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Development of Cantaloupe (*Cucumis melo*) Lines Carrying *Vat* Gene with Favorable Fruit Traits

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Abstract

The most popular Iranian cantaloupe ‘Samsoori’ is highly susceptible to devastating viruses transmitted by *Aphis gossypii*. A dominant gene (*Vat*) causing resistance to the aphid and viruses was detected in ‘Ginsen Makuwa’ in spite of its low fruit quality. They were crossed and the segregating offspring were assessed for combining favorable traits with *Vat* gene. In the F_2 population, moderate to high broad-sense heritability estimates were found for measured traits including, fruit weight (0.78) and soluble solid content (SSC) (0.7). The F_3 families were significantly different from each other for earliness, fruit shape indices, cavity, flesh thickness, SSC, and fruit numbers per plant. Resistant and susceptible plants were determined by genotyping 210 plants in F_4 generation using a dominant DNA marker for the resistant allele of *Vat* gene. Out of 15 selected F_3 families, four were susceptible, three were homozygote resistant and six showed segregation in their progeny for the *Vat* gene. Selection assisted by *Vat* gene marker was a very useful and applied approach for the identification of healthy plants along with phenotypic selection.

Introduction

Melon (*Cucumis melo* L.; Cucurbitaceae; 2n = 2x = 24), is a cross-pollinated horticultural crop with a wide diversity in fruit shape, flavor traits, and climate adaptation. Iran is an important center of variation and also rank in the top five production countries in the world for melons (FAO 2019). Many high-quality cantaloupe landraces or indigenous cultivars are cultivated in large acreages in Iran due to their unique flavor and shape and early concentrated fruiting. Despite consumer preference for local cultivars, the majority of them are susceptible to biotic stresses such as fungal and viral diseases, which cause annual colossal yield loss. Thus, in recent years, a number of farmers prefer to pay for commercial hybrid seeds provided by seed companies to assure healthier plants with higher yields.

Viral diseases are the most devastating diseases of melons, which cause a severe damage to the fruit and lower its yield and quality. The most prevalent viruses in melons are *cucurbit aphid borne yellow virus* (CABYV), *cucumber mosaic virus* (CMV), *cucurbit yellow stunting disorder virus* (CYSDV), *zucchini yellow mosaic virus* (ZYMV), *papaya ringspot virus* (PRSV), and *watermelon mosaic virus* (WMV). The most effective approach to control viral diseases is by developing resistant cultivars (Prohens-Tomás and Nuez 2008). There is a line of research describing the virus resistance in melon. A breeding line derived from PI 414723 with resistance to three potyviruses (WMV, ZYMV, PRSV) and powdery mildew. All four resistances displayed dominant monogenic inheritance and a genetic linkage was observed between resistance to WMV and ZYMV (Anagnostou et al. 2000). It was displayed that the resistance to CMV is oligogenic, where different loci confer resistance to different CMV strains, but not necessarily quantitative (Essafi et al. 2009). Diaz et al. (2011) constructed an integrated genetic map associated with economically important traits in melon including four virus resistance genes.

Furthermore aphids are also important pests that harm crops either directly through the feeding of the phloem texture or indirectly through the transmission of viral diseases (Ng & Perry, 2004). Most plant viral infections can be partially controlled by elimination of their vectors; however, this is not a recommended method due to environmental concerns by pesticide application. Therefore, the use of resistant cultivars is the best approach for controlling aphids and whiteflies. The only aphid species that can colonize effectively on melon is *Aphis gossypii* which is the vector of CMV, ZYMV, PRSV, and WMV (Dogimont et al., 2014). By means of a RIL population developed from a cross between Vedrantais and PI161375, two minor QTLs were found for resistance to biotype B of *Bemisia tabaci*, and a major dominant gene (*Vat*) for resistance to *Aphis gossypii* (Boissot et al., 2010). It was shown that the virus aphid transmission (*Vat*) gene is located on linkage group 5, and confers double resistance to *Aphis gossypii* as well as the viruses transmitted by it. The amino acid sequences of this gene for resistance and susceptible alleles were well characterized, and the DNA sequence of the resistance allele was determined (Dogimont et al., 2014).

The availability of suitable molecular markers encourages plant breeders to use them for marker-assisted selection and speed up the breeding process (Sousaraei et al., 2018). This method can overcome the limitations associated with phenotypic selection (Foolad & Panthee, 2012), especially for virus resistance that phenotypic selection is often not straight forward or costly; the use of molecular markers can be a very beneficial tool.

Cultivar ‘Samsoori’ is the main commercial cantaloupe cultivar in Iran. It is well-known for its early concentrated fruiting, striped and fine netted rind, and desirable juicy flavor. However, this cultivar is highly susceptible to viruses and its sugar content is not satisfactory. In a breeding program, ‘Samsoori’ for improvement was crossed with the Korean melon ‘Ginsen Makuwa’ which shows high resistance to viruses and has high sugar content. The main objective of this study was to identify the offspring in a F_3 generation of a cross between ‘Samsoori’ and ‘Ginsen Makuwa’ carrying *Vat* resistant allele (Boissot et al., 2016) with desirable traits such as high soluble solid content (SSC), proper shape and fruit flavor, early harvest, and high yield. However, initially it was necessary to clarify the efficiency of the molecular marker to distinguish the resistant and susceptible allele of *Vat* gene in the segregating population.

Materials And Methods

Plant material

The melon genotypes used in this study were ‘Samsoori’, ‘Ginsen Makuwa’, and the derivative progenies of their cross. The fruit of 'Samsoori' is round to oblate with leathery rind and a clear vein tract and thorough netting. It has full slip from its vine once ripe, and its flesh is juicy green. In contrast, the fruit of 'Ginsen Makuwa' is oval shape with a very thin rind and a ridged surface, no netting and yellowish-white color. The flesh of fruit is crunchy texture and white color (Fig. 1).

For crossing Samsoori was used as the pistillate and Ginsen Makuwa as the staminate parent, here thereafter referred to as P_1 and P_2, respectively. The initial cross was made in the spring of 2017, at the greenhouse of Auraihan campus, Pakdasht, Iran. Ten seeds were germinated and grew to maturity and self-pollinated to create the F_2 population (n = 350). In the spring of 2018, each parent's seed, F_1s, and F_2s were sown in potting trays. Within a month after sowing the seed, the plants were hardened off and transplanted to the field, in a randomized complete block design experiment in three replications. Each block consisted of one row (10 seedlings) of P1, P2, and F_1 and five rows of F_2 generation. During the flowering time, each plant in the F_2 generation was self-pollinated. Thus, on the day of anthesis, male flowers of the same plant were used for pollinating androgynous flowers, which both were covered in the previous evening.

Phenotypic Evaluation of P1, P2, F1 and F2 generations

At the end of the growth season, matured fruits were harvested. Earliness of fruits was recorded as the number of days from the date of transplanting of seedlings to the field until the harvest date. Fruit yield in each plant was calculated with summing up the weight of all fruits from each plant. Other traits such as fruit length, width, flesh thickness, and seed cavity were measured for each fruit after longitudinal cutting. Soluble solid content (SSC) was measured using a handheld Refractometer (Master-20PM, Atago, Japan) from fruit juice extracted by squeezing a piece from the middle of each fruit.

Phenotypic selection of desirable plants in F2

During the growth season, the healthy plants of F_2 population were labeled. Disease free plants with early mature fruit, high SCC (brix index), acceptable fruit shape (round shape with thorough netting and clear vein tracts), desirable flesh features (green, juicy and thick) were selected within the F_2 population. Out of 150 plants in the F_2 population, 20 were selected. Since each plant had at least one fruit developed from self-pollinattion, they produced 20 different F_3 families.

Phenotypic evaluation of F3 families

In spring 2019 the seedling of 20 F_3 families as well as parental genotypes (in total 22 treatments) were evaluated for different traits in the experimental station of Aburaihan campus, Pakdasht, Iran. The seedlings were transplanted in randomized complete block design with three replications. Each plot consisted of a row with ten plants. Important traits were measured the same way as the previous generation, and during flowering time, all F_3 plants were self-pollinated to produce F_4 generation. Based on the collected data, a total of top rated 70 plants were selected within and between rows of F_3 families. Subsequently, three seeds of each selected F_4 plant were sown in the greenhouse to maturity, and their leaf tissue was used for DNA extraction and genotyping.

Parental and Cultivar Survey for the *Vat* gene

It was necessary to verify the reliability and efficiency of the molecular marker before applying it for selection of resistant plants in F_4 families. Thus, six Iranian native cultivars and 11 other genotypes (Table 1), were clarified for the presence or absence of the *Vat* gene. Then F_4 families were tested by extracting DNA from all three plants of 70 families beside 'Ginsen Makuwa', 'Samsoori' and F1 generation as genetic controls.

Table 1
Name and characters of genotypes tested for the presence or absence of the *Vat* gene.

Name of genotype	type	Supplied from
Samsoori	Local cultivar	Varamin - Iran
Saveh	Local cultivar	Savezh - Iran
Shah-abadi	Local cultivar	Isfahan - Iran
Rish-baba	Local cultivar	Kashan - Iran
Garmak	Local cultivar	Isfahan - Iran
Tile-torogh	Local cultivar	Mashhad - Iran
Cory (Galia)	Commercial hybrid	Seminis Co.
Kogane 9-go Makuwa	Oriental accession	IPK genebank
Kanro Kiku Makuwa	Oriental accession	IPK genebank
Ginsen Makuwa	Oriental accession	IPK genebank
Kogane Sennari Makuwa	Oriental accession	IPK genebank
Charentais Fom-1	Breeding line	donated by M. Pitrat
Charentais Fom-2	Breeding line	donated by M. Pitrat
Isabelle	Breeding line	donated by M. Pitrat
Ananasi	Commercial cultivar	Market
Crenshaw	Commercial cultivar	Market
Honey Dew	Commercial cultivar	Market

DNA was extracted from leaves of each genotype by CTAB method (Doyle & Doyle, 1990). A pair of specific primers (forward primer 5' CCTCAGTTCTTCAACATTTGATTTCTC 3' and reverse primer 5' CCATCACATTTATAAACCCGAAGATG 3')

were used for amplifying a part of the resistant allele of *Vat* gene (Dogimont et al., 2014). The PCR product of this primer pair is 121bp, and no amplification is expected for the susceptible allele. The PCR reaction was carried out by LightCycler® 96 Real-Time PCR machine (Roche Diagnostics, Mannheim, Germany). HiFi master mix with Hot Start *Taq* polymerase enzyme (Custombiotech, Roche) was used for PCR reaction. The 20µl reaction contained 2 µl of template DNA (30 ng/µl), 4 µl of HiFi Master-mix (5X), and 1µl of each primer (10 µM). The amplification condition comprised pre-incubation (15 min at 95ºC) for activation of *Taq* polymerase enzyme, followed by 40 cycles of 95ºC for 15s, 61ºC for 20s, and 72º for 20s. To test the specificity of PCR, the melting curve of PCR products were obtained and compared to each other. Gel electrophoresis of PCR products was conducted on 2% agarose gel containing TAE at 100 V for 2 h. The safe stain was used for staining of DNA. Amplified bands were visualized under ultraviolet light.

Data analysis

The analysis of variance of phenotypic data was carried out using SAS software version 9.0 (Cary, NC). Outlier data points were detected by visually inspecting the scattered plots or using any data point with larger two standard deviations from the mean, and were removed from the dataset for further analyses. Comparison of the means of different treatments was carried out using the Duncan's Multiple Range test.

The following equation was used for calculating broad-sense heritability in F_2 population (Brown et al., 2014).

$$H_b = \frac{Vg}{V_{F2}} \quad (1)$$

Where H_b is broad-sense heritability, V_{F2} is the phenotypic variance of F_2 population. Vg was calculated with the following formula:

$$V_g = V_{F2} - \frac{V_{P1} + V_{P2} + 2V_{F1}}{4} \quad (2)$$

Results And Discussion

Phenotypic evaluation of P1, P2, F1, and F2

Analysis of variance among different generations showed significant differences for most traits. Table 2 shows the mean comparison of different generations for measured traits. There are significant differences for all traits except yield that was not significantly different among different generations. Substantial diversity for different traits was expected as the parents belonged to different groups; 'Samsoori' is fit to cantalupensis while 'Ginsen Makuwa' is an oriental melon belonging to conomon cultivar group (Pitrat, 2017). The phenotypic characteristics of F_1 progeny were intermediated (Fig. 1) and in F_2 progeny were segregating between parents.

Table 2
Mean comparison of different generations for the measured traits as well as the minimum and maximum values of the F_2 plants and broad sense heritability.

	Generation				Minimum	Maximum	Broad Sense
Traits	P_1	P_2	F_1	F_2	in F_2	in F_2	Heritability
Fruit number per plant	2.06c	5.66a	3.01bc	4.05b	1	12	0.57
Yield (kg/plant)	1.33a	1.37a	1.56a	1.7a	0.21	4.81	0.7
Fruit weight (kg)	0.72a	0.23c	0.52ab	0.45b	0.14	1.67	0.78
Earliness (days)	99.2bc	104.3a	97.4c	101.4ab	87	121	0.80
Fruit length (mm)	131a	104b	128a	122a	77	208	0.71
Fruit width (mm)	112a	80c	108ab	100b	64	176	0.73
SSC%	10.2c	12.2b	14.6a	12.3b	7	18.3	0.70
Flesh thickness (cm)	2.3a	1.4d	1.9bc	1.8c	1.1	3.4	0.64
Cavity width (cm)	6.8a	4.2c	5.7b	5.3b	3.4	9.5	0.64

P_1: 'Samsoori', P_2: 'Ginsen Makuwa', Earliness: Days from transplanting to harvest, SSC (%): soluble solid content. Means with different superscript letters are significantly different from each other according to Duncan multiple range test ($p \leq 0.01$).

Moderate to high values for broad-sense heritability for most traits show that this population has the potential for improvement via genetic selection. For most traits in the F_2 population, transgressive segregation was observed, as the range of data in F_2 population is broader than the difference between parents. This result may be explained by the fact that parental genotypes are genetically distantly related from each other.

As an index for sweetness between two parents, a significant difference was observed for soluble solid content (SCC%). A wide range in F_2 population for this trait provides an opportunity for the selection of genotypes with higher sugar content. Low sugar content in Iranian landraces was reported before (Pouyesh et al., 2017); therefore 'Ginsen Makuwa' with high sugar content in addition to its virus resistance appears to be a right complementary parent for improvement of 'Samsoori'.

Although the disease severity index could not be measured in the field accurately, but the difference of the parents for the level of incidence and segregating of the trait among offsprings was quite obvious.

Evaluation of traits in F3 generations

Analysis of variance (ANOVA) revealed highly significant differences among treatments for all measured traits (Table 3). In order to select the best families, means of different traits was compared among 22 treatments. Table 4 illustrates the characteristics of different F_3 families, along with parental genotypes. The number of fruits per plant in 'Samsoori' was on average 2.5 while for 'Ginsen Makuwa' it was 8.1. Fruit weight of 'Samsoori' was higher than that of 'Ginsen Makuwa'. In F_3 families, a wide range was observed for these two traits. Also, there are two families that are significantly had higher SSC than that of 'Samsoori' which means selection for this trait has been led an increase in the sweetness of progenies. The response to selection was more evident for fruit shape (round or oval, netting and vein tract), flesh color, and texture. In Table 5 the genotypes were categorized based on fruit-related traits. The flesh color, vein tract, netting, and flesh texture

are differentiated by nominal scale, and the shape index is the ratio of fruit length to width. Considerably the frequency of desirable plant type (round fruit with netting and vein tract, juicy green flesh) in most families is higher than unfavorable ones. The length to width ratio for all families is near 1 in contrast to this ratio for 'Ginsen Makuwa', which is 1.37. These findings confirm the idea that netting skin, vein tract, flesh color, and fruit shape are controlled with oligo-genes with high heritability (Dogimont, 2011).

Table 3
Mean squares of ANOVA for different traits measured in 22 treatments (20 F_3 families along with P_1 'Samsoori' and P_2 'Ginsen Makuwa' as controls). The experiment was conducted as a randomized complete block design with three replications.

S.O.V.	df	Earliness	Fruit weight	Fruit length	Fruit width	Cavity	Flesh thickness	SSC	Yield	No. of fruits per plant
Replication	2	31.08	36090*	2.46*	2.50**	0.77**	0.021	3.00**	4.87**	7.9**
Treatments	21	21.13*	77831**	2.76**	3.32**	1.03**	0.19**	4.00**	0.542	4.7**
Error	42	10.45	10076	0.60	0.44	0.15	0.056	0.36	0.495	1.11
C.V. (%)		3.16	14.6	6.9	6	6.6	8.8	6.07	29	29

Table 4
Means of parents (P1: 'Samsoori', P2: 'Ginsen Makuwa') and F3 families measured for different traits.

Parents and F3 families	Earliness	Fruit Weight (g)	Fruit Length (cm)	Fruit Width (cm)	Cavity (cm)	Flesh thickness (cm)	SCC %	Yield (kg/p)	No. of fruits per plant
P1	101.26^{ab}	1012.50^{a}	11.32^{abc}	12.98^{a}	6.81^{ab}	3.18^{a}	9.40^{c}	2.7a	2.5cd
P2	103.75^{ab}	353.68^{g}	11.58^{abc}	8.41^{g}	4.25^{g}	2.11^{e}	14.02^{a}	2.9a	8.1a
B4101	96.97^{b}	448.13^{fg}	9.47^{d}	9.17^{ij}	4.82^{fg}	2.51^{cde}	10.16^{c}	1.9a	4.3bcd
B682	106.62^{a}	938.21^{ab}	12.65^{a}	12.52^{ab}	6.38^{abc}	3.01^{ab}	8.88^{c}	2.45a	2.6cd
B692	102.79^{ab}	940.74^{ab}	11.34^{abc}	12.41^{abc}	6.52^{ab}	2.84^{abc}	9.86^{c}	2.6a	2.7cd
B133	104.57^{ab}	706.96^{cde}	10.75^{bcd}	11.13^{cdefg}	6.27^{abcd}	2.47^{cde}	10.83^{b}	2.01a	2.8cd
B872	103.19^{ab}	606.60^{def}	10.70^{bcd}	10.61^{fgh}	5.66^{bcdef}	2.59^{bcd}	8.85^{c}	2.15a	3.5bcd
BT13102	99.40^{ab}	540.45^{defg}	10.41^{cd}	10.19^{ghi}	5.21^{ef}	2.65^{bcd}	11.92^{b}	1.9a	3.4bcd
B13103	102.11^{ab}	843.66^{abc}	12.57^{a}	11.74^{abcdef}	6.09^{abcde}	2.87^{abc}	10.30^{c}	2.7a	3.2bcd
B242	104.23^{ab}	940.23^{ab}	12.00^{ab}	12.04^{abcde}	5.67^{bcdef}	3.18^{a}	8.88^{c}	3.3a	3.7bcd
BT521	98.79^{ab}	617.03^{def}	10.72^{bcd}	10.79^{efgh}	5.80^{bcde}	2.46^{cde}	9.62^{c}	2.05a	3.4bcd
BT732	100.49^{ab}	655.32^{cdef}	10.29^{cd}	10.82^{defgh}	5.41^{def}	2.74^{abc}	9.92^{c}	1.8a	2.7cd
B172	100.03^{ab}	738.54^{bcde}	11.24^{abc}	11.35^{bcdefg}	6.32^{abcd}	2.57^{bcde}	9.36^{c}	2.4a	3.3bcd
B971	104.81^{ab}	526.92^{efg}	9.69^{d}	10.43^{fgh}	5.83^{bcde}	2.48^{cde}	9.81^{c}	1.9a	3.6bcd
BT743	105.50^{ab}	767.88^{bcd}	11.65^{abc}	12.03^{abcde}	6.19^{abcd}	2.89^{abc}	9.06^{c}	2.5a	3.3bcd
B592	98.90^{ab}	564.64^{defg}	9.67^{d}	10.57^{fgh}	5.73^{bcde}	2.54^{bcde}	9.66^{c}	1.9a	3.4bcd
B123	103.54^{ab}	745.59^{bcde}	12.66^{a}	11.48^{bcdefg}	5.93^{abcde}	2.77^{abc}	9.09^{c}	2.1a	2.7cd
B1192	100.54^{ab}	719.72^{bcde}	11.61^{abc}	11.40^{bcdefg}	5.86^{bcde}	2.81^{abc}	9.46^{c}	2.3a	3.2bcd
B1482	100.43^{ab}	853.69^{abc}	11.76^{abc}	12.15^{abcd}	7.20^{a}	2.81^{abc}	8.75^{c}	2.04a	2.2d
B392	105.43^{ab}	667.76^{cdef}	11.79^{abc}	10.93^{defgh}	5.86^{bcde}	2.59^{bcd}	9.51^{c}	2.5a	3.7bcd
B721	98.09^{ab}	462.36^{fg}	9.62^{d}	9.72^{hi}	5.51^{cdef}	2.19^{de}	10.31^{c}	2.8a	5.7b
B251	103.21^{ab}	634.21^{cdef}	10.88^{bcd}	10.63^{fgh}	5.68^{bcdef}	2.57^{bcde}	10^{c}	3.5a	5.4bc

Means with different superscript letters are significantly different from each other based on Duncan multiple range test at $p \leq 0.01$

Table 5

Classification of F3 families considering fruit related traits. Data for flesh color, vein tract, netting and flesh texture are the frequencies of their nominal scale (percentage of all fruits measured in each treatment). Also, the shape index is the ratio of fruit length to width calculated based on data from Table 4.

Parents and F3 Families	Flesh color		Vein tract		Netting		Flesh texture		Shape index
	Green	Others [a]	Yes	No	Yes	No	Juicy	others	
P1	100	0	100	0	100	0	100	0	0.87
P2	0	100	0	100	0	100	0	100	1.38
B4101	83	17	90	10	52	48	97	3	1.03
B682	100	0	98	2	95	5	88	12	1.01
B692	88	12	100	0	66	34	92	8	0.91
B133	94	6	100	0	95	5	87	13	0.96
B872	100	0	96	4	74	26	93	7	1
BT13102	47	53	89	11	46	54	91	9	1.02
B13103	90	10	96	4	44	56	91	9	1.07
B242	68	32	89	11	73	27	81	19	0.99
BT521	53	47	97	3	73	27	95	5	0.99
BT732	91	9	99	1	82	18	82	18	0.95
B172	100	0	98	2	85	15	91	9	0.99
B971	100	0	98	2	88	12	85	15	0.92
BT743	58	42	85	15	77	23	94	6	0.96
B592	26	76	93	7	79	21	97	3	0.91
B123	47	53	98	2	78	22	91	9	1.10
B1192	65	35	99	1	84	16	87	13	1.01
B1482	85	15	100	0	79	21	87	13	0.96
B392	75	25	97	3	96	4	80	20	1.07
B721	34	66	95	5	49	51	96	4	0.98
B251	96	4	85	15	90	10	95	5	1.02

a: others colors were those colors different from green such as yellow, yellowish white, etc. b: flesh texture was determined by tasting. In addition to juicy texture, other textures such as mealy and crispy were also detected.

Genotyping of *Vat* gene in different populations

Screening of different genotypes

The specific primer pair developed by Dogimont et al. (2014) for the resistant allele of *Vat* produced 121bp fragment. Figure 2 depicts the amplification curve of this primer pair with Real time PCR for different genotypes. HRM analysis showed the unique DNA fragment had been amplified only in ‘Ginsen Makuwa’ and "Karno Kiku Makuwa" among 17 tested genotypes (see material and methods). None of other genotypes include Iranian cultivars showed amplification curve and hence they had no resistant allele of *Vat*. These results is in congruity with the findings of a previous study in which ‘Ginsen Makuwa’ displayed double resistance toward *A. gossypii* and CMV (Boissot et al., 2016).

Marker assisted selection of resistant plants in F_4 populations

Figure 3 shows the melting peak of amplified fragment in control parental genotypes in which the melting peak of resistant allele in ‘Ginsen Makuwa’ and F_1 generation are clearly different from primer dimer peaks in ‘Samsoori’. This experiment was carried out several times to screen the F_4 population. After amplification, the melting curve was obtained for all samples in a plate (Fig. 4). For some plants there were only the resistant allele of *Vat* while for others no amplification was observed or a minor primer-dimer peak was observed. Figure 5 shows the electrophoresis of PCR product of the primer for control parental genotypes and some F4 generation plants.

In total 178 plants were genotyped in F_4 generation. Table 6 shows the results of classification of plants as resistant and susceptible in each family of F_3. If the original selected plant in F_2 generation was homozygote then all descended F_4 plants are expected to be homozygote and whether the selected plant in F_2 was heterozygote, plants in F_4 generation will be diverse according to Mendelian single gene segregation. Out of 13 F_3 families, six showed segregation regarding the *Vat* gene. Hence, they have been descended from a heterozygote plant in F2 plant. The ratio of resistant and susceptible plants descended from these plants corresponded the expected ratio of 9/16 for resistant and 7/16 for susceptible plants on the basis of *Chi* square test.

Table 6
Genotyping of plants in F4 generation for *Vat* gene. It revealed three F2 plants were homozygote resistant, four plants were susceptible and six plants were heterozygote.

Row	Selected F_2 plant (or F_3 family)	No. of resistant Plants in F_4 generation	No. of Susceptible plants in F_4 generation	X^2 statistic for fitting Mendelian ratio	Deduced F_2 genotype of the selected plant
1	BT 743	7	0	-	*Vat Vat*
2	B872	13	0	-	*Vat Vat*
3	B172	41	0	-	*Vat Vat*
4	B1192	0	15	-	*vat vat*
5	B971	0	16	-	*vat vat*
6	B682	0	13	-	*vat vat*
7	B13103	0	4	-	*vat vat*
8	BT 732	4	4	0.13ns	*Vat vat*
9	B392	9	3	1.71ns	*Vat vat*
10	B251	13	9	0.07ns	*Vat vat*
11	B133	14	4	3.39ns	*Vat vat*
12	BT 13102	4	2	0.26ns	*Vat vat*
13	BT 521	1	2	0.64ns	*Vat vat*

Conclusion

The current research made a major contribution to marker assisted selection for aphid and virus resistance in melon. The marker related to *Vat* gene was easy, fast and reliable enough to be substituted phenotypic selection. Also, it costs less because evaluating of virus resistance in plants via artificial inoculation is complicated and time-consuming, though by marker selection large number of plantlets could be screened in a short time.

The study also was an advancement in breeding of Iranian cantaloupe landraces with valued characters for virus resistance and improving sweetness. Further characterization of F_5 families (lines) is necessary for developing and selection of best lines which can be introduced as new cultivars or be included in commercial hybrid production.

Declarations

Funding -

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Conflict of interest -

The authors declared that they have no conflicts of interest that could have appeared to influence the work reported in this paper.

Availability of data and material -

The authors confirm that the data supporting the findings of this study are available within the article and any supporting data could be requested from the correspond author, ML; but the developed lines are restricted by funder.

Code availability -

Not applicable.

Author contributions-

ML and HR were involved in planning and supervised the work, PJ and HA contributed in collecting the data, RA supported lab experiments, FA performed statistical analysis, ML and HR processed the experimental data and wrote the paper with input from all authors.

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Figures

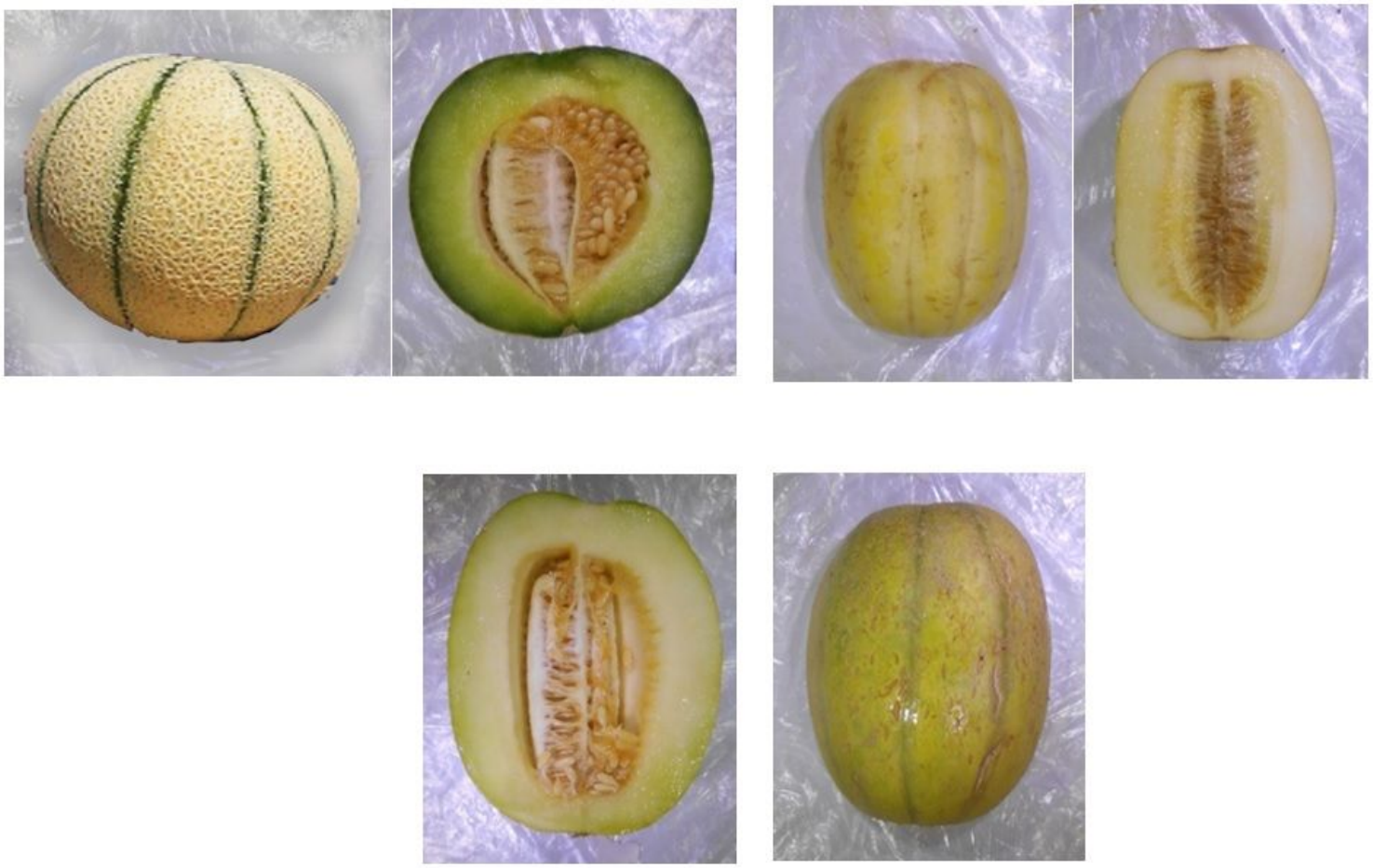

Figure 1

Fruit shape of ‘Samsoori’ (P1, top left), ‘Ginsen Makuwa’ (P2, top right), and their F1 generation (bottom).

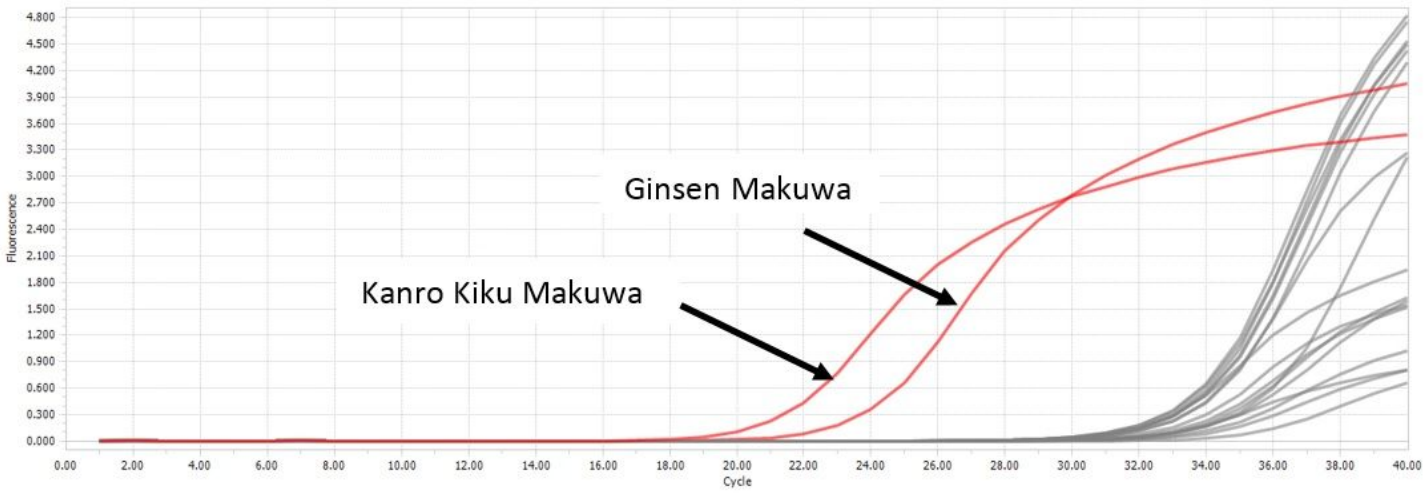

Figure 2

Amplification curve in Real time PCR for a part of resistant allele of the Vat gene. The primer pair sequence used for PCR was according to a previous study (Dogimont et al. 2014). All amplification curves after cycle 33 are primer dimers (see Figure4 and 5)

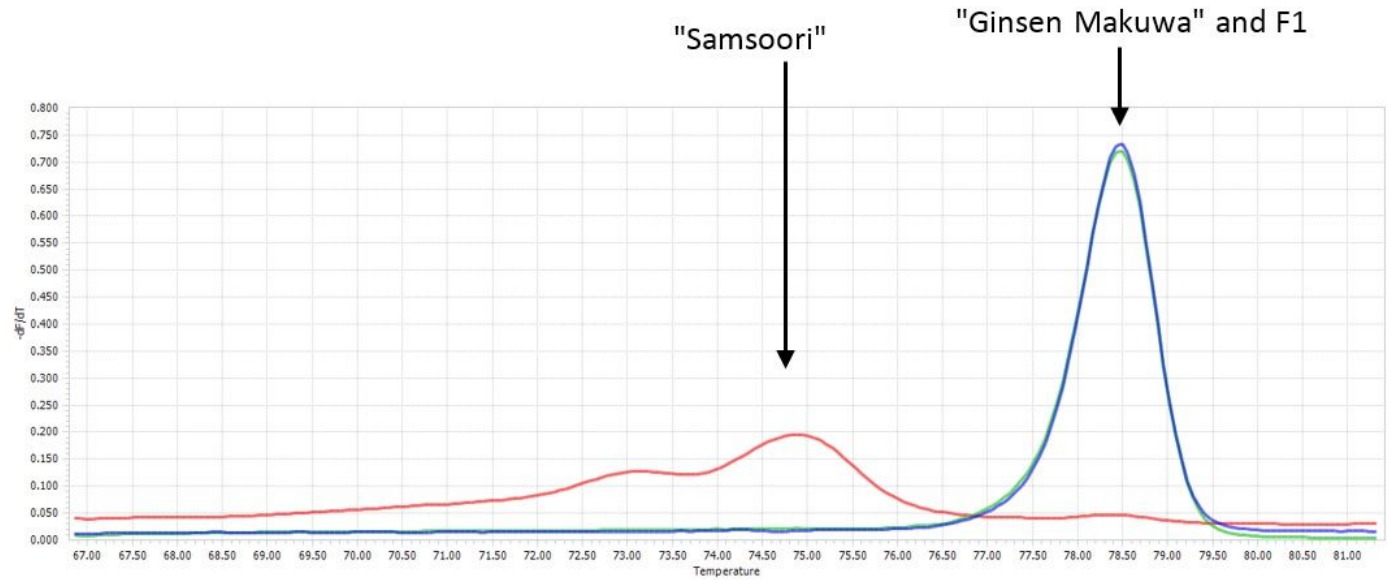

Figure 3

Melting curves of amplified PCR products with the primer pair specific to resistant allele of Vat in control parental genotypes of ‘Ginsen Makuwa’ (blue color), ‘Samsoori’ (red color) and F1 (green color). The electrophoresis of PCR product showed that the peak related to ‘Samsoori’ is primer dimers.

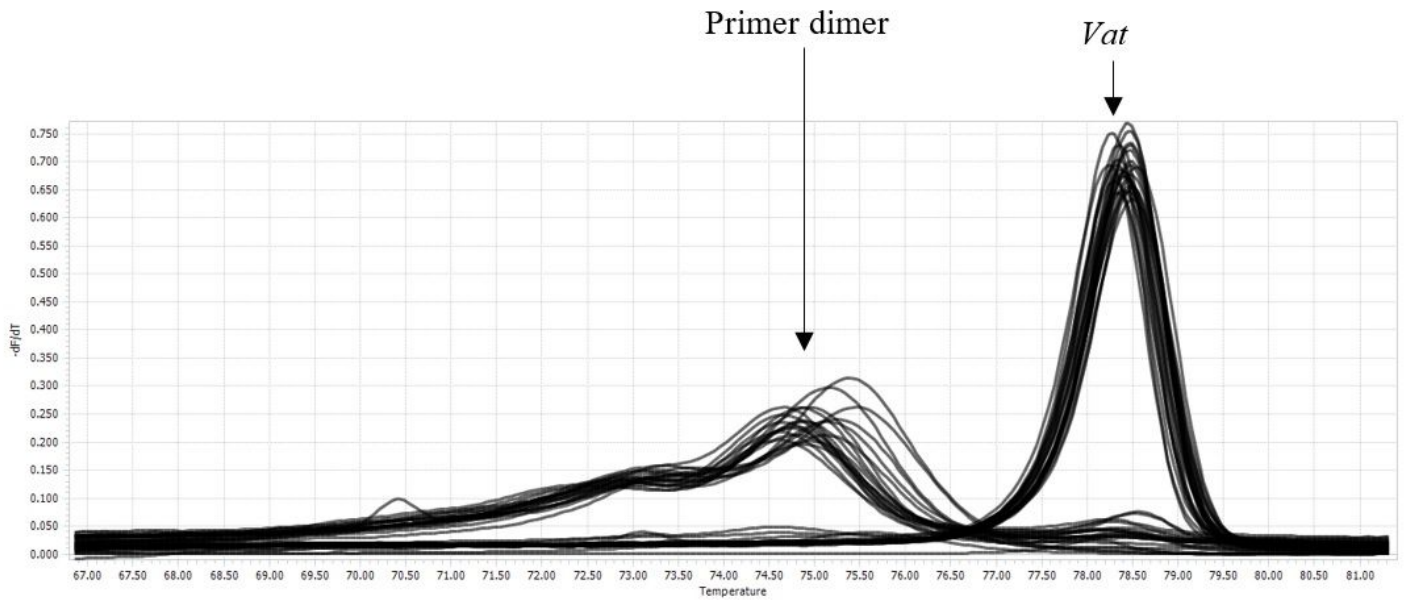

Figure 4

Melting curve of amplified PCR products with the primer pair specific to resistant allele of Vat in F4 generation plants. In all amplification experiments ‘Samsoori’, ‘Ginsen Makuwa’ and F1 were included. In all experiments, in each plate (96 well), three wells were assigned for non-DNA template controls.

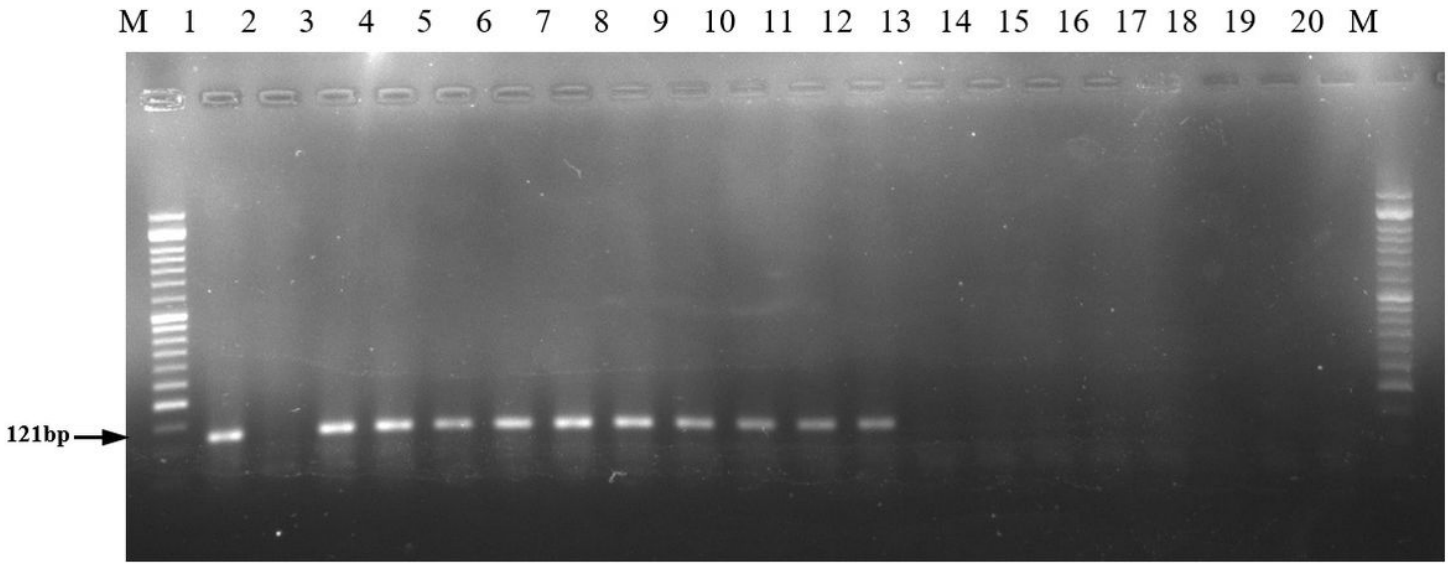

Figure 5

Gel electrophoresis of amplified fragment of resistant allele of Vat. M: size marker, 1: ‘Ginsen Makuwa’, 2: ‘Samsoori’, 3: F1, 4-12: different plants in F4 population which are carrying resistant allele of Vat, 13-18: different plants in F4 population which are carrying susceptible allele of Vat, 19-20: non-template DNA controls.