8	0.78	1						
Gz 168 Bas. S	0.75	0.7	0.75	0.83	0.78	<mark>0.81</mark>	. 1	l				
Gz 168 Cer. S.	0.74	0.69	0.7	0.81	0.75	0.82	. 0.86	i 1				
Gz 168 Far. S.	0.79	0.73	0.79	0.77	0.82	0.76	0.83	0.82	: 1			
Sd 12 Bas. S.	0.79	0.73	0.77	0.81	0.82	0.79	0.87	0.86	0.89	1		
Sd 12 Cer. S.	0.74	0.67	0.76	<mark>0.81</mark>	0.75	0.78	0.78	8 0.87	0.8	0.86	1	
Sd 12 Far. S.	0.75	0.78	0.75	0.82	0.76	0.75	0.81	0.86	0.8	0.87	0.84	1

Table 10. Genetic diversity coefficients among wheat varieties and seed categories

0.85

0.80

Mr1 (Misr 1), Gm11 (Gemmeiza 11), Gz168 (Giza 168), Sd 12 (Sids 12), Bas.S. (Basic seed), Cer.S. (Certified seed) and Far. S. (farmer seed)

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# التحقق من النقاوة الوراثية وتأشرها بمصادر البذرة لأربعة أصناف من قمح الخبز محمد السيد سعيد قمورة <sup>1</sup>- أحمد عبدالغني علي<sup>2</sup>- حسن عودة عواد<sup>2</sup>- محمود إبراهيم العميرى<sup>1</sup> 1- قسم تكنولوجيا البذور - معهد المحاصيل الحقلية - مركز البحوث الزراعية - مصر 2- قسم المحاصيل - كلية الزراعة - جامعة الزقازيق - مصر

تلعب درجات التقاوى وأصناف المحصول دوراً هاماً في إنتاج القمح في مصر والتأثير على النقاوة الوراثية للتراكيب الوراثية في القمح. لذلك يهدف هذا البحث الى دراسة تأثير مصادر التقاوي والاصناف والتفاعل بينهما على المحصول ومكوناته. أجريت تجربتان حقليتان بمحطة البحوث الزراعية - مركز البحوث الزراعية - الجيزة – جمهورية مصر العربية خلال الموسم الشتوي لاعوام 2014- 2015 و 2015- 2016 في الميعاد الأمثل للزراعه 15 نوفمبر. واستخدمت في هذه الدراسة أربعة أصناف من القمح " مصر1، جميزه 11، جيزة 168 وسدس 12" مع ثلاث درجات تقاوى لكل صنف " بذور الأساس- البذور المعتمدة وبذور المزارع". تم تقدير المحصول ومكوناته ونسبة الشوارد في التجربة الحقلية، بالإضافة إلى إجراء إختبار الفينول وإختبار ISSR بمعمل قسم بحوث تكنولوجيا البذور. أوضحت النتائج وجود إختلافات معنوية بين التراكيب الوراثية للقمح ودرجات التقاوى لمعظم الصفات. أعطى الصنف جميزة 11 أعلى القيم في معظم الصفات المدروسة " محتوى ورقة العلم من الكلوروفيل، مساحة ورقة العلم، طول السنبلة، عدد السنابل/م²، عدد حبوب السنبلة، وزن الألف حبه ومحصول الحبوب/فدان"، بينما سجل الصنف مصر 1 أقل القيم لتلك الصفات. بالنسبة لتاثير درجات التقاوي على المحصول ومكوناته، أعطت بذور الأساس أعلى القيم في معظم الصفات، ماعدا إرتفاع النبات حيث سجلت بذور المزارع أعلى قيمة لهذه الصفة. لوحظت تأثيرات غير معنوية لدرجات التقاوى على محتوى ورقة العلم من الكلوروفيل. أوضحت نتائج تأثير التفاعل بين التراكيب الوراثية ودرجات التقاوي أن تفاعل الصنفان جميزة 11 وسدس 12 مع كل من درجتي الأساس والمعتمدة أعطى أعلى القيم لمعظم الصفات، بينما أعطى التفاعل بين الصنف مصر 1 وبذور المزارع أقل القيم لتلك الصفات. كانت هناك تأثيرات غير معنوية للتراكيب الوراثية على نسبة الشوارد، بينما كان لدرجات التقاوى تأثيرات عالية المعنوية، حيث سجلت بذور المزارع أعلى القيم. وأظهرت أصناف القمح إختلافات لونيه لإختبار الفينول، بينما أخذت درجات التقاوى نفس التفاعل لكل صنف وفيما يتعلَّق بتحليل ISSR -PCR أستخدمت ثمانية بادئات للتمييز بين أصناف القمح الأربعة ودرجات تقاويها. وأظهرت النتائج وجود 97 علامة جزيئية منهم 31 علامة جزيئية مختلفة بنسبة %68.04 . وأظهر البادئ HB-12 أعلى نسبة إختلاف بين الأصناف موضع الدراسة بنسبة 95%، يبنما أظهر الدادئ HB-09 أقل نسبة اختلاف % 28.57

أستاذ المحاصيل المساعد – كلية الزراعة – جامعة الزقازيق

المحكمــون :

<sup>1</sup> ـ أ.د. عفساف طلبسسه

<sup>2-</sup>د. محمد عبد السلام طه

أستاذ المحاصيل – كلية الزراعة – جامعة عين شمس.