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# Morphological and molecular genetic diversity of exotic melon germplasm

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**Morphological and molecular characterization helps identify desirable traits in exotic germplasm, expediting crop breeding. Genetic variability assessment of several domestic melon germplasm has been already analyzed in Bangladesh. There is a lack of information regarding the assessment of genetic variability in exotic melon germplasm. The study evaluated five exotic melon germplasm using morphological and molecular markers for their morphological, genetic, and yield performance. Five germplasms exhibited morphological variations in all studied characteristics. Variations were recorded among the germplasm in terms of flowering, harvesting, branching, fruit size, weight, yield, and quality. The findings indicated that the ME18 genotype germplasm achieved the greatest fruit weight (3.87), brix index (10.80), and yield (9.43). The ME26 germplasm demonstrated similar fruit weight (3.73), brix index (10.30), and yield (8.60). The study found lower-than-expected heterozygosity (0.264 vs 0.473), indicating selection pressure. Five primers generated 24 alleles across 5 germplasm. CMGA104 and CMCTT144 had 6 alleles, CMAG59 had 5, CMTA134a had 4, and CMTA170a had 3. Allele size ranged from 90 bp (CMAG59) to 415 bp (CMGA104). The polymorphism Information content values ranged from 0.301 to 0.689. The study suggests further analysis for genetic enhancement and the existence of exotic melon germplasm.**

**Key words:** Melon, morphological variations, microsatellite DNA marker, genomic DNA, genetic diversity.

## INTRODUCTION

Melon (*Cucumis melo* L.) is a cucurbitaceous (2n = 2x = 24) short-duration summer fruit crop that originated in Africa, India or Persia (Garcia-Mas et al., 2012). It is the ninth most important cultivated horticultural crop in terms of global production (Fita et al., 2012; Kong et al., 2014). Melon is one of the popular fruits due to its high vitamins, minerals, health-promoting antioxidants, ascorbic acid, carotene, folic acid, and potassium content (Lester and Crosby, 2002; Lester and Eischen, 1996). It is also beneficial to human health and facilitates to protect humans from hidden hunger. In Bangladesh, it is cultivated as a minor fruit crop and contributes 1.13% to

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the total fruit production of the country (Bangladesh Bureau of Statistics and Informatics Division (SID), 2016). Besides different natural adverse conditions (biotic and abiotic stress, tunnel preparation, unavailability of hormones) lack of superior melon varieties are one of the vital problems for melon cultivation and production worldwide including Bangladesh. Utilization of diverse crop germplasm with higher and sustainable production potential is a prerequisite for agricultural productivity to meet the growing population's food demand. Molecular markers and marker-assisted selection have been used efficiently for diversity study, germplasm curation, and improvement (Dhillon et al., 2009; Malik et al., 2014). Diverse variation is found in different morphological characteristics of melon fruits such as shape, color, size, texture, taste, yield, maturity time, and harvesting time. Among the identified 19 intraspecific horticultural groups *C. melo*, is the most important commercial melons grown in America, Europe, and Asian countries like Bangladesh (McCreight, 2006). In Bangladesh, a total of 131 germplasm of melon were collected and conserved in the Plant Genetic Resources Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI).

Besides these collected germplasm, diverse exotic germplasm could be used as an important reservoir of genetic variability for melon breeding in Bangladesh. Genetic variability assessment and suitable genotype selection are the prime benchmarks for genetic improvement of crop species. Morphological trait-based evaluation of genetic diversity has several restrictions as the majority of morphological attributes are significantly influenced by the developmental stage of the plant and environmental factors. Molecular markers play a crucial role in understanding genetic diversity and the relationship of species since these markers are highly polymorphic and exhibit genetic distinction without any environmental impact and help to significantly lead to target traits without the need for direct control of the phenotype (Baruah et al., 2019; Pandey et al., 2018). Kaçar et al. (2012), demonstrated high levels of polymorphism associated with SSR loci (simple sequence repeats) in *C. melo.* Thus, morphological and SSRs together can be effectively used for the genetic characterization of melon germplasm. Bangladesh has limited melon germplasms (a total of 131 germplasm of melon) and among these, several could be used as a source of genetic material from the point of view of its exploitation for the improvement of yield in general, and fruit quality. However, inbred lines derived from landraces, wild relatives, and exotic cultivars belonging to various horticultural groups are an important reservoir of genetic variability for melon breeding in Bangladesh.

The present study evaluates the morphological, genetic, and yield performance of some exotic melon germplasms collected from South Korea to test their adaptability in Bangladeshi environments. The information gained in this study will be highly useful for the effective utilization and conservation of exotic melon germplasm and enrich Bangladesh as well as world germplasm centres.

## MATERIALS AND METHODS

### Plant

The experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the fiscal year (2021-2022). Seeds of 15 (fifteen) exotic germplasms (SCNU1154, EML61, IML206, Enga, ME5, ME6, ME14, ME18, ME23, ME25, ME26, ME35, Cat697, Sweet honey, Red diamond) were collected from the Department of Horticulture, Sunchon National University, South Korea. Germplasms were evaluated at the Experimental Farm of Sher-e-Bangla Agricultural University (30° 55´ N, 75° 48´ E, and 248 m amsl).

### Seedling growth and morphological traits evaluation

Seeds are shown in plastic rectangle (Figure 1A and B) trays with a mixture of clay soil and farmyard manure (1:1). 25 days old seedlings were transplanted in the main field for further growth and development (Figure 1C). Standard agronomic melon culture practices were followed properly (Koffi et al., 2008). The seedlings were transplanted in previously prepared pits (50 cm depth and 20-25 cm diameter) at 3 m (row to row) and 0.60 m (vine to vine) spacing. A complete randomized block design (CRBD) with three replications was followed in the experiment**.** A basal dose of N, P, K (5:10:5 g of Urea) at 140 and 100 kg $ha^{-1}$ respectively was applied. The fertilizer was kept in circular rings 8-10 cm apart from the root around each plant for side dressing and at basal application they were mixed with the soil in each pit. Water was provided with the help of a watering can. Owing to environmental variations like temperature, rainfall, and humidity, all seedlings (exotic germplasms) were not properly full-grown in the field. Data on different morphological characters, viz. root system, habit, stem, tendril type, flowering type, fruit type, fruit color, fruit shape, and fruit firmness (Malek et al., 2012) were recorded and harvesting of fruits (Figure 2) was performed on yellow color or breaking stage with the help of rust-proof still scissors.

### Genomic DNA extraction and purification

Fresh, disease free, and insect-free leaves from 20 to 25-day-old seedlings of each melon germplasm were used for DNA extraction. CTAB (Cetyl Trimethyl Ammonium Bromide) procedure (Doyle et al., 1996) with some modifications was used for extraction of DNA from 100 mg leaf sample. A nano-Drop Spectrophotometer (Thermo Fisher Scientific, USA) was employed for DNA quantification between the readings at 260 and 280 nm. The concentration of the DNA was calculated at 260 nm and used for further analyses.

### Primer selection and DNA amplification

Previously published five SSR markers (Molla et al., 2017) were selected in the present study for genetic diversity assessment (Table 1). DNA amplification was performed in a final reaction volume of 10 µl containing 1 µl forward primers (10 p.m.), 1 µl reverse primer (10 p.m.) and 1 µl (40 ng) genomic DNA, 1.5 µl 10 × buffer, 0.6 µl $MgCl_2$ (25 mM), 0.18 µl dNTP (25 mM), Taq polymerase 0.3 µl (5U/µl), 4.42 µl $ddH_2O$. The PCR cycling program was set as initial denaturation at 94°C for 4 min followed by 35 cycles at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min. 1.5% agarose

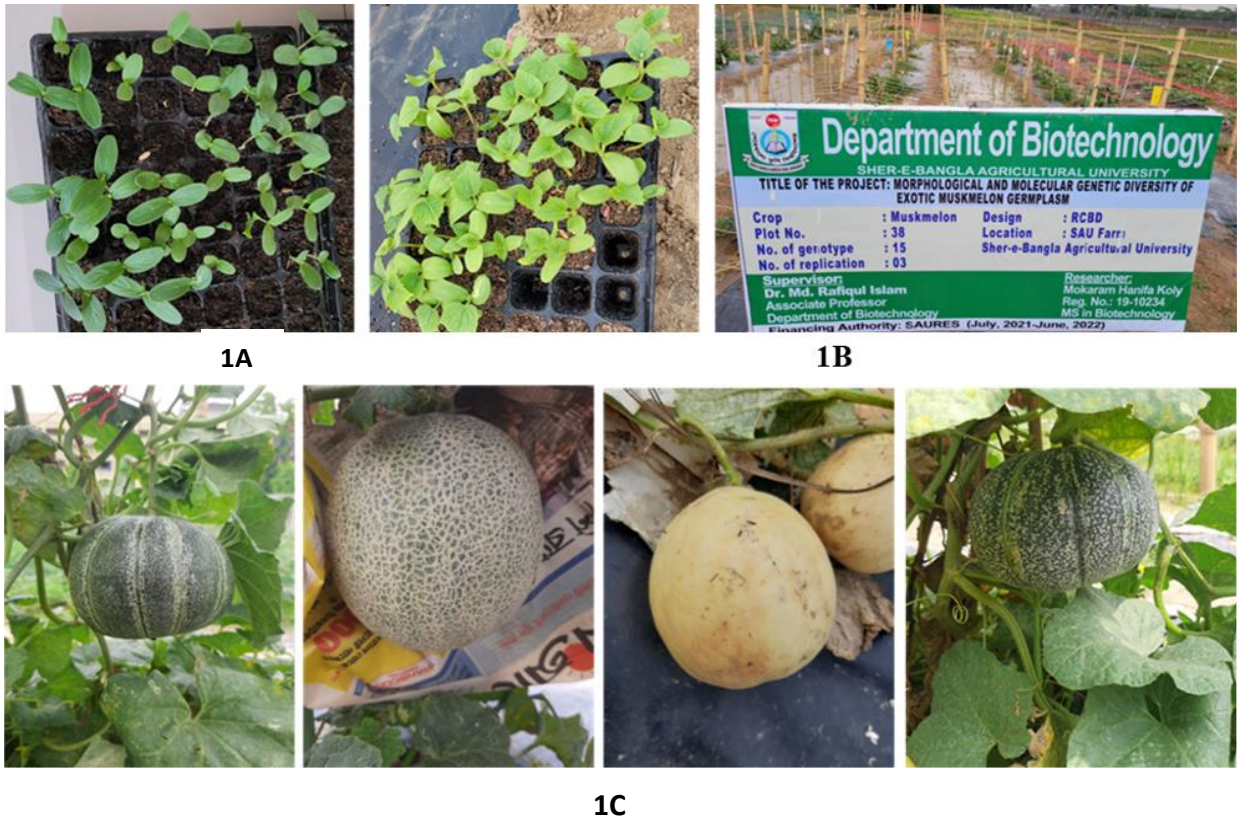


**Figure 1.** View of the field experiment. (A) A representative sample of growing seedlings, (B) Seedling transplanted to the field, and (C) Growth and development of muskmelon.

gel having 1 × TBE buffer was employed for the separation of amplified DNA fragments and DNA bands were observed under the Thermo Scientific gel documentation system.

### Statistical analysis

Data of all morphological and biochemical parameters of exotic melon germplasm was subjected to analysis of variance (ANOVA) using Randomized Complete Block Designs (RCBD) following procedures of MINITAB 19 statistical software packages (Minitab Inc., State College, Pennsylvania, USA).

## RESULTS

The seedlings of fifteen exotic germplasm of melon seedlings were transplanted in the Sher-e-Bangla Agricultural University farm but all seedlings (collected from South Korea) were not properly grown in the field. In Bangladesh, February to March, 2022 average temperature 32.1°C, rainfall 75.6 mm, and humidity 86% (https://weatherspark.com/h/y/111858/2022/Historical-Weather-during-2022-in-Dhaka-Bangladesh) (Malek et al., 2012) whereas in South Korea is 24.5°C, 672.8 mm and55.4% (https://www.statista.com/topics/8726/weather-in-south-korea/#editorsPicks) respectively. Due to environmental variation, only five of the fifteen exotic seedlings survived which were screened throughout the crop growth period and produced fruit (Figure 2).

The result indicated a significant correlation and interaction between the environment and genotype especially those related to yield and yield contributing characters. Therefore, the five germplasms were employed for subsequent morphological, quantitative, and molecular characterization.

### Qualitative morphological traits

Significant ($p<0.05$) variations among the germplasms for all the morphological traits (vegetative, floral, fruit etc.,) were observed (Figure 2 and Table 1). Ten qualitative traits (discontinuous variables) were analyzed where fruit characters exhibited the highest level of variation (Table 1). Four fruit shapes were observed whereby globular shapes were found in two germplasms followed by oval–roundish, round/elongated, and spherically elongated in each single germplasm. Four distinct fruit skin colours were observed (light green, greenish white, light yellow, deep yellow, and light yellow) in each of the germplasm. Firmness is an important qualitative trait for maintaining the quality and shelf life of melon fruits. The majority (three among the five) of the germplasm showed crispy

**Table 1.** Vegetative and floral traits of five exotic muskmelon germplasm.

| Character | ME5 | ME18 | ME23 | ME26 | Cat697 |
|---|---|---|---|---|---|
| Root system | Extensive | Extensive | Extensive | Extensive | Extensive |
| Habit | Trailing annual | Trailing annual | Climbing annual | Trailing annual | Climbing annual |
| Stem | Aerial | Aerial | Aerial | Aerial | Aerial |
| Tendril type | Simple | Simple | Simple | Simple | Branched |
| Flowering type | Solitary | Solitary | Solitary | Solitary | Solitary |
| Fruit type | Simple | Netted | Pepo | Simple | Simple |
| Fruit color | Light green | Green whitish | Light yellow | Deep yellow | Light yellow |
| Fruit shape | Oval – roundish | Globular | Round/ elongated | Globular | Spherically elongate |
| Fruit firmness | Hard | Crispy | Crispy | Crispy | Hard |

ME= Melon Exotic.


**Figure 2.** Harvested fruits of five muskmelon germplasm **a.** ME5, **b**. ME18, **c.** ME23, **d.** ME26 and **e.** Cat697.

firmness whereas the remaining two had hard firmness. A short (15 days) to medium (25–30 days) shelf life was observed of all the five germplasms. Variations are also found in fruit color (white, yellow, and orange) among the germplasm (Figure 2). The highest yield/plant was exhibited in ME26, while ME5 recorded the lowest yield. In addition, fruits from all of the germplasms are non-splitting types (Figure 2). Little or no variation was found in other traits (leave, stem, tendril) and growth habits of the five-germplasm studied (Table 1). All germplasms had five petals and sepals yellow and greenish in color respectively. There was no variation observed in the case of the root system, flowering type, and stem among the germplasms.

**Table 2.** Quantitative traits of five muskmelon germplasms.

| Characters | ME5 | ME18 | ME26 | ME23 | Cat697 | Mean | Std. | CV% |
|---|---|---|---|---|---|---|---|---|
| Days of first flowering (days) | 36.33[a] | 28.33[b] | 30.33[b] | 29.67[b] | 34.67[a] | 31.87 | 2.81 | 8.82637 |
| Plant height (cm) | 117.70[d] | 120.16[c] | 152.00[a] | 135.85[b] | 154.36[a] | 136.02 | 14.02 | 10.3057 |
| Number of primary branches | 11.33[a] | 9.33[b] | 12.33[a] | 11.33[a] | 12.00[a] | 11.27 | 0.95 | 8.43757 |
| Length of primary branches (cm) | 55.73[b] | 51.80[c] | 57.17[a] | 59.13[a] | 40.80[d] | 52.93 | 5.95 | 11.2496 |
| Harvesting time (days) | 73.48[b] | 61.23[d] | 78.64[a] | 68.33[c] | 64.33[d] | 69.20 | 5.71 | 8.24703 |
| Fruit length (cm) | 22.03[b] | 19.90[c] | 21.33[b] | 27.63[a] | 19.93[c] | 22.17 | 2.61 | 11.7528 |
| Fruit diameter (cm) | 35.03[a] | 25.47[c] | 26.43[c] | 32.13[b] | 27.5[c] | 29.31 | 3.34 | 11.4041 |
| Number of fruit/plants | 3.33[a] | 2.67[b] | 3.33[a] | 3.33[a] | 3.00[a] | 3.13 | 0.24 | 7.76911 |
| Fruit weight (kg) | 2.83[b] | 3.87[a] | 3.73[a] | 2.93[b] | 2.83[b] | 3.14 | 0.33 | 10.3542 |
| Brix Index | 9.33[a] | 10.80[a] | 10.30[a] | 8.30[b] | 8.03[a] | 9.35 | 0.99 | 10.5491 |
| Yield/plant (Kg) | 7.38[b] | 9.43[a] | 8.60[a] | 7.43[b] | 6.73[b] | 7.92 | 0.88 | 11.1625 |

Values for the same letter are not significantly different ($p<0.05$) as per Tukey's pairwise comparison.

### Quantitative morphological traits

Significant variation was exhibited for various quantitative traits among the germplasms (Table 2). ME5 required the highest days of first flowering (36.33) which was statistically similar to Cat697 (34.67) whereas ME18 had the lowest days of flowering (28.33) which was statistically similar to ME23 (29.67) and ME26 (30.33) (Table 3).

The highest plant height was observed in Cat697 (154.36 cm) which was statistically similar to M26 (152.00 cm). The lowest plant height was found in ME18 (120.16 cm). All the germplasms expressed excellent branch length: ME5-55.73, ME18-51.80, ME26-57.17, ME23-59.13, Cat697-40.80 cm and fruit length: ME5-22.03, ME18-19.90, ME26-21.33, ME23-27.63, Cat697-19.93 cm. The highest number of fruit/plant (3.33) was counted at ME5 which was statistically similar to all other germplasms. Statistically similar highest brix index (10.80, 10.30 respectively) was observed in ME18, ME26, and M5, respectively whereas the lowest was found in Cat697 (8.03), and M23 (8.30), respectively. Considerable variation was observed in fruit diameter where M5 showed the highest (35.03 cm) and M18 showed the lowest (25.47 cm) fruit diameter. Almost similar fruit weight was recorded from all of the five germplasm. At 61.23 days after planting (DAP) the highest yield/plant (9.43) was harvested from ME18 whereas the lowest yield/plant (6.73) was recorded from Cat697 at 64.33 DAP.

### Molecular characterization

All SSR markers were polymorphic and a total of 24 alleles were identified in five germplasm with an average of 4.8 alleles per locus (Figure 3 and Table 3). Two primer pairs CMGA104 and CMCTT144 amplified the highest number of alleles that is 6 each and primer CMTA170a amplified the lowest number of alleles that is 3 (Table 3). The length of the amplified fragments ranged from 90 to 410 bp and the allele frequencies ranged from 0.291 for locus CMGA104 allele to 0.530 for locus CMAG59. The polymorphic information content (PIC) value ranged from 0.301 (CMAG59) to 0.689 (CMGA104) with a mean value of 0.470 across all accessions (Table 3). The observed heterozygosity (Ho) ranged from 0.000 (CMGA104, CMTA170a) to 0.478 (CMTA134a) with an average Ho at 0.264 (Table 3).

## DISCUSSION

Morphological and molecular characterization and assessment of germplasm facilitated the exploitation, and advancement of crop genetic resources which could serve as a source of genes for the development of cultivars in response to climate change. The collection of germplasm from different ecological conditions is an important way to produce a high genetic variation and selection of high-quality and high-yielding cultivars for breeding programs. In the present study, 15 exotic germplasms of melon were assessed for their genetic diversity and adaptation in Bangladeshi environmental conditions, based on some qualitative and quantitative morphological characteristics. Furthermore, SSR markers-based genetic diversity analysis was also performed for the assessment of germplasms. The survival of only five Korean melon germplasm among the fifteen in Bangladesh indicated a close relation between the crop existence and the environment which may be influenced by climate change. Morphological characterization of melon germplasms showed significant variation among different phenotypic traits especially in fruit characteristics. The result agreed with earlier findings where different melon germplasm showed different morphological traits (Ajuru and Okoli, 2013; Molla et al., 2017). Morphological parameter is an important indicator for the study and

**Table 3.** Variability of SSR markers used for genetic analysis of exotic muskmelon germplasm.

| Locus | NA | AS (bp) | MAF | OH | EH** | PIC |
|---|---|---|---|---|---|---|
| CMAG59 | 5 | 90, 130, 150, 190, 410 | 0.530 | 0.472 | 0.305 | 0.301 |
| CMGA104 | 6 | 95, 135, 150, 200, 250, 415 | 0.291 | 0.000 | 0.691 | 0.689 |
| CMCTT144 | 6 | 90, 140, 150, 192, 245, 410 | 0.302 | 0.369 | 0.543 | 0.541 |
| CMTA170[a] | 3 | 130, 190, 410 | 0.528 | 0.000 | 0.452 | 0.449 |
| CMTA134[a] | 4 | 130, 159, 200, 410 | 0.481 | 0.478 | 0.374 | 0.372 |
| Mean | 4.8 | - | 0.426 | 0.264 | 0.473 | 0.470 |

NA=Number of Allele, AS= Allele sizes, MAF= Major Allele Frequency, OH= Observed Heterozygosity, EH= Expected Heterozygosity, PIC=Polymorphic Content.

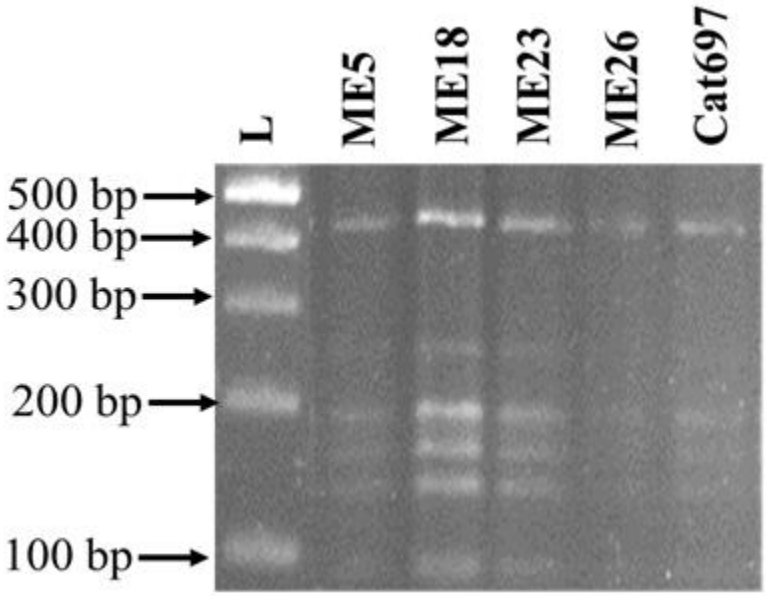


**Figure 3.** SSR profiles of five muskmelon genotypes at the locus; L: 500 bp DNA ladder

classification of any crop germplasm (Koffi et al., 2008). Breeders primarily and generally, depend upon the extent of morphological variation for utilization and conservation of crop germplasm. Thus, the study of morphological parameters of Korean melon germplasms could be a valuable indicator for the conservation, classification, and assessment of melon germplasm. SSR markers are an important tool for genetic diversity analysis and determining polymorphic associations among various melon accessions (Fergany et al., 2011; Kaçar et al., 2012). In this study, five highly informative primers were used for molecular characterization of melon germplasm and found that all SSR markers were polymorphic, and the average polymorphic content (PIC) was 0.47 which showed close similarity to the report of Hu et al. (2015). The mean number of alleles in the present study was 4.8 which is lower than the mean number of alleles reported by Monforte et al. (2004), they reported 6.3 alleles on 27 wild and cultivated melons. Lower mean alleles of exotic melon germplasm might be due to less diversity among the accessions used in this study. The level of genetic variability that exists in any population can easily be determined using the heterozygosity level of that population. The lower the heterozygosity among germplasm the lower their genetic variability (Hu et al., 2015). Despite the significant morphological variation observed among the studied germplasms and allogamous reproductive system of melon the observed mean heterozygosity (Ho = 0.264) was lower than the expected one (He = 0.473) that is the 24 alleles of the 5 SSR loci were not adequate to discriminate all the 5 germplasm of melon. For example, the M18 and ME23 germplasm pair was genetically closely related for the loci analyzed which indicated an excess in homozygosis of melon accessions or poor relation in the diversity based on morphological characters and molecular markers.

Therefore, use of an additional higher number of primers can be used for the analysis of genetic variation among germplasms.

Overall, the results of this investigation added important information about the survival and variation of exotic melon germplasm. Both morphological and molecular features are important for the sustainable conservation and existence for the better understanding of exotic melon genetic resources. Based on the morphological and molecular characteristics ME18 and ME26 germplasms were selected suitable as exotic germplasms for cultivation in our country. Thus, the present study revealed that variation investigated at the morphological traits among the studied germplasm, ME18 was more due to environmental conditions. The present study also concluded that SSR is a better technique for the study of genetic diversity. However, a higher number of variable primers and other modern techniques should be employed for further high level of investigation of genetic diversity among the germplasms.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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