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Variability and Molecular Characterization of Ten Onion Genotypes

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMAA designed the study, performed the field experimental work and the statistical analysis. Authors MAHM and SEIE performed the molecular experimental work, the molecular analyses and wrote the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Releasing new varieties mainly depend on evaluation the genotypes in the breeding programs. So, the objectives of this work were evaluating ten Egyptian onion genotypes for producing onion bulbs from sets, assessing the magnitude of genetic variability, heritability and genetic advance and estimating the genetic diversity among the genotypes using molecular markers.
Place and Duration of Study: Study was conducted at Giza Research Station, Giza Governorate, Egypt, at consecutive two seasons.
Study Design and Methodology: The onion genotypes were grown from sets in RCBD with three replications. The mean performance and genetic components of twelve yield related traits were estimated as well as the genetic diversity among these genotypes by using RAPD and ISSR techniques.
Results: The results showed that the high means of leaf blade length and number of leaves/plant were obtained by Giza 20. The high means of fresh leaf blade weight/plant and culls yield were recorded by Shandaweel 1. The high means of total yield, marketable yield and average bulb weight

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were given by Composite 13, while Giza 20 x Ori gave the high mean of total weight loss (%). Composite 8 and H.Y. 28 recorded the high means of dry leaf weight and number of complete ring/bulb, respectively. The high means of bulb total soluble solids content (%) and bulb dry matter content (%) were recorded by Z218 white. Heritability in broad sense ranged from 0.002 for leaf number/plant to 0.67 for fresh leaf weight/plant. The difference between PCV and GCV percentages ranged between 1.72% for bulb total soluble solids content and 20.76% for number of complete ring/bulb. The genetic advance mean ranged from 0.04 for leaf number/plant to 76.65 for marketable yield. At the molecular level, RAPD and ISSR markers were used to assess the degree of polymorphism among the genotypes. The markers showed genetic diversity remarkably. The ISSR markers gave diversified results than RAPD.

Conclusion: It could be concluded that the new promising genotype composite 13 had the high response to produce bulb onions from sets. Meanwhile, the genotypes H.Y. 28 and Z 218 white had the high bulb quality traits. These results give an insight into the genetic polymorphism and the possibility of their further use in breeding programs.

Keywords: Onion; RAPD; ISSR; polymorphism; yield components; heritability; genetic advance.

1. INTRODUCTION

Onion (*Allium cepa* L., 2n= 2x=16) often called as “queen of kitchen”, is a monocot bulb crop belonging to the family *Alliaceae* of the class *Monocotyledones* [1]. It is one of the oldest known and an important vegetable crop grown all over the world. It is a cross pollinated, seed propagated and biennial important spices as well as vegetable crop throughout the world. The genus *Allium* consists of 750 species and onion (*Allium cepa* L.) is the only cultivated species in this genus [2]. It has been grown in Egypt for thousands of years. Onion contains a lachrymatic agent, a strong antibiotic in addition to fungicidal, bacterial, anticholestrol, anti-cancer and antioxidant components such as quercetin [3]. In addition, it has been reported to be rich in phytochemicals especially flavonols which are medicinal [4]. Onion occupies the 4th position in the world level after tomato, cabbage and watermelon with a global annual production of 25 million tons [5]. Central Asia is the primary center of its origin and the Mediterranean area is the secondary center for large types of onions [6]. In fact, successful onion production largely depends on the selection of varieties that are adapted to different conditions imposed by different environments, modern production technology and quality seeds. Inferior seeds may decrease production by 15-25%. Yield could be regarded as a complex character dependent on a number of agronomic characters and is influenced by many factors that could be genetic or environmental [7]. To breed for higher yield and quality, it is important to have impressive genotype collection. To achieve these goals, it is essential to evaluate the genotype from the available gene pool. Thus, evaluations of local onion genotypes have been carried out worldwide [8]. Most of these characterizations are based either on morphological, agronomical or physical and chemical measurements. Wide variations in bulb characteristics were observed among the cultivated genotypes by several investigators [9,10,11]. Despite the global culinary and economic significance, genetic research of onion has greatly lagged that of other major vegetable crops. This has been due in part to the difficulty of developing and propagating genetic stocks, compounded by a lack of sequence resources. The small size of the community engaged in *Allium* genetic research has in the past paradigm contributed to the research between onions and other major crops. Currently, the very powerful PCR-based techniques have emerged which are very fast, reliable and require minimal amounts of tissue for investigation. Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphism (SNP) and Simple Sequence Repeats (SSR) have a large number of applications like characterization of gene pool, DNA fingerprinting, phylogenetic analysis and evaluation of genetic diversity within and between species and populations [12]. Molecular markers have been previously used to detect genetic diversity in onion germplasm [13-16]. These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objectively. The objectives of this work were (a) to evaluate the performance of ten Egyptian onion genotypes planted from sets during autumn season, (b) to assess the magnitude of genetic variability, heritability and genetic advance associated with important traits of onion and (c) to evaluate the genetic diversity among the genotypes using molecular markers.

2. MATERIALS AND METHODS

The present study was conducted at Giza Research Station, Giza Governorate, Egypt, during 2015/2016 and 2016/2017. The name and pedigree of these genotypes are presented in Table 1. Seeds of 10 onion genotypes developed by Onion Research Section, Field Crops Research Institute, Agricultural Research Center, Giza were sown in the nursery on the 15th of January of each growing season to produce onion sets with a rate of 30 kg/fed. Sets were harvested on May 25th in both seasons, then cured for 3 weeks and dry foliage was trimmed. Sets were sized to the proper size (8-16 mm diameter) and stored in natural ventilation conditions until planting in the permanent field. Sets of 10 onion genotypes were planted 7 cm apart on both sides of ridges 3 m long and 0.65 m wide on September 5th and September 10th for both annual seasons, respectively. The soil of the experimental field was clay loam. The plot size was 2 x 3 m (1/700 feddan). Each plot consisted of 3 ridges (6 rows). All cultural practices concerning sets or onion production from sets were applied. The experimental design used in this experiment was the randomized complete blocks design in three replicates. After 90 days from planting, 10 randomly selected plants were taken from each plot to measure leave blade length (cm), leaves number/plant, fresh leaves weight (g)/plant and dry leaves weight (g)/plant. At harvest time, all plants in the experimental plot were harvested after 50% top down, cured for 3 weeks, then dry foliage was removed and bulbs uprooted. Total yield was calculated on basis of yield for the experimental plot in tons/fed. Marketable yield (ton/fed) was determined as the weight of single bulb yield for each experimental plot. Culls yield (ton/fed) includes bulbs of less than 3 cm diameter, doubles, bolters, off-color and scallions. Average bulb weight (g) was calculated by dividing aggregate weight of single bulbs by its number of bulbs. Percentage of double bulbs was estimated by dividing number of double bulbs by the total number of bulbs × 100. Percentage of bolters was estimated by dividing number of bolter bulbs by the total number of bulbs × 100.

2.1 Internal Bulb Characteristics

At harvest, a random sample of 10 bulbs was taken from each plot, and cross sectioned to record number of entire rings which completely encircle the growing centers. Bulb total soluble solids percentage (TSS %) was determined by using a hand refractometer. Bulb dry matter content percentage (DM%) was determined on a random sample of bulbs sliced and dried in an oven at 70°C for 72 hours. 50 bulbs of marketable yield of each plot were randomly chosen and placed in common burlap bags and kept under normal storage conditions. Storability was calculated according to Wills et al. [17].

2.2 Statistical Analysis

The analysis of variance was carried out separately for each season, and then a combined analysis for the two seasons was performed [18]. Bartelet test was done prior to the combined analysis [19]. Significance of difference among means was tested using LSD method. Estimates of phenotypic and genotypic variance were obtained from the combined analysis for the ten genotypes. The expected mean squares were calculated according to Snedecor and Cochran [20]. Broad sense heritability (H^2bs) was calculated according to Johnson et al. [21] as follows:

$$H^2bs = (б^2_g / б^2_{ph})\ 100$$

Where $б^2_g$ is the genotypic variance = (MSg – MSgy)/ry

$б^2_{ph}$ is the phenotypic variance = $б^2_g + б^2_{gy} + (б^2e/r)$

Where:

$$б^2_e = MSe,\ б^2_{gy} = (MSgy – MSe)/r$$

r = replications, Y= years

The phenotypic coefficient of variation (PCV) was calculated as:

$$PCV = (б^2_{ph} / X)\ 100$$

The genotypic coefficient of variation (GCV) was calculated as:

$$GCV = (б^2_g / X)\ 100.$$

Where: X = Grand mean of all genotypes.

Predicted genetic advance under selection (GS) in absolute units and as percentage of grand mean (GS%) was computed according to Falconer [22] as follows:

$$GS = K_x H^2bs_x б_{ph}.$$

Where: K is the selection differential and equals 2.06 at selection intensity of 5%.

$$GS\% = (GS/ X)\ 100.$$

Table 1. Name, origin and pedigree of onion genotypes

Genotype	Origin	Method of development
H. Y. 28	USA	Selection from an introduced cv. from USA
Z 218 white	USA	Selection from an introduced cv. from USA
Giza 20 x Ori	Egypt	Selection from single cross between Giza 20 with introduced cv. from Israel.
Composite 8	Egypt	Selection from single cross between two Egyptian and 10 American cultivars.
Composite 13	Egypt	Selection from single cross between two Egyptian and 10 American cultivars.
Composite 16	Egypt	Selection from single cross between two Egyptian and 10 American cultivars.
Shandaweel 1	Egypt	Selection from bulb samples collected from Sohag province
Giza 6 Mohassan	Egypt	Selection from cv. Giza 6 which selected from Upper Egypt strain (Saiedi).
Giza red	Egypt	Selection from Behairy red strains.
Giza 20	Egypt	Selection from Egyptian Deltan types (Behairy) which collected from different provinces of delta regions.

2.3 DNA Extraction

Genomic DNA was isolated from onion leaves harvested at 15 days old seedlings using DNA extraction kit (Quigen Inc., Cat. no. 69104, USA). The DNA quality and quantity were checked on 0.8% agarose gel and DNA concentration was normalized to ~5 ng/µl for PCR.

2.4 RAPD- PCR Analysis

RAPD-PCR reactions were conducted using 5 arbitrary 10-mer primers with the 5′ → 3′ sequences as shown in Table 2.

Table 2. Names and sequences of primers used for RAPD- PCR analysis

Primer name	Sequence 5→3
OP-A03	AGTCAGCCAC
OP-A15	TTCCGAACCC
OP-A16	AGCCAGCGAA
OP-B06	TGCTCTGCCC
OP-B10	CTGCTGGGAC

2.4.1 Polymerase chain reaction (PCR) conditions

The reaction conditions were optimized. A mixture (25 µl total volume) consisting of dNTPs 0.4 µl, $MgCl_2$ 2.0 µl, 10 x buffer 3.0 µl, Primer (10 um) 2.0 µl, Taq (5 u/µl) 0.2 µl Template DNA (50 ng/µl) 2.0 µl and H_2O (dd) 15.4 µl was prepared. Amplification was carried out in a PTC- 200 thermal cycler (MJ Research, Watertown, USA) programmed as follows: Denaturation, 94°C for 2 minutes, then for 40 cycles. Each cycle consisted of 1 minute at 94°C, 1 minute at 37°C, 2 minutes 30 seconds at 72°C, followed by a final extension time of 12 minutes at 72°C then 4°C (indefinite). Gel electrophoresis was applied according to Sambrook et al. [23]. Agarose (1.2%) gel containing ethidium bromide was used for resolving the PCR products. The run was performed for one hour at 80 volt in Pharmacia Submarine (20 x 20 cm). Bands were detected on UV– transilluminator and photographed by Gel documentation 2000, Bio- Rad.

2.5 ISSR Analysis

ISSR primers were screened, five of them were chosen for amplification (Table 3). Polymerase chain reaction (PCR) was performed in a 25 µl volume containing 5 ng of genomic plant DNA, 10 mM Tris-HCl, pH 8.3, 3.5 mM MgCl2, 200 µM of each dNTP, 0.5 µM primer and 0.625 U of *Taq* DNA polymerase , Template DNA (50 ng/µl) 2.0 µl and up to 25 µl H2O (dd). Amplification was carried out in a PTC- 200 thermal cycler (MJ Research, Watertown, USA) programmed as follows: an initial denaturation at 95°C for 5 min followed by; 42 cycles of 90 s at 95°C, 2 min at 55°C and 60 s at 72°C were performed; a final extension at 72°C for 7 min and a subsequent incubation at 4°C followed. PCR products were loaded onto 1.2% agarose gel containing ethidium bromide. Bands were detected on UV–transilluminator and photographed by Gel Documentation 2000; Bio- Rad.

Table 3. ISSR primers and their sequences

Primer name	Sequence 5→3
SH6	CGCGATAGATAGATAGATA
SH7	GACGATAGATAGATAGATA
SH8	AGACAGACAGACAGACGC
SH9	GATAGATAGATAGATAGC
SH10	GACAGACAGACAGACAAT

2.6 RAPD and ISSR Data Analysis

To analyze the data, the presence and absence of bands were scored as 1 or 0, respectively. Similarity coefficients were calculated according to Dice matrix [24]. Construction of the dendrogram tree was performed using the un-weighted pair group method based on arithmetic mean (UPGMA) in the 'SPSS' program version 10.

3. RESULTS AND DISCUSSION

A plant breeding program for any crop aims at improving the existing types or evolving a new type which is superior to existing ones. Evaluation of genotypes for assessing the extent of variability is the first step in any crop improvement program. Mean performance serves as an important criterion in eliminating the undesirable types in a selection program. The results of the present investigation revealed that significant differences in growth, yield and quality characteristics exist among the various genotypes of onion.

Data of individual and combined analysis of variance for growth, bulb quality and storability of onion during the two seasons are presented in Table 4. The results showed significant differences among the genotypes for all characters except bulb total soluble solids content percentage. The G × E interactions were significant for all characters except total weight loss (%) indicating the presence of genetic variability among the genotypes and heterogeneity between the environments. Also, Mohanty [25] recorded that the genotype × environment interactions were significant for all characters except bulb diameter. Variability expressed in coefficient of variation (CV) ranged from 4.91 for bulb dry matter content (%) to 35.31 for total weight loss (%).

The combined analysis of two years' data showed there was a significant difference for the leaf blade length, number of leaves/plant, fresh leaves blade weight/plant and dry leaves blade weight/plant due to genotypes (Table 5). The highest mean of leaf blade length (73.00 cm) was observed in Giza 20 whereas the lowest one (50.67 cm) was recorded by Z 218 white. The difference in leaf blade length on onion is mainly attributed to the genetic potential but also to environmental factors, especially temperature and photoperiod [26]. These results of current investigation are in agreement with Abo et al. [27]. The number of leaves was more in the genotype Giza 20 (12.83) and it was found on par with the genotypes Shandaweel 1 and Composite 13 (12.33) whereas lowest leaves (9.50 and 9.67) were recorded on Composite 16 and Z 218 white (Table 5), respectively. The variation in number of branches per plant might have been due to their own genetic makeup and also due to environmental factors, especially when onion grown by sets. Boukary et al. [5], and Dwivedi et al. [28] observed the difference in production of leaves between varieties of onion and attributed this difference mainly to the cultivar, but other researchers confirmed that environmental conditions affecting plant growth contribute to the development of leaves on plant [29]. In respect to fresh leaves blade weight/plant, the highest mean value was observed by Shandaweel 1 (159.8 g), while the lowest mean value was recorded by Composite 16 (64.17 g). Regarding the dry leaves blade weight/plant, the highest mean value was observed by Composite 8 (7.98 g), while the lowest mean value was recorded by Composite 16 (4.5 g) on par with the genotypes Z 218 white (4.78 g) and Giza red (4.97 g).

Yield is the foremost important parameter for any agricultural or horticultural crop. Means of total yield, marketable yield, culls yield and total weight loss (%) traits are presented in Table 6. Data revealed that Composite 13 gave the high mean for total yield (19.1 t/fed), while Giza 20 recorded the lowest one (9.92 t/fed). Composite 13 had highest marketable yield accounting 5.58 t/fed and it was found on par with Composite 8 (5.48 t/fed), while the low marketable yield was recorded by Giza 20 (0.44 t/fed). Regarding culls yield, it was found that the high mean value was observed by genotype Shandaweel 1 (14.54 t/fed) and it was on par with genotype Composite 13 (13.52 t/fed). The low mean value of culls yield was recorded by genotype Giza 20 (9.47 t/fed) and it was on par with genotype H.Y. 28 (9.51 t/fed). In respect to total weight loss (%), Giza 20 x Ori gave the high mean value (22.9%), while genotype H.Y. 28 recorded the low mean value (10.74%).

Means of average bulb weight, number of complete rings/bulb, bulb total soluble solids content (%) and bulb dry matter content (%) are presented in Table 7. The results showed that the highest mean of average bulb weight (156.4 g) was recorded by the genotype Composite 13, while the lowest one (103.2 g) was observed by the genotype Giza 20. Regarding the number of complete rings/bulb, genotype H.Y. 28 gave the highest mean (5.62), while Giza 20 gave the lowest mean (2.93). The genotype Z 218 white recorded the highest mean (14.27%) for bulb total soluble solids content (%), while genotype Giza 20 x Ori gave the lowest one (12.83%). In respect to bulb dry matter content (%), the highest mean (18.13%) was observed by the genotype Z 218 white, whereas the genotype Giza 20 x Ori gave the lowest one (15.52%). Significant differences between onion genotypes grown by transplants and variability were previously detected by [30], [31], [32], [25], [33], [34], [35], [36], [37], [38] and [8]. Singh [39] reported that variability among the genotypes may be due to their differences in genetic backgrounds or environmental condition.

3.1 Genetic Parameters

Estimates of phenotypic (PCV) and genotypic (GCV) coefficient of variations, broad sense heritability (H^2bs), genetic advance under selection in absolute units (GA) and genetic advance expressed as a percentage of grand mean (GAM) for the studied traits are presented in Table 8. The results showed that the high estimate of PCV was observed by marketable yield (71.75%), while the low PCV estimate was observed by Bulb total soluble solids content (5.61%) and Bulb dry matter content (5.69%). The high estimate of genetic coefficient of variation (GCV) was noticed in marketable yield (51.63%), while the low GCV estimate attended Bulb total soluble solids content (3.89%) and Bulb dry matter content (2.43%) followed by Leaf number/plant in the combined analysis (0.54). The relatively high genetic coefficient of variation for some traits indicated that these traits might be more genetically predominant and would be possible to achieve further improvement in them. In general, the estimates of phenotypic coefficient of variation (PCV) were higher in magnitude than genotypic coefficient of variation (GCV) for all studied characters but the gap between PCV and GCV was narrow for Bulb total soluble solids content (%) and leaf blade length, indicating little influence of environment in the expression of these characters. Thus, selection for the improvement of such characters based on phenotype would be rewarding in the present genotypes. As observed from the combined analysis, values of heritability in broad sense (H^2bs) ranged from 0.002 to 0.67. High estimates of (H^2bs) were obtained for fresh leaf weight/plant (0.67), leaf blade length (0.62), average bulb weight (0.56), total weight loss (0.53) and Marketable yield (0.52), while low estimates were observed for leaf number/plant (0.002) and dry leaf weight/plant (0.07). High heritability estimates for some traits indicated that they were slightly affected by environmental factors and hence these traits may be improved by selection. Estimates of genetic advance (GA) based on 5% selection intensity ranged from 0.01% for leaf number/plant to 44.12% for fresh leaf weight/plant. Estimates of genetic advance mean ranged between 0.04 for leaf number/plant and 76.65 for marketable yield. High estimates of heritability along with high GCV% and GA estimates were observed for marketable yield and fresh leaf weight/plant which might be attributed to additive gene action in regulation of their expression. This indicates that simple selection processes for these traits would certainly result in improvement in the studied genotypes. Low estimates of heritability along with low GCV% and GA estimates were recorded for leaf number/plant and dry leaf weight/plant. This indicated that these traits might be governed by non-additive gene action and the interaction between genotypes and environment, and hence these traits may be improved by development of hybrid varieties. Genetic parameters of leaf blade length, fresh leaf weight/plant, marketable yield, number of complete rings/bulb, bulb total soluble solids (%) and total weight loss (%) showed high proportion of genetic contribution to phenotypic expression or narrow differences between genotypic and phenotypic coefficients of variation with moderate to high broad sense heritability along with high genetic advance as percentage of mean. Therefore, these traits could be used for enhancing simple selection programs for further improvement of onion production from sets.

3.2 Molecular Markers

3.2.1 RAPD- PCR analysis

Five selected RAPD primers were used to differentiate among ten onion genotypes (Fig. 1). A total of 60 bands were recorded, 28 of them were polymorphic (46.7%) and 32 were monomorphic (53.3%). The number of amplified

Table 4. Mean squares of individual and combined analysis of variance for growth, yield and bulb quality of onion during 2015/2016 and 2016/2017 seasons

Character	2015/2016			2016/2017			Combined			
	(G)	(E)	C.V. %	(G)	(E)	C.V. %	(G)	G × E	(E)	C.V. %
	d.f.= 9	d.f.= 18		d.f.= 9	d.f.= 18		d.f.=9	d.f.=9	d.f.=36	
Leaf blade length(cm)	152.90*	30.204	8.10	152.23*	26.03	7.78	237.93*	67.20*	28.12	7.95
Leaf number/plant	9.71*	1.085	8.75	5.00 ns	2.84	15.85	7.36*	7.34*	1.96	12.44
Fresh leaf blade weight/plant (g)	3377.64*	322.90	13.99	3018.17*	322.90	17.70	5239.50*	1156.31*	322.90	15.63
Dry leaf blade weight/plant(g)	6.50*	1.178	25.98	8.00*	1.51	17.00	7.70*	6.80*	1.34	20.32
Total yield (t/fed.)	52.88*	2.285	10.09	12.80*	2.05	11.19	46.73*	18.95*	2.17	10.60
Marketable yield (t/fed.)	18.55*	0.808	25.04	3.83 *	0.24	28.56	16.85*	5.53*	0.53	27.27
Culls yield (t/fed.)	23.38*	2.436	13.69	8.21*	2.03	12.90	18.04*	13.54*	2.23	13.32
Average bulb weight (g)	1320.10*	419.297	13.91	1318.83*	221.08	12.84	1974.72*	664.22*	320.19	13.61
Number of complete rings/bulb	4.25*	0.868	24.16	5.49*	0.99	23.59	5.30*	4.45*	0.93	23.87
Bulb total soluble solids content (%)	1.60 ns	0.889	6.96	2.92*	1.10	7.60	1.41 ns	3.11*	0.99	7.29
Bulb dry matter content (%)	2.87*	0.633	4.77	3.42*	0.76	5.04	3.66*	2.63*	0.70	4.91
Total weight loss (%)	51.24*	8.685	15.46	71.10 ns	57.12	56.32	84.80*	37.54ns	32.90	35.31

*(G) and (E); genotype and error mean square, C.V.; Coefficient of variation, (d.f.);Degree of freedom, * and n.s.: Significant and no significant at 0.05 probability level*

Table 5. Mean of individual and combined analysis for growth characters of onion during 2015/2016 and 2016/2017 seasons

Genotype	Leaf blade length (cm)			Number of leaf /plant			Fresh leaves blade weight/plant (g)			Dry leaves blade weight/plant (g)		
	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.
H. Y. 28	66.00 bc	67.00 abc	66.50 ab	12.33 b	11.00 ab	11.67ab	151.3 a	105.7 bc	128.5 bc	4.23 bcd	7.80 abc	6.02 bc
Z 218 white	51.67 d	49.67 d	5067 c	10.33 cd	9.00 b	9.665 c	95.33 b	89.00 cd	92.17 d	4.07 cd	5.50 cd	4.78 c
Giza 20 x Ori	62.67 c	69.00 ab	65.83 ab	9.333 d	12.33 a	10.83bc	101.7 b	105.3 bc	103.5 d	2.33 d	7.77 abc	5.05 c
Composite 8	69.67 abc	69.33 ab	69.50 ab	12.00 bc	11.00 ab	11.50ab	99.00 b	86.00 cd	92.50 d	6.87 a	9.10 a	7.98 a
Composite 13	69.00 abc	69.00 ab	69.00 ab	12.33 b	12.33 a	12.33ab	164.0 a	116.0 bc	140.0 ab	2.60 d	9.13 a	5.87 bc
Composite 16	66.33 bc	61.33 bc	63.83 b	10.67 bcd	8.33 b	9.50 c	86.00 b	42.33 e	64.17 e	4.17 bcd	4.83 d	4.50 c
Shandaweel 1	72.00 abc	67.67 abc	69.83 ab	14.33 a	10.33 ab	12.33ab	165.0 a	154.7 a	159.8 a	6.13 ab	7.60 abc	6.87 ab
Giza 6 Mohassan	69.00 abc	75.00 a	72.00 a	10.33 cd	11.33 ab	10.83bc	105.0 b	112.7 bc	108.8 cd	3.67 cd	6.73 bcd	5.20 c
Giza red	75.00 ab	59.00 c	67.00 ab	12.33 b	10.00 ab	11.17abc	150.0 a	70.33 de	110.2 cd	4.87 bc	5.07 d	4.97 c
Giza 20	77.33 a	68.67 ab	73.00 a	15.00 a	10.67 ab	12.83 a	167.3 a	133.0 ab	150.2 ab	2.83 cd	8.80 ab	5.82 bc
LSD at 0.05%	9.427	8.751	6.209	1.787	2.891	1.641	30.82	30.82	21.04	1.862	2.109	1.357

Values followed by the same letters are not significant different from each other at P=0.05 according to Duncan' s multiple range test

Table 6. Mean of individual and combined analysis for bulb yield of onion during 2015/2016 and 2016/2017 seasons

Genotype	Total yield (t/fed.)			Marketable yield (t/fed.)			Culls yield (t/fed.)			Total weight loss (%)		
	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.
H. Y. 28	9.64 f	13.11 abc	11.38 ef	1.52 ef	2.22 bc	1.87cd	8.12 d	10.90 bc	9.51 d	15.05 d	6.43 b	10.74 c
Z 218 white	10.73 ef	12.74 bc	11.74 de	2.52 de	1.24 de	1.88cd	8.21 d	11.50 b	9.86 cd	20.49 bcd	10.53 ab	15.51abc
Giza 20 x Ori	13.32 de	13.53 abc	13.43 cd	3.43 cd	1.19 de	2.31bc	9.89 cd	12.34 ab	11.12cd	26.05 a	19.74 a	22.90 a
Composite 8	19.55 b	13.49 abc	16.52 b	8.12 a	2.85 b	5.48 a	11.43 bc	10.64 bc	11.04cd	24.24 ab	8.39 ab	16.32 abc
Composite 13	23.17 a	15.03 ab	19.10 a	6.79 a	4.36 a	5.58 a	16.38a	10.66 bc	13.52ab	20.45 bcd	17.44 ab	18.94 ab
Composite 16	14.58 cd	10.99 cd	12.79cde	5.11 b	1.07 de	3.09 b	9.47 cd	9.92 bc	9.70 d	15.07 d	9.81 ab	12.44 bc
Shandaweel 1	17.27 bc	15.80 a	16.53b	2.38 de	1.61 cd	1.99cd	14.89 a	14.19 a	14.54 a	17.88 cd	20.39 a	19.13 ab
Giza6Mohassan	15.28 cd	13.46 abc	14.37c	4.22 bc	1.12 de	2.67bc	11.06 bcd	12.34 ab	11.70bc	14.91 d	12.54 ab	13.72 bc
Giza Red	15.31 cd	10.85 cd	13.08 cde	1.52 ef	1.05 de	1.28de	13.79 ab	9.80 bc	11.80bc	14.94 d	12.17 ab	13.55 bc
Giza 20	11.01 ef	8.82 d	9.92 f	0.28 f	0.61 e	0.44 e	10.73 cd	8.21 c	9.47 d	21.57 abc	16.71 ab	19.14 ab
LSD at 0.05%	2.593	2.454	1.723	1.542	0.847	0.849	2.677	2.445	1.752	5.05	12.96	6.72

Values followed by the same letters are not significant different from each other at P=0.05 according to Duncan' s multiple range test

Table 7. Mean of individual and combined analysis for bulb quality of onion during 2015/2016 and 2016/2017 seasons

Genotype	Average bulb weight (g)			Number of complete rings/bulb			Bulb total soluble solids content (%)			Bulb dry matter content (%)		
	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.	2015/2016	2016/2017	Comb.
H. Y. 28	154.8abcd	143.4 a	149.1 ab	5.03 a	6.20 a	5.62 a	13.23 abc	14.87 a	14.05abc	17.70 ab	18.00 abc	17.85 ab
Z 218 white	124.2 d	108.2 bcd	116.2 cd	4.30 ab	2.23 d	3.27 c	14.47 ab	14.07 abcd	14.27 a	17.83 a	18.43 ab	18.13 a
Giza 20 x Ori	127.6 cd	125.1 abc	126.3bcd	5.30 a	4.63 abc	4.97ab	13.13 abc	12.53 cd	12.83 c	16.00 cde	15.03 d	15.52 e
Composite 8	161.6abcd	135.6 ab	148.6 ab	3.73 ab	4.57 abc	4.15bc	13.40 abc	15.00 a	14.20 ab	16.90abcd	17.23 abc	17.07bcd
Composite 13	178.8 a	134.0 ab	156.4 a	1.80 c	4.80 abc	3.30 c	13.40 abc	12.40 d	12.90 bc	15.60 de	16.40 cd	16.00 de
Composite 16	167.8 ab	98.72 cde	133.3 abc	5.30 a	2.93 cd	4.12bc	12.47 c	14.67 ab	13.57abc	14.90 e	18.80 a	16.85bcd
Shandaweel 1	166.1 abc	128.6 ab	147.4 ab	4.23 ab	5.87 a	5.05ab	13.93 abc	13.67abcd	13.80abc	16.57abcd	17.53 abc	17.05bcd
Giza6 Mohassan	124.9 d	112.4 bcd	118.6 cd	2.60 bc	3.30 bcd	2.95 c	14.67 a	12.80 bcd	13.73abc	17.17 abc	16.83 bc	17.00bcd
Giza Red	139.5 bcd	91.52 de	115.5 cd	3.10 bc	4.90 ab	4.00bc	14.13 abc	13.27abcd	13.70abc	17.67 ab	17.20 abc	17.43abc
Giza 20	126.2 d	80.16 e	103.2 d	3.17 bc	2.70 d	2.93 c	12.70 bc	14.50 abc	13.60abc	16.27bcde	17.20 abc	16.73 cd
LSD at 0.05%	35.13	25.51	20.95	1.598	1.704	1.128	1.617	1.795	1.166	1.365	1.492	0.976

Values followed by the same letters are not significant different from each other at P=0.05 according to Duncan' s multiple range test

Table 8. Estimation genetic components of 12 yield related characters in onion during 2015/2016 and 2016/2017 seasons

Character	2015/2016						2016/2017						Combined					
	GCV %	PCV %	D^z	h^2 (bs)	GA	GAM	GCV %	PCV %	D^z	h^2 (bs)	GA	GAM	GCV %	PCV %	D^z	h^2 (bs)	GA	GAM
Leaf blade length(cm)	40.90	50.90	10.0	0.80	11.82	17.41	9.89	10.86	0.97	0.83	12.18	18.58	7.99	10.18	2.19	0.62	8.64	12.95
Leaf number/plant	14.45	15.12	0.67	0.88	3.29	27.69	7.97	12.14	4.17	0.43	1.15	10.79	0.54	12.93	12.39	0.002	0.01	0.04
Fresh leaf blade weight/plant (g)	24.84	26.12	1.28	0.90	62.6	48.73	29.53	31.25	1.72	0.89	58.43	57.57	22.68	27.66	4.98	0.67	44.12	38.37
Dry leaf blade weight/plant(g)	31.89	35.24	3.35	0.82	2.48	59.53	20.33	22.57	2.24	0.81	2.73	37.77	6.75	25.95	19.20	0.07	0.21	3.63
Total yield (t/fed.)	27.40	28.01	0.61	0.95	8.28	55.29	14.81	16.16	1.35	0.84	3.58	28.01	15.49	23.43	7.94	0.44	2.93	21.14
Marketable yield (t/fed.)	67.76	69.29	1.53	0.95	4.91	136.70	63.17	65.29	2.12	0.94	2.18	126.10	51.63	71.75	20.12	0.52	2.04	76.65
Culls yield (t/fed.)	23.17	24.48	1.31	0.89	5.15	45.25	12.98	14.96	1.98	0.75	2.56	23.23	7.72	19.70	11.98	0.15	0.70	6.24
Average bulb weight (g)	11.77	14.25	2.48	0.68	29.53	20.06	16.52	18.11	1.59	0.83	36.00	31.01	11.24	14.95	3.71	0.56	22.92	17.43
Number of complete rings/bulb	27.54	30.87	3.33	0.79	1.95	50.69	29.08	32.12	3.04	0.82	2.28	54.35	9.28	30.04	20.76	0.10	0.24	5.92
Bulb total soluble solids content (%)	3.58	5.38	1.80	0.44	0.66	4.92	5.65	7.15	1.50	0.62	1.27	9.22	3.89	5.61	1.72	0.48	0.76	5.57
Bulb dry matter content (%)	5.18	5.86	0.68	0.77	1.57	9.43	5.46	6.18	0.73	0.77	1.71	9.93	2.43	5.69	3.26	0.18	0.36	2.14
Total weight loss (%)	19.16	21.03	1.87	0.83	7.08	36.03	16.09	36.28	20.19	0.19	1.97	14.73	17.27	23.77	6.50	0.53	4.21	25.91

D^z = The difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %)

bands per primer ranged from 9 to 16 bands. A maximum number of 16 bands were amplified with primer OP-A16, while a minimum number of 9 bands were amplified with the primer OP-B06. The high polymorphism was found in primer OP-A15 (64.3%) and the low in primer OP-B06 (22.2%) (Table 9). Unique markers and their molecular sizes generated by RAPD analysis are presented in (Table 9). RAPD analysis revealed three unique markers which distinguished genotype Giza 6 Mohassan, two of them in primer OP-A03 at 731 and 564 bp, while the third band at 1031 bp was given by primer OP-A15. RAPD markers have proven to be a powerful tool for molecular genetic analysis and plant breeding programs to assess genetic diversity for the development of improved varieties.

3.2.2 ISSR analysis

Five selected ISSR primers were used to differentiate among ten onion genotypes (Fig. 2). The five primers amplified different numbers of bands and revealed various levels of polymorphism. A total of 78 bands were

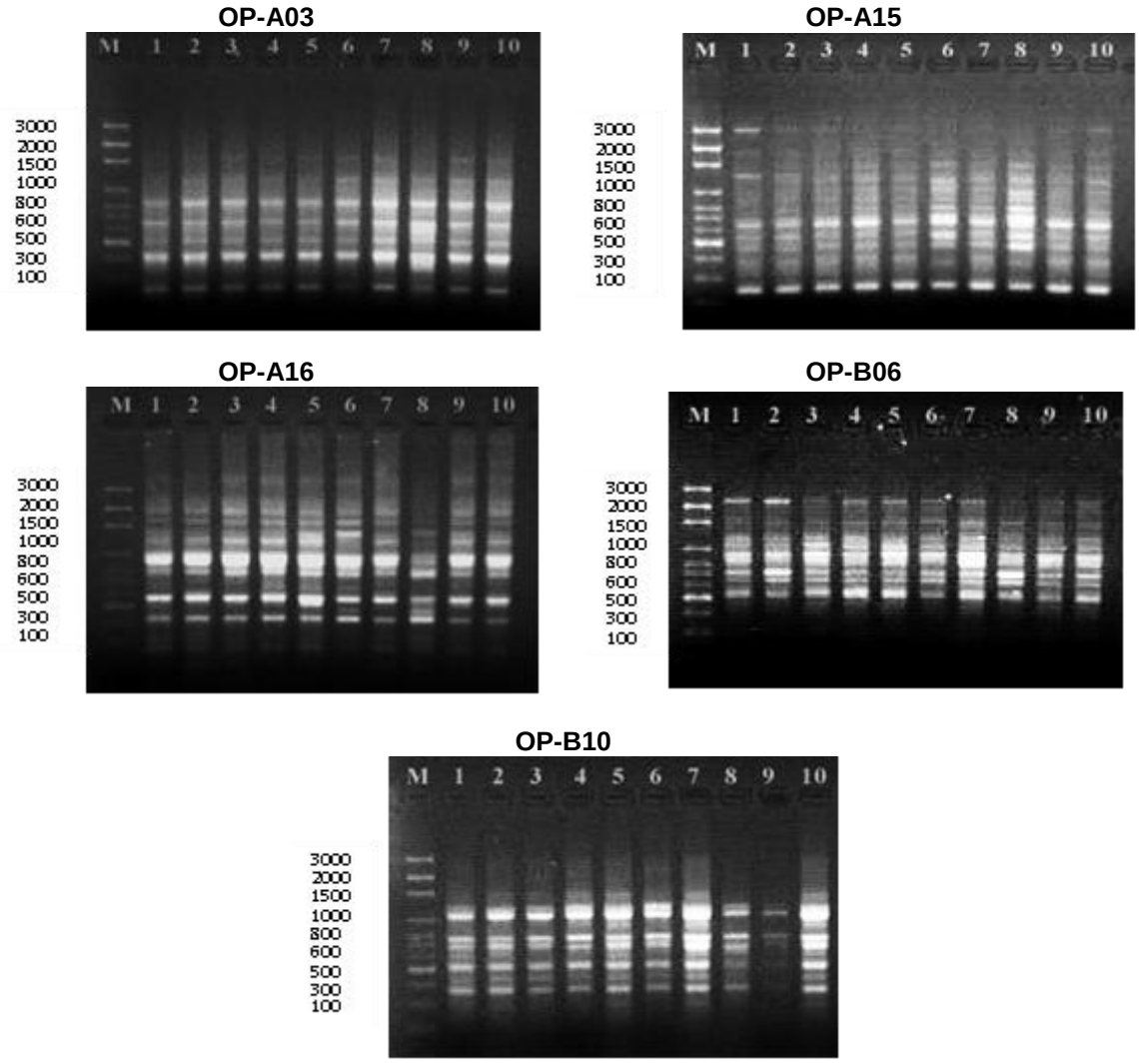

Fig. 1. RAPD fingerprinting of ten onion genotypes. (Line 1) H.Y. 28, (Line 2) Z 218 white, (Line 3) Giza 20 x Ori, (Line 4) Composite 8, (Line 5) Composite 13, (Line 6) Composite 16, (Line 7) Shandaweel 1, (Line 8) Giza 6 Mohassan, (Line 9) Giza Red, (Line 10) Giza 20, and M (DNA marker)

Table 9. Levels of polymorphism and unique genotypes specific bands for ten onion genotypes by RAPD- PCR analysis

Primer name	Number of total bands	Polymorphic bands	Monomorphic bands	Polymorphism %	Unique bands	
					Genotype	MW (bp)
OP-A03	10	4	6	40	Giza6Mohassan	737, 564
OP-A15	14	9	5	64.3	Giza6Mohassan	1031
OP-A16	16	8	8	50	-	-
OP-B06	9	2	7	22.2	-	-
OP-B10	11	5	6	45.5	-	-
Total	60	28	32	46.7		

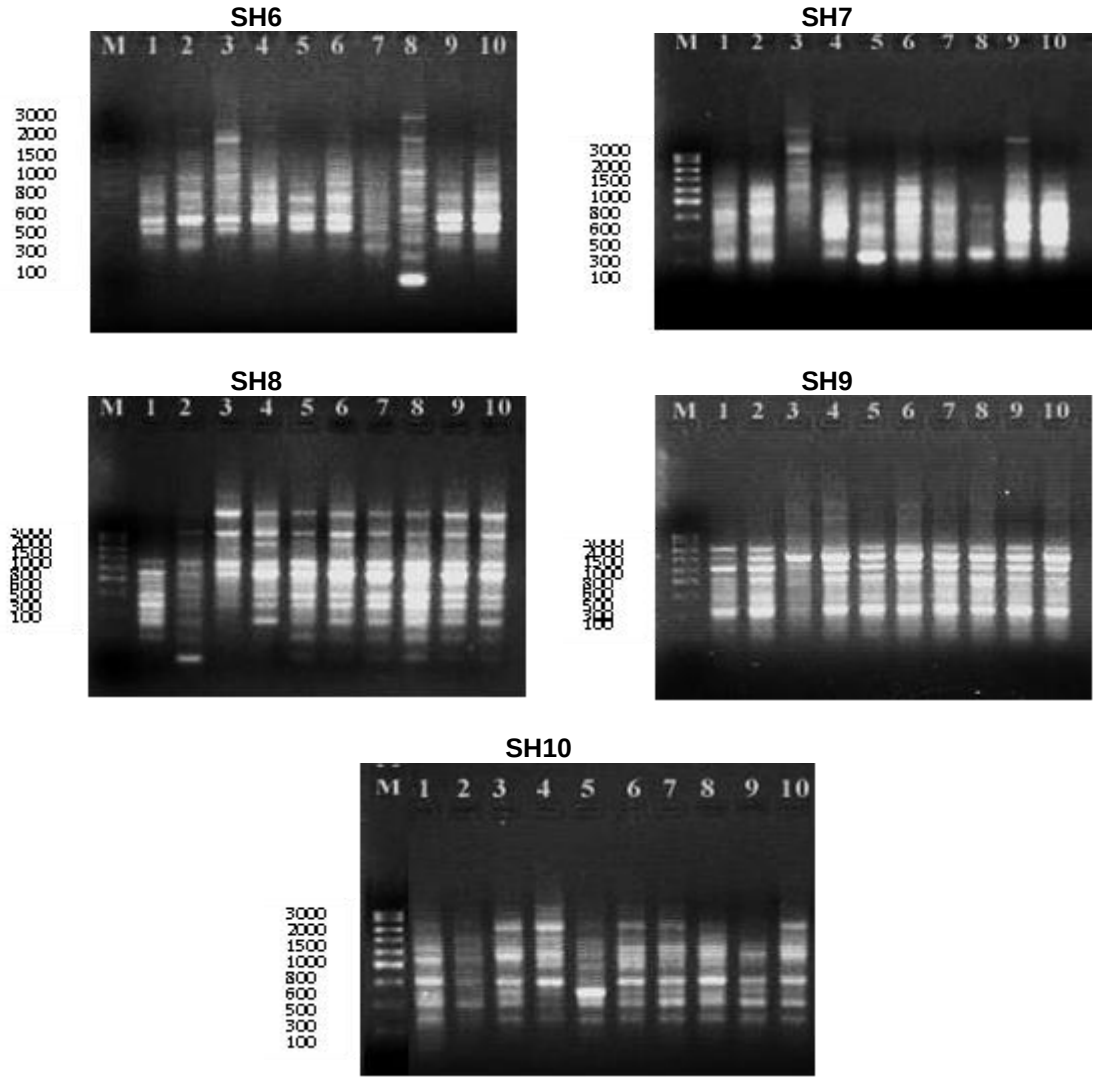

Fig. 2. ISSR fingerprinting of ten onion genotypes. (Line 1) H.Y. 28, (Line 2) Z 218 white, (Line 3) Giza 20 x Ori, (Line 4) Composite 8, (Line 5) Composite 13, (Line 6) Composite 16, (Line 7) Shandaweel 1, (Line 8) Giza 6 Mohassan, (Line 9) Giza Red, (Line 10) Giza 20, and M (DNA marker)

observed and 65 (83.3%) of them were polymorphic. These primers yielded 19, 22, 14, 11 and 12 bands, respectively (Table 10). The percentage of polymorphism was 100%, 100%, 64.3%, 72.7% and 58.3%, respectively. Primer SH7 yielded the largest number of bands (22

bands). Thirteen unique bands were observed. The high number of unique bands was recorded by primers SH6 and SH7 which produced five markers. Primer SH10 does not produce any marker. Genotype Giza 20 x Ori was distinguished by six markers, three of them with primer SH7 at 1429, 1172 and 906 bp, one with primer SH8 at 1806 bp and two with primer SH9 at 970 and 482 bp. The ISSR primer method is reported to produce more complex markers [40], which is advantageous when differentiating closely related genotypes.

3.3 Genetic Diversity

From the genetic similarity index (Table 11), the pair-wise genetic similarity coefficients indicated that the genotypes Shandaweel 1 and Composite 13 are closest (91%) to one another followed by Composite 13 and Giza 20 (89%). The results suggest that the genotypes are more similar to each other. The similarity due to Composite 13 is a selection in single crossing between Egyptian Deltan onion cultivars and 10 American cvs. So, in crop improvement program, these genetically similar parents could not be chosen in the crossing program for purposes of creating genetic variability. On the other hand, the lowest similarity (67%) was found between Giza 6 Mohassan and Giza 20 x Ori. The dissimilarity is owing to Giza 6 Mohassan having developed from Upper Egypt strain (Saiedi), whereas Giza 20 x Ori is a selection from single cross between Giza 20 (Lower Egypt type) with introduced cv. Ori. This means that these genotypes are the most dissimilar in their genetic level. Therefore, these dissimilar parents should be chosen in crop improvement program for creating genetic variability.

Table 10. Levels of polymorphism and unique genotypes specific bands for ten onion genotypes by ISSR analysis

Primer name	Number of total bands	Polymorphic bands	Monomorphic bands	Polymorphism %	Unique bands Genotype	MW (bp)
SH6	19	19	0	100	Giza 6 Mohassan	1435, 824, 216, 156
					Giza 20	637
SH7	22	22	0	100	Z 218 white	765
					Giza 20 x Ori	1429, 1172, 906
					Composite 8	378
SH8	14	9	5	64.3	Giza 20 x Ori	1806
SH9	11	8	3	72.7	Giza 20 x Ori	970, 482
SH10	12	7	5	58.3	-	-
Total	78	65	13	83.3		

Table 11. Genetic similarity index among ten onion genotypes based on RAPD and ISSR markers

Genotype	H.Y.28	Z218 white	Giza20x Ori	Composite 8	Composite 13	Composite 16	Shandaweel 1	Giza 6 Mohassan	Giza Red
Z 218 white	.84								
Giza 20 x Ori	.76	.75							
Composite 8	.82	.80	.79						
Composite 13	.78	.78	.76	.84					
Composite 16	.74	.77	.74	.84	.86				
Shandaweel 1	.78	.79	.77	.85	.91	.85			
Giza 6 Mohassan	.72	.72	.67	.73	.76	.79	.80		
Giza Red	.82	.81	.82	.84	.87	.84	.82	.77	
Giza 20	.82	.81	.78	.87	.89	.83	.85	.74	.87

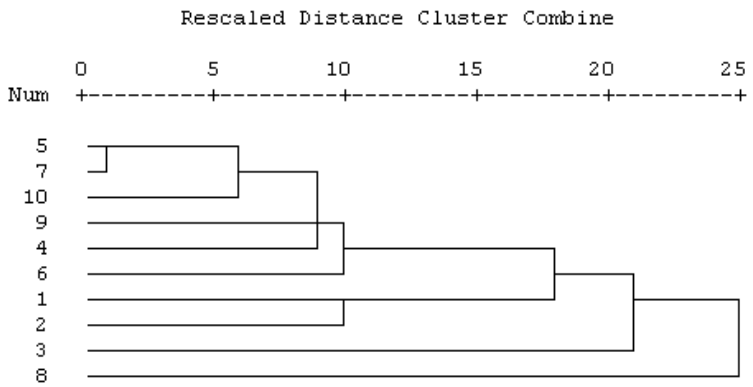

Fig. 3. An UPGMA cluster dendrogram (Jaccard coefficient) showing the genetic relationships among ten genotypes of onion based on RAPD and ISSR markers

A dendrogram based on UPGMA cluster analysis (Fig. 3) of the RAPD and ISSR data showed two clearly distinct groups of the 10 genotypes. Giza 6 Mohassan was in a separated cluster, while all other genotypes were grouped in the second cluster. The second cluster divided into two sub-clusters. One of them included the genotype Giza 20 x Ori, while the other one included the rest of the genotypes. These results give an insight into the genetic polymorphism and the possibility of their further use in breeding programs.

4. CONCLUSION

The data establish conclusively that the new promising genotype composite 13 had the best ability to produce bulb onions from sets, especially for total and marketable yield as well as average bulb weight, while the genotypes H.Y. 28 and Z 218 white ranked best for high bulb quality traits i.e. number of complete rings, Bulb total soluble solids and dry matter content. Concerning storability, H.Y. 28 had the least percentage of total bulb weight loss (%). These results provide insight into the genetic polymorphism inherent to these particular onion sets, illuminating the possibility of their further use in breeding programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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