Evaluation of qualitative traits in modified Iranian Red Rey onion and comparison genetic resistance with primary mass selection and Red Azar-shahr cv to Fusarium oxysporum using laboratory and molecular markers

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Abstract

Background The bulb onion (Allium cepa L.) is grown on all continents except Antarctica, and is prized by essentially all of the world’s cultures for its flavor and health-enhancing attributes. Onion breeders focus primarily on bulb characteristics such as color, shape, soluble-solids content, pungency and flavor, storage ability, and health-enhancing attributes, as well as plant characters such as resistances to diseases. The use of breeding approaches, offers great promise for population improvement and hybrid development addressing changes in consumer preference and production environments. The aim of this study is to evaluate the storage and qualitative feature of modified Red Rey Iranian Onion.

Method Firstly, the modified population was obtained by the selection of superior bulbs, cultivation, its self-pollination and consequently the identification of the best families and implement open pollination between them. In next level, the Red Rey Iranian modified with basic population and Red Azar-shahr cultivar (comparative) was crossed.

Results Our results showed that the selection procedure has leading to improvement in variety of traits in population. Also, the modified Red Rey is significantly superior to the base mass in qualitative traits such as: bulb stiffness, bulb dry matter, TSS, total sugar and glucose; So that the percentage of dry bulb content increased from 10.4% in the basal mass to 11.1% in the modified Red Rey; while spouring and rotting, minerals, and dry matter, vitamin C and fructose-reducing sugar was not affected by genotype. In the second step, resistances to Fusarium wilt disease (laboratory and molecular markers) were evaluated. Based on the results of phenotypic evaluation, the modified Red Rey had the lowest rate and level of infection and the highest score. According to the results of genotypic evaluation, there is a very high genetic affinity between resistant and susceptible cultivars.

Keywords Breeding · Genetic affinity · Phenotypic evaluation · Qualitative characteristic · Red Rey onion

Introduction

Allium cepa L. is belonging to the family Alliaceae that is the third most important cultivated vegetable crop with a global yield of dry bulbs 88 million tons. Iranian onion is exported including fresh white, red, and yellow onion with the taste various ranges. Because of nativeness, cultivation and cross-pollination history, Iranian onion has a very rich variety and genetic reserves. Traits of native populations are often undesirable due to their genetic erosion and frequent cultivation so improvement of native cultivars is a priority. Ray population in Tehran and Alborz provinces is often cultivated in an area of about 1000 hectares. In the results of previous experiments all morphotypes of Red Rey, especially No. 13, had high status in terms of yield per hectare. The non-uniformity of this population, time of maturity and relative thickness of the bulb neck was a major problem for the population, which required the cultivar improvement [1]. Local populations in vegetables such as onions are always
considered as an important gene treasury in breeding. Onion populations have a high degree of heterozygosis that persists through amphimixis. It is essential to make accurate selection of native bulbs when seeds are produced to maintain the desired traits. Improvement has been done for traits such as quality, yield, uniformity, resistance to diseases, adaptation to different latitudes and agronomic operations. Historically, the method of selecting pollinator-free populations or segregated families has been considered, although using a periodic selection method-breeding yield can be achieved. In a study of [2] the local population breeding was carried out by the method of producing pollinator-free cultivars from 1999. The obtained showed that if the planting date and suitable density were observed, this genotype had a high production potential and given the white color and high percentage of the dry matter, the bulb is suitable for processing. The mentioned genotype has a long shelf life and is desired in terms of uniformity in the shape and color of the bulb. The study results on Iranian onion cultivars such as Red Rey showed that this population has a relatively high storage potential.

The results of previous study showed that the improved population was significantly superior to the base population in different traits, mainly bulb performance, neck diameter thinner and earlier maturity, but no superiority was observed in qualitative and storage traits [4]. Result of Modified mass in onion Qom Sefid cultivar indicated that yield, maturity, storage, uniformity of shape, size, and single center by mass selection method, was relatively superior to the base mass.

One of the most important fungal diseases of onion in the world is Fusarium head rot disease caused by the soil pathogen Fusarium oxysporum f. sp. cepa which damages the crop and reduced by about 23% in the field and storage [6]. 18 onion varieties were studied and it was found resistance to the disease during the growing period, while Spanish onion and Mustang hybrids were resistant. Other studies have shown that estimates of heritability for such traits and coefficient of medium to high genotypic variation with moderate genetic yield. By simple selection, the population can be modified for the mentioned traits. It should be noted that the estimation of heritability of traits depends on the studied population, estimation method, location and agro-nomic conditions.

Therefore, this research was carried out with the aim of studying of qualitative and storage feature of bulb in Red Rey Iranian modified Onion with maintaining and improving total yield. In addition, evaluation genetic resistance to F. oxysporum f. sp. cepa was investigated thorough laboratory and molecular markers.

### Materials and methods

These 2 experiments activities was accomplished in two assays during 2012–2016 in the field of Seedling and Seed Breeding Institute of Karaj located in 35.48° N 50.58° E.

### Experiment 1: selecting best families and evaluation of storage and qualitative traits

At first step, Seed Morphotype No. 13, as the best among the Red Rey morphotypes was planted in spring 2012 (base population). Suitable bulbs were harvested up to 500, kept in the cold store (Oct. 2012), cultivated in (March 2013), 300 superior plants were selected and then netting was created on them for self-seeding (June 2013). Selfed seeds were prepared separately from each branches and stored. 100 self-pollinated seeds were cultivated in Mid-April 2014. Bulbs of the 5 best families were selected and stored until March 2014 (yield, early maturity and bulb uniformity) and then planted in the field (March 2015) (Figure S1). At second step, pollination occurred between plants in spring 2016; seed is now sown in July 2016, named as modified seeds. Finally, modified Red Rey seed along with primary seed (base) and Red Azar-shahr (compara-tive) were used in completely random block design in 3 replicates [7]. Investigation of important breeding traits including storage characteristics was calculated. Weight loss percentage was measured approximately on the 60th day of storage at 4–10 °C, RH 50–60%. All bulbs were weighed then subtracting the weight of stored bulbs from the beginning of storage. All germinated and rotten bulbs were separated and weighed from the bulbs of each package then subtracted the weight of germinated and rotten bulbs from the initial weight of each package and was recorded as a percentage. Bulb stiffness was measured with a Model 11 FTO pantrometer with an 8 mm tip in the bulb’s equatorial diameter. The amount of bulb minerals including total sulfur (preparation of ash and extract from dry sample and spectrophotometer), total phosphorus (preparation of ash and extract from dry sample and spectrophotometer at 450 nm), total potassium (preparation of ash and extract from dry sample and film Photometry) were measured. The percentage of dry matter of the bulb was obtained by weighing 50 g of bulb and kept in an oven at 72 °C for 48 h. Measurement of vitamin C in bulbs was calculated by dichlorophenlindophenol method by mixing 20 ml of onion juice with 20 ml of 6% metaphosphoric acid and 25 ml of the obtained solution with 100% metaphosphoric acid. Then 10 ml of this solution was taken and titrated with dichlorophenylphenol solution. The titration was continued until the reddish-brown
solution changed color. TSS value was measured using a manual refractometer (CHD) made in China. Total sugar and reducing sugars (glucose and fructose) were determined by Novaspec spectrophotometer made by Pharmacia Biotech, UK.

Experiment 2: resistances to Fusarium wilt disease (laboratory and molecular markers)

Isolates *F. oxysporum* f. sp. *cepa* were incubated in the dish containing the Potato Dextrose Agar and suspension obtained was adjusted to $4 \times 10^7$/ml. Three onion bulbs were artificially inoculated in the laboratory with the invasive isolate and placed germination room. After the required time, the extent of the disease-induced lesion on the head and roots was investigated using the scoring method. To evaluate the genetic resistance of onion lines and masses, first the genomic DNA of the studied cultivars was extracted using Thermo scientific kit. To design the target primers, the nucleotide sequence of the target genes was obtained from (www.ncbi.nlm.nih.gov). PCR products appeared as seen by the presence of related band and without smear. For investigation of genetic mechanism of resistance, the sources of 15 genes that were documented to be effective in resistance to fungal diseases, especially Fusarium, was selected. An attempt was made to use an onion-related sequence to design the primer for all genes. After obtaining gene sequences by the NCBI Primer Designing Tool, primers were designed for all genes.

Data analysis

In this study, SPSS V.19 software was used to analyze data. Also, analysis of variance of traits and comparison of their means were performed using Duncan’s multiple range tests at 5% level.

Result

Experiment 1: selecting best families and evaluation of storage and qualitative traits

In the former study by authors concerning selected families, yield (The best families in terms of statistical rank of 68 and 94), early maturity (top are 57 and 56) and bulb uniformity (best number of bulb uniformity is number 6) had been investigated [7].

Evaluated storage traits (Red Rey modified seed along with primary seed (base) and Red Azar-shahr modified)

The results revealed that the percentage of weight loss in the Red Azar-shahr with 22.67% has a minimum range. In the primary population, it has a superior priority of 25.73% and in the modified cultivar with 24.67%; so it can be said that, a significant improvement has not been made compared to the primary population and Red Azar-shahr. According to the results of analysis of variance, no significant difference was observed in the percentage of rotten and germinated bulb.

Evaluated qualitative traits (Red Rey modified seed along with primary seed (base) and Red Azar-shahr modified)

The results indicate that there is no significant difference between the genotypes in the amount of mineral elements, Vitamin C and fructose level. In terms of bulb stiffness, it seems that in the modified Red Rey has a rank of “a” with 7.60 N/cm and is not in the same level with the primary mass cultivar “b” with 7 N/cm. This trait with an average of 6.67 N/cm in Red Azar-shahr has the grade of “c”. The percentage of dry bulb content is relatively higher in the modified mass than the primary mass. The modified Red Ray improved TSS with (11.40%) compare with Red Azar-shahr. As shown in Table 1, total bulb sugar ranks a, b and c, respectively, in the modified Red Ray, base mass and Red Azar-shahr.

Experiment 2: resistances to Fusarium wilt disease (laboratory and molecular markers)

The infected surface, rate and score were significantly affected by the treatment at 1%. Our funding showed that the highest rate of infection was observed in Red Azar-shahr (100%) and the lowest in the modified mass (0%). According to Table 2, the maximum infection score was seen in the modified cultivar and the lowest in the Red Azar-shahr. Also, infected surface was related to the highest in Red Azar-shahr and lowest modified mass. According to the general aim of the study, it was tried to determine the mechanisms used by resistant cultivars to resist Fusarium wilt or, conversely, the mechanisms that have caused susceptibility in cultivars. Based on the amplification of Flavonol synthase gene (Fig. 1), Anthocyanidin synthase gene (Fig. 2), Trypsin inhibitor gene (Fig. 3), Pentose phosphate phosphatase gene (Fig. 4), SERK1 and MYB1 genes (Fig. 5), WRKY1 gene (Fig. 6), Pectin methyl esterase gene (Figure S3), two samples on the left section are linked to susceptible cultivars and the portions on the right are closed to treatment in resistant cultivars. In LOX2 and Invertase genes, intermediate segment are bound to the susceptible cultivars and both side of treatment samples are connected with resistant cultivars (Figure S4). In Thioni and LTP genes, two samples next to the size indicator (100 bp) are associated to sensitive cultivars whereas the other two reigns are treatment (resistant cultivars) (Figure S2).
Discussion

Acquaviva red onion” showing higher sweetness with respect to the two above mentioned fresh consumption, soluble solid content and pungency [8]. Based on the results of phenotypic evaluation of this study, the lowest rate and surface of infection were observed in the modified mass and then the basal mass, respectively. Also, the highest score was seen in the modified mass and then the basal mass. Regarding to the results of genotypic evaluation, there is a very high genetic affinity between resistant and susceptible cultivars. Transcription factors MYB, bHLH2, and WRKY1 are mainly involved in pathways associated with stress hormones such as jasmonic acid and abscisic acid. There are numerous studies of their role in resistance to fungal and bacterial diseases such as Xanthomonas and Botrytis [9]. MYBs are also implicated in the metabolism

Table 1  The results of the mean comparison of qualitative traits in improved population of the basic and control cultivar

| SOV         | DF | Bulb stiffness | Total sulfur | Total phosphor | Total potassium | Dry matter | Vitamin C | TSS | Total sugar | fructose | Weight lost | sprouting and rotting |
|-------------|----|----------------|--------------|----------------|----------------|------------|-----------|-----|-------------|----------|-------------|-----------------------|
| Red Rey modified | 2  | 7.60a          | 0.47a        | 0.447a         | 2.210a         | 7.86a      | 9.80b     | 9.06a| 49.97a      | 19.83a   | 24.67a      | 15.23a                |
| Base seed   | 2  | 7.00b          | 0.48a        | 0.0431a        | 2.060a         | 7.86a      | 9.80b     | 9.06b| 44.33b      | 25.73a   | 16.70a      | 17.53a                |
| Red Azar-shahr – |   | 6.67c          | 0.57a        | 0.385a         | 2.237a         | 9.64b      | 9.06b     | 9.06b| 41.67c      | 22.67a   | 13.73a      | 13.73a                |

Means within each column followed by the same letter are not different according to the Duncan test

Table 2  The results of the mean comparison of storage traits in improved population of the basic population and control cultivar

| SOV         | DF  | Infected rate | Infected score | Infected surface |
|-------------|-----|---------------|----------------|-----------------|
| Red Rey modified | 2   | 0c            | 5a             | 0c              |
| Base seed   | 2   | 16.67b        | 4.8a           | 6.167b          |
| Red Azar-shahr – | –   | 100a          | 33b            | 100a            |

Fig. 1 Flavonol synthase gene amplification
of secondary metabolites [10]. Cycling of pentose phosphate and glycolysis pathways in response to disease is well known, and given that phosphoenolpyruvate phosphatase is one of the key enzymes in the glycolysis pathway. It was expected that the cause of the difference in disease resistance may be related to this gene. Chalcone isomerase is also involved in the response to ultraviolet light, fungal and bacterial diseases [11]. Lox2 is one of the key genes in the production of the plant hormone jasmonic acid, which plays an important role in the response to live stress [12]. Antimicrobial proteins are found in all groups of organisms and have different roles in various organisms against microbial diseases. These proteins are found in onions and many onion families. CaAMP1 is one of these protein-hosts [13]. Flavonol synthase and flavonol accumulation due to overexpression of this enzyme is one of the known mechanisms in response to biotic and abiotic stresses [14]. SERK1 is a kinase protein that has a known role in transmitting the message of various diseases including Fusarium wilt [15]. Pectinases are enzymes that help microbes’ pathogenesis by breaking down the plant.
cell wall. In contrast, pectinase blockers such as pectin methylesterase can help plants against microbes [16]. Glutathione transferases important for various physiological processes, especially during abiotic stress, the cell maintains oxidation–reduction balance, and their effect on oxidative stresses [17]. They break down peptides host cell are broken down by proteolytic bacteria and subsequently used either in growth. Protein inhibitors such as trypsin inhibitor are one way to counteract the host against plant pathogens [18]. Anthocyanins are plant pigments that are also involved in microbial resistance. Invertases are enzymes that convert sucrose in cells into fructose and glucose, which their role in disease resistance has been proven [19]. The lachrymatory factor synthase gene is a very important gene in onions, because of its pungent odor and tears when chopping onions; also, it causes a lot of changes in the sulfur-containing metabolites in the cell. It has been proven that sulfur compounds in cell defense play an important role against pathogens [20]. The importance of thionine gene in resistance to Fusarium wilt disease has been demonstrated [21]. According to the results of DNA amplification by PCR, it seems that the difference between sensitive and resistant cultivars is not related to the presence or absence of a gene. In other words, the results of studying the presence of genes among susceptible and resistant cultivars show that among the studied genes, all genes present in susceptible cultivars are also present in resistant cultivars. However, the two genes, antimicrobial peptide and bHLH transcription factor, were not amplified, which may be related to the presence of polymorphisms in the primer binding region. Also, the presence of bands other than the main band can be due to duplication in the genome. This is the case with the ANS, OTI, LOX2 and invertase genes. Since this condition was seen in both resistant and susceptible cultivars, they can not be attributed to the resistance factor in the resistant cultivar or the susceptibility factor in the susceptible group. However, because not all of the genes involved in resistance have been studied in this study, it may be due to resistance in other genes. Therefore, to find out the cause of resistance, it is better to use methods that include the whole genome or the whole transcript. Many experiments proved that two organisms, despite their great genetic affinity, show completely different reactions to the same trait, due to the small differences in the sequence and expression of their genes [22, 23]. Compared to the highly FBR resistant Welsh onion or Japanese bunching onion (Allium fistulosum L.), in which quantitative trait loci (QTLs) for FBR resistance were located in chromosome 8 [24], cultivated onion possesses FBR resistance QTLs on chromosomes 1, 6B, and 8 [25].

### Conclusion

In general, considering the results of this research in the molecular part, it showed that firstly, there is a very high genetic similarity between two resistant and sensitive cultivars. Because all the genes found in the resistant are also present in the susceptible and, conversely, all the genes that are not amplified in the resistant and are not amplified in the susceptible. Second, resistance probably depends on the sequence of these genes and the extent to which they are expressed, not on their presence or absence.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06679-1.

### Declarations

**Ethical approval** “This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.” “The authors declare that there is no conflict of interest regarding the publication of this article.” “All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national). Informed consent was obtained from all patients for being included in the study.”

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