
Molecular, Physiological and Phenotypic Evaluation of Resistance of Iranian Bean Cultivars to *Fusarium Oxysporum* f.sp. *phaseoli* the Causal Agent of Fusarium Wilt

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Abstract: In this study order to identify resistant cultivars in two separate experiments by two methods inoculation of also each method has one experiment for control treatment in that randomized complete block design with three replications and 12 treatments, including different cultivars of beans. By two methods at a concentration of 106 spores in ml the Spore suspension inoculation of root dipping and root drench were inoculated. After four weeks were of inoculation, the plants in a greenhouse with a temperature of 25-35°C were maintained. Disease severity was assessed by the scale of 1-9. The results showed that infection with both inoculation methods, especially the method of dipping the roots in suspensions spores pathogen cause changing in morphological traits including reduced plant height, fresh and dry weight of roots and shoots of bean plant also effect on pigments. In this study evaluate different bean varieties reaction to pairs of specific primers SU2 and morphological traits, was diagnosed sensitive. In order to evaluate the results of diagnostic morphological, resistance molecular analysis was also performed. Molecular diagnosis uses marker SCAR and two primers PCR and RAPD were used. With the evaluation of measured characteristics disease severity and tested SCAR marker The cultivars Naz, Sayad, WA and Sadri were resistance and cultivars E9, Akhtar and Jegari were semi-sensitive a cultivars Khomein, Capsules, Aej, shekofa and talash were identified as susceptible cultivars.

Keywords: Marker, Disease, Greenhouse, morphological, Fusarium

1. INTRODUCTION

Among grain crops, pulses (food legumes) rank third after cereals and oilseeds in terms of total world production. Pulses are rich in proteins and represent an important source of dietary protein for humans and animals. The proteins are generally composed of high amount of lysine, while the amount of methionine and cysteine is less. However, consumption of legumes and cereals results in a balanced diet of energy and protein. Legumes are also an important source of some essential minerals [1]. Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes on earth and provides many nutrients, high levels of proteins, unique carbohydrates, and essential vitamins for millions of people worldwide [2]. *Leguminosae* (*Fabaceae*), is the second largest family of flowering plants, comprising about 750 genera and more than 20,000 species and among them only 15% have been explored for rhizobial diversity. Legumes of economic importance are widely grown in India under various agro-climatic conditions, and the presence of native rhizobia has, therefore, been anticipated. Legumes are an important source of food and feed proteins [3]. Common bean (*Phaseolus vulgaris* L.) is the most widely grown and consumed grain legume in the world. Despite being a tropical-season legume, it has been adapted to a wide range of environments from Canada to South America, northern Europe to Southern Africa [4]. Living inconspicuously within plant tissues, fungal endophytes play important roles in plant community ecology [5,6,7,8,9,10]. and can have negative effects on insect pests and plant pathogens [11,12,13]. Little is known about other fungal endophytes naturally occurring in common bean seeds. A recent search for seed borne bacterial endophytes in the common bean yielded over 50 species, including the new species *Rhizobium endophyticum* [14]. Genetic disease resistance is the most biologically safe, socially acceptable, effective, and environmentally friendly way to control bacterial, fungal, and viral plant pathogens [15]. In recent years, BC420, SU91, and SAP6 have been widely used in MAS of common bean despite being dominant markers [16,17,18]. There is accumulating evidence that AMF can reduce disease incidence and propagule

number of several soilborne pathogens including *Aphanomyces*, *Fusarium*, *Phytophthora*, *Pythium*, and *Verticillium* species in the plant and mycorrhizosphere [19,20,21,22, 23, 24,25,26]. Genetic disease resistance is the most biologically safe, socially acceptable, effective, and environmentally friendly way to control bacterial, fungal, and viral plant pathogens [27]. Molecular markers for disease resistance are powerful tools for analyzing the genome and are comprehensively applied in mapping genes and MAS [28]. DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more efficiently than other marker systems [29]. Various types of markers have been used for these studies, the most common being allozymes, seed proteins, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphic DNA (AFLPs), microsatellites (SSRs), and inter-simple sequence repeats (ISSRs) etc. The major applications of these markers have been the assessment of genetic diversity, molecular linkage mapping, marker-assisted selection and positional cloning of genes or gene clusters [30].

The goal of this study which are done on three types of beans with the use of disease severity marker and measuring characteristics in green – house to show resistant and sensitive types and then the result of scar molecular marker which its relationship and bond has proved with resistance to *Fusarium*, will be investigated.

2. MATERIAL AND METHODS

In this research the reaction of different types of beans to *Fusarium*, using some morphological and physiological characteristics in green – house will be done in addition the reaction of different types of bean with the use of SCAR was used for evaluating resistance rate of phenotypical marker, and physiological.

3. DISCRIMINATION, PURIFICATION AND RECOGNITION OF THE DISEASE

In observation of bean farms in (shiraz – Fars – Iran), in July 2016, the beans which were in doubt of *Fusarium* languor were sampled then after transferring to lab and antiseptic with 1% HCL, the cultivation of 20 cm of Plant crown on the medium (PDA) the infection factor discriminated. The purification of the samples done with separated spores and recognition of *Fusarium oxysporum* of infected samples with use of the monograph [31].

4. SPORE SUSPENSIONS

For the tuber, greenhouse, and field pathogenicity tests, spore suspensions were created in a similar manner. Macro- and micro conidia were harvested from 2-week-old cultures grown on PDA at 25°C by adding 3 ml of sterile water to the plates and scraping the surface of the agar plate with a sterile glass slide. The resulting conidial or mycelia suspension was filtered through eight layers of cheesecloth to remove mycelia fragments [32].

5. THE PHENOTYPICAL INVESTIGATION OF RESISTANT TYPES OF BEANS

In this investigation different types of bean were used. types of beans were cultivated in 1:1 loam which was sterilized by autoclave in green – house circumstances in shiraz Azad university of agriculture. statistical patterns were considered in 3 random blocks with three spores in each vase. the vases were kept in green – house with 16 hours of lighting cycle in 32 degree of Celsius and 8 hours of darkness in 21 degree of Celsius. when the plants became jugum, inoculation were done for inoculation, first the bean bushes taken off the vases then got washed in distilled water 1/3, then roots were cut and put in 1×10⁶ ml/CFU for five minutes (dip root method) after wards the inoculated bushes were planted in sterilized loam, then, after 10 day, for growing improvement were Fertilization By N.P.K (8,28,16), so after 3 to 4 weeks the symptoms of disease appeared.

6. GRADING THE SEVERITY OF DISEASE

Disease severity scale of 1 to 9 were identified. So that numbers between 1.00 to 3.00 represents the resistant genotypes, genotypes 3.1 to 6 represents the average and between 6.1 and 9 are sensitive genotype. Table (1) [33].

Table 1. discriminating the reaction of different types of bean to *Fusarium* using severity disease

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| Disease symptoms | The amount of damages | Scale |
|--|-----------------------|-------|
| Without symptoms, healthy | 0 | 1-3 |
| Wilting of and yellowing of the plant Partial. | % 10 | 3.1-6 |
| Wilting of and yellowing of the plant average. | % 25 | 5 |
| Wilting of and severe yellowing and change colors vessel plant | % 50 | 6.1-9 |
| Plant death | % 75 | 9 |

7. DISCRIMINATING THE RATE OF CHLOROPHYLL

For this discriminating , 0/05 chlorophyll from leaves were weighed and 0/5 ml of DMSO added and for 3 hours were kept in own in 80 degree celcuse, then in 470 nanometer carotenoid , 645 nanometer (a chlorophyll) and 665 nanometer (b chlorophyll) spectro photo merered and the result got from below formula [34] .

$$\text{Carotonoid (mg/ml) : } (1000 A_{470} - 3.27 [\text{Chl a}] - 104 [\text{chl b}]) / 227$$

$$[\text{Chl b}] : 22.90 E^{a 645} - 4.68 E^{b 663}$$

$$[\text{Chl a}] : 12.70 E^{663} - 2.69 E^{645}$$

$$\text{Total chl [Chl a + b] : } 20.21 E^{645} + 8.02 E^{663}$$

8. WET AND DRY SHOOT AND ROOT WEIGHT MEASUREMENT

Isolated bushes to cut the root and stem crown of the district of separately to two decimal places was measured by balance was put in a paper bag And put the the sample into the oven at 70 ° C for 48 hours were measured weight dry bushes.

9. EVALUATION OF RESISTANCE BEAN GENOTYPES OF USING THE MOLECULAR MARKER SCAR

Genomic DNA was isolated from young leaves, as described by Doyle and Doyle [35]. Quality and quantity of DNA spectrophotometry and electrophoresis using methods agarose gel were measured. The polymerase chain reaction (pcr) using thermo cycler instruments quanta biotech Model Auto qb96 with 1 uL DNA, 2 uL of buffer 10 x ,0.7 uL mgcl2 (50uM) , 0.5uL dNTPs (10 uM) , 0.3the enzyme taq DNA polymerase (5u/ ul) and 1 uL of each primer Su20 (10 uM) making company Sina gene (Iran) the final volume was 20 uL with the program according to table 2 .Then PRC product in order to create specific band on agarose gel (2.5%) electrophoresis became next with the help gel imaging device UVidoc model GAS9000 of them photographed was .And molecular markers Scar in relation to the resistance genes to *Fusarium oxysporum f.sp. phaseoli* shown in Table 3

Table2. Timesheet and temperature cycling Primer SU20 (for30 cycles)

| | Primary temperature. | Denaturing | Connector | Stretch | Final temperature. |
|----------------------|----------------------|------------|-----------|---------|--------------------|
| Temperature(Celsius) | 94 | 94 | 53 | 72 | 72 |
| Time (min) | 5 | 1 | 1 | 1 | 5 |

Table3: molecular markers Scar in relation to the resistance genes to *Fusarium oxysporum f.sp. phaseoli* in beans

| SCAR Name | Marker of Origin | Size(bp)/ orention | Sequences of SCARS | Tagged Locus | LG | Reference |
|-----------|------------------|--------------------|---|--------------|----|------------------------------|
| SU20 | U20 | 750 | F: ACA GCC CCC ATT GTG AAT TGT AT R: ACA GCC CCC ACA CTT ATG GCA | A55 | 10 | Brick, 2006 Fall, 2001 |

10. RESULTS AND DISCUSSION

Evaluation of bean varieties reaction to the fungus using measured traits

Comparing the mean measured traits by using Duncan's multiple range test was performed at the level of five percent probability that relevant results.

As it observed the highest rate of height related to jegari, Naz and Akhtar which in infection rate don't have a meaning full difference and the lowest rate of height of bused related to Khomein , shekofa , E9 and WA, the most density of chlorophyll (a) related to Naz , sayad, sadri, Akhtar and E and from (b) sayad , WA , Naz. the most density of cartonoied related to Naz, sayad , WA , sadri , jegari , Akhtar and 9. the most total rate of chlorophyll related to E9 and sayad, eyj and shekofa had lowest weight of wet root. there was not meaning full difference from view of dried weight of root and wet weight of stick . According to results, the high-test rate of pigmentation were seen in sayad , Naz and E9 . finally, the most rate of height of bushes and wet weight and dried sticks and root were observed in Naz and Akhtar . And in discriminated features of controlling groups, the most height of bush related to Naz, sadri , jegari and Akhtar and capsule. the most density of chlorophyll (a) related to Naz, jegari, talash , Akhtar and E9 and from view of chlorophyll (b) they had no meaning full difference . Akhtar had the highest rate of chlorophyll (b) and cartonoied . sayad , sadri , jegari , Akhtar and eyj, khomein and E9 had the most weight of wet weight . the wet weight of stick were the highest in sadri, khomein, talash. khomein and eyj had also most weight of dried root . sadri and khomein had the highest weight of dried stick .

Table4. Plant foot insemination methods and control

| plant foot insemination methods | | | | | | | | | |
|---------------------------------------|---------------------|----------------------|---------------------|--------------------|---------------------------|---------------------|---------------------|--------------------|----------------------|
| Bush Sample | | Density chlorophyll | | Density cartenoied | Total Density chlorophyll | Average | | | |
| Sample | height | | | | | Root | | Stick | |
| | | a | b | | | wet | dry | wet | Dry |
| talash | 6.77 ^{de} | 5.96 ^{bcde} | 2.83 ^{bc} | 4.45 ^{ab} | 4.45 ^{abc} | 3.76 ^{abc} | 0.40 ^b | 3.06 ^{ab} | 0.46 ^{cd} |
| shokofa | 6.55 ^{de} | 5.00 ^{cde} | 1.92 ^c | 3.46 ^{bc} | 3.46 ^{bc} | 2.51 ^{bc} | 0.46 ^b | 2.69 ^{ab} | 0.37 ^d |
| jegary | 11.99 ^a | 5.21 ^{cde} | 2.19 ^c | 4.01 ^{ab} | 4.01 ^{bc} | 4.17 ^{abc} | 0.94 ^{ab} | 3.05 ^{ab} | 0.97 ^a |
| Akhtar | 11.66 ^a | 8.26 ^{ab} | 2.73 ^{bc} | 4.44 ^{ab} | 4.44 ^{abc} | 6.16 ^a | 1.21 ^a | 3.28 ^{ab} | 0.92 ^{ab} |
| Naz | 10.83 ^{ab} | 6.63 ^{abc} | 3.16 ^{abc} | 4.78 ^a | 4.78 ^{abc} | 4.71 ^{abc} | 0.73 ^{ab} | 2.35 ^{ab} | 0.66 ^{abcd} |
| Eyj | 7.94 ^d | 5.31 ^{cde} | 2.79 ^{bc} | 2.77 ^c | 2.77 ^c | 2.10 ^{bc} | 0.76 ^{ab} | 2.36 ^{ab} | 0.74 ^{abcd} |
| Khomein | 6.94 ^{de} | 3.45 ^{de} | 1.83 ^c | 2.86 ^c | 2.86 ^c | 3.67 ^{abc} | 0.97 ^{ab} | 2.88 ^{ab} | 0.76 ^{abcd} |
| E9 | 7.71 ^{de} | 6.23 ^{abcd} | 2.79 ^{bc} | 4.58 ^a | 6.41 ^a | 4.86 ^{ab} | 0.99 ^{ab} | 2.74 ^{ab} | 0.92 ^{ab} |
| capsule | 9.21 ^c | 3.19 ^e | 1.98 ^c | 2.67 ^c | 2.67 ^c | 1.92 ^c | 0.60 ^b | 1.46 ^b | 0.34 ^d |
| Sadri | 10.10 ^{bc} | 6.47 ^{abc} | 2.53 ^{bc} | 4.07 ^{ab} | 4.07 ^{bc} | 3.04 ^{bc} | 0.46 ^b | 3.45 ^a | 0.59 ^{abcd} |
| Sayyad | 6.77 ^{de} | 8.87 ^a | 4.36 ^a | 5.09 ^a | 5.09 ^{ab} | 4.17 ^{abc} | 0.80 ^{ab} | 2.78 ^{ab} | 0.87 ^{abc} |
| WA | 6.33 ^e | 5.81 ^{bcde} | 3.85 ^{ab} | 4.53 ^{ab} | 4.39 ^{abc} | 3.32 ^{bc} | 0.56 ^b | 3.79 ^a | 0.55 ^{bcd} |
| plant insemination methods in control | | | | | | | | | |
| talash | 8.13 ^c | 12.21 ^{ab} | 4.33 ^a | 5.28 ^{ab} | 15.13 ^{ab} | 4.45 ^{bc} | 0.84 ^{bc} | 4.75 ^{ab} | 0.69 ^{abcd} |
| shokofa | 7.10 ^c | 7.25 ^c | 2.57 ^a | 4.04 ^b | 10.13 ^b | 2.93 ^c | 0.63 ^c | 2.87 ^c | 0.41 ^d |
| jegary | 12.33 ^a | 10.97 ^{ab} | 3.03 ^a | 4.78 ^b | 14.01 ^{ab} | 6.70 ^{ab} | 0.96 ^{abc} | 2.04 ^c | 0.54 ^{cd} |
| Akhtar | 12.83 ^a | 13.31 ^a | 5.04 ^a | 6.54 ^a | 18.36 ^a | 6.63 ^{ab} | 0.74 ^c | 1.92 ^c | 0.57 ^{cd} |
| Naz | 13.05 ^a | 11.48 ^{ab} | 3.56 ^a | 5.37 ^{ab} | 15.04 ^{ab} | 4.49 ^{bc} | 0.70 ^c | 1.70 ^c | 0.50 ^d |
| Eyj | 8.66 ^{bc} | 10.61 ^{abc} | 3.29 ^a | 4.92 ^b | 10.64 ^b | 7.94 ^a | 1.37 ^a | 3.35 ^{bc} | 1.00 ^a |
| Khomein | 8.38 ^{bc} | 10.34 ^{abc} | 3.72 ^a | 5.62 ^{ab} | 11.65 ^b | 7.94 ^a | 1.28 ^{ab} | 6.34 ^a | 0.87 ^{abc} |
| E9 | 8.38 ^{bc} | 11.63 ^{ab} | 4.39 ^a | 5.53 ^{ab} | 11.15 ^b | 6.86 ^{ab} | 0.83 ^{bc} | 2.41 ^c | 0.62 ^{bcd} |
| capsule | 12.44 ^a | 9.47 ^{bc} | 4.60 ^a | 5.36 ^{ab} | 10.45 ^b | 4.74 ^{bc} | 0.58 ^c | 1.90 ^c | 0.53 ^d |
| Sadri | 11.11 ^{ab} | 8.79 ^{bc} | 3.19 ^a | 5.41 ^{ab} | 10.21 ^b | 5.71 ^{ab} | 0.85 ^{bc} | 5.72 ^a | 0.93 ^{ab} |
| Sayad | 7.33 ^c | 10.49 ^{abc} | 3.58 ^a | 4.61 ^b | 10.24 ^b | 6.81 ^{ab} | 0.69 ^c | 1.67 ^c | 0.52 ^d |
| WA | 7.10 ^c | 9.57 ^{bc} | 4.49 ^a | 5.37 ^{ab} | 10.33 ^b | 4.74 ^{bc} | 0.70 ^c | 1.89 ^c | 0.58 ^{cd} |

Evaluating the reaction of different types of beans to Fusarium with the method of Root Dipping in spore suspension and Soil Drenching of spore suspension

According to this table different types in %1 probability from points of height of the bushes , chlorophyll a , cartonoied density , total chlorophyll density , wet weight of sticks , wet of roots ,

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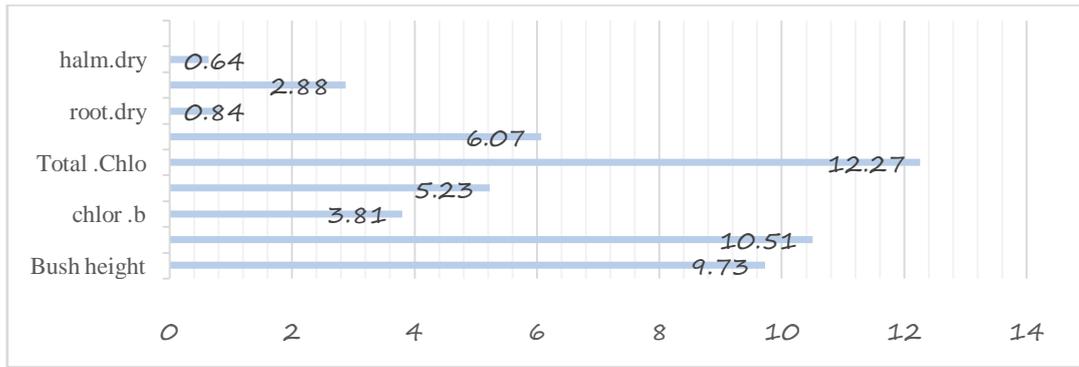
dried weight of root and dried weight of sticks have a meaningful difference and from chlorophyll b density don't have a meaningful difference . (Table 5)

Table5. Compare method of Root Dipping in spore suspension and Soil Drenching of spore suspension

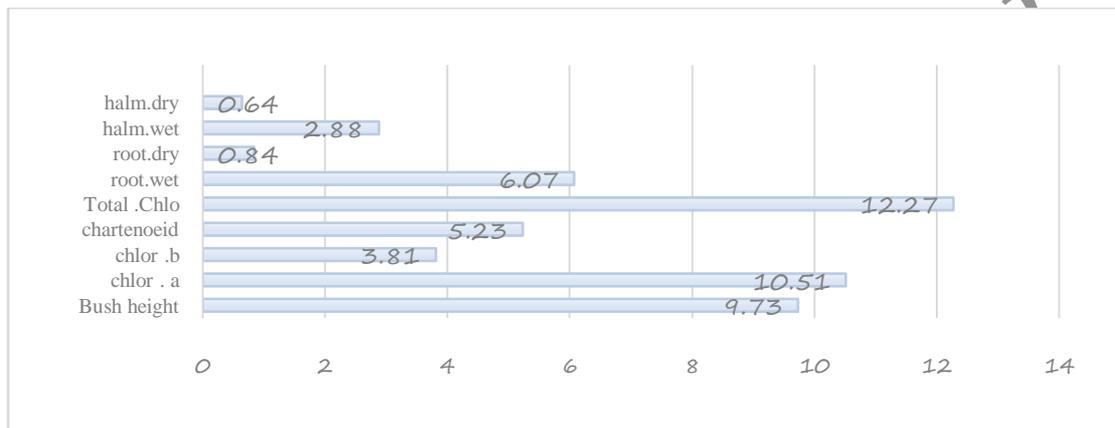
| Root Dipping in spore suspension | | | | | | | | | |
|------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------------|---------------------|---------------------|--------------------|---------------------|
| Bush Sampal | | Density chlorophyll | | Density chartenoeid | Total Density chlorophyll | Average | | | |
| Sampal | height | | | | | Root | | Stick | |
| | | a | B | | | Wet | dry | wet | Dry |
| talash | 6.44 ^{de} | 3.00 ^{cd} | 1.05 ^{ab} | 3.54 ^b | 3.54 ^{bc} | 4.66 ^a | 0.67 ^{bc} | 2.11 ^{ab} | 0.56 ^{bcd} |
| shokofa | 5.88 ^e | 2.04 ^d | 1.10 ^{ab} | 2.22 ^{cd} | 2.22 ^{de} | 2.08 ^{bc} | 0.47 ^{cde} | 2.32 ^a | 0.33 ^d |
| jegary | 9.94 ^{ab} | 3.94 ^{bcd} | 2.37 ^{ab} | 2.86 ^{bc} | 2.86 ^{cd} | 3.66 ^a | 0.67 ^{bc} | 2.06 ^{ab} | 0.64 ^{bc} |
| Akhtar | 10.66 ^a | 5.05 ^{abc} | 2.22 ^{ab} | 3.82 ^b | 3.82 ^{bc} | 4.87 ^a | 1.16 ^a | 2.70 ^a | 0.94 ^a |
| naz | 10.55 ^a | 7.40 ^a | 2.97 ^{ab} | 5.11 ^a | 5.11 ^a | 3.81 ^a | 0.49 ^{cde} | 1.48 ^{bc} | 0.45 ^{cd} |
| eyj | 7.21 ^{de} | 1.60 ^d | 0.56 ^b | 1.26 ^d | 1.26 ^e | 0.94 ^c | 0.23 ^e | 0.84 ^c | 0.33 ^d |
| Khomein | 6.44 ^{de} | 1.13 ^d | 0.71 ^{ab} | 1.17 ^d | 1.17 ^e | 1.41 ^c | 0.33 ^{de} | 0.95 ^c | 0.35 ^d |
| E9 | 7.66 ^{cd} | 3.40 ^{cd} | 1.90 ^{ab} | 3.56 ^b | 3.56 ^{bc} | 3.86 ^a | 0.88 ^b | 2.66 ^a | 0.76 ^{ab} |
| capsule | 8.99 ^{bc} | 1.54 ^d | 1.00 ^{ab} | 1.44 ^d | 1.44 ^e | 1.40 ^c | 0.45 ^{cde} | 1.44 ^{bc} | 0.48 ^{cd} |
| Sadri | 9.33 ^{ab} | 3.08 ^{cd} | 1.76 ^{ab} | 3.06 ^{bc} | 3.06 ^{cd} | 3.92 ^a | 0.60 ^{bcd} | 2.39 ^a | 0.45 ^{cd} |
| Sayyad | 6.55 ^{de} | 6.43 ^{ab} | 3.14 ^a | 3.78 ^b | 3.78 ^{bc} | 3.80 ^a | 0.67 ^{bc} | 2.09 ^{ab} | 0.66 ^{bc} |
| WA | 6.49 ^{de} | 3.34 ^{cd} | 1.88 ^{ab} | 3.86 ^b | 4.43 ^{ab} | 3.54 ^{ab} | 0.41 ^{cde} | 2.59 ^a | 0.36 ^d |
| Soil Drenching of spore suspension | | | | | | | | | |
| talash | 7.94 ^{cd} | 7.54 ^{bc} | 2.71 ^{abc} | 4.17 ^{bc} | 4.16 ^{bc} | 1.71 ^e | 0.35 ^c | 4.32 ^a | 0.54 ^a |
| shokofa | 6.55 ^d | 7.50 ^{bc} | 3.12 ^{abc} | 4.37 ^{bc} | 4.37 ^{bc} | 2.74 ^{cde} | 0.69 ^{ab} | 2.30 ^{bc} | 0.50 ^a |
| jegary | 10.94 ^{ab} | 8.25 ^{ab} | 2.94 ^{abc} | 4.67 ^{bc} | 4.67 ^{bc} | 5.26 ^{ab} | 0.76 ^a | 1.26 ^c | 0.35 ^a |
| Akhtar | 12.11 ^a | 8.73 ^{ab} | 2.72 ^{abc} | 4.28 ^{bc} | 4.32 ^{bc} | 5.36 ^a | 0.68 ^{ab} | 2.28 ^{bc} | 0.57 ^a |
| naz | 11.88 ^a | 8.57 ^{ab} | 2.63 ^{abc} | 4.10 ^c | 4.10 ^c | 3.54 ^{bcd} | 0.45 ^{bc} | 1.30 ^c | 0.38 ^a |
| eyj | 7.99 ^{cd} | 6.90 ^{bc} | 2.88 ^{abc} | 4.28 ^{bc} | 4.28 ^{bc} | 5.67 ^a | 0.75 ^{ab} | 1.83 ^c | 0.54 ^a |
| Khomein | 6.99 ^{cd} | 5.54 ^c | 2.42 ^{bc} | 3.92 ^c | 3.92 ^c | 2.27 ^{de} | 0.36 ^c | 3.40 ^{ab} | 0.52 ^a |
| E9 | 7.72 ^{cd} | 7.06 ^{bc} | 1.85 ^c | 4.24 ^{bc} | 4.30 ^{bc} | 4.97 ^{ab} | 0.69 ^{ab} | 2.03 ^c | 0.54 ^a |
| capsule | 11.49 ^a | 6.97 ^{bc} | 2.83 ^{abc} | 4.05 ^c | 4.05 ^c | 3.19 ^{cde} | 0.57 ^{abc} | 1.64 ^c | 0.63 ^a |
| Sadri | 9.05 ^{bc} | 8.90 ^{ab} | 3.68 ^{ab} | 4.94 ^{ab} | 4.94 ^{ab} | 2.68 ^{cde} | 0.35 ^c | 3.90 ^a | 0.60 ^a |
| sayad | 6.94 ^{cd} | 10.25 ^a | 4.29 ^a | 5.44 ^a | 5.44 ^a | 5.15 ^a | 0.58 ^{abc} | 1.52 ^c | 0.63 ^a |
| WA | 6.44 ^d | 8.18 ^{ab} | 3.32 ^{abc} | 4.39 ^{bc} | 4.39 ^{bc} | 4.26 ^{abc} | 0.59 ^{abc} | 1.47 ^c | 0.45 ^a |

As it is considered .the most height of bushes related to Naz , Sadri , jegari and Akhtar and the least height of bushes related to sayad , WA , khomein , eyj , shekofa and talash . most density of chlorophyll(a) related to Naz , sayad and Akhtar which least of them were WA , sadri , jegari , E9 , khomein , capsule , eyj , shekofa and talash . from chlorophyll (b) they didn't have meaningful difference and were placed all in one statistical group . Naz had the most density of cartenoied and khomein . capsule , eyj and shekofa had the least . the most a mount of total chlorophyll related to Naz and WA and the least to khomein , capsule , eyj and shekofa . types , Naz , sayad , WA , sadri , jegari , Akhtar , E9 and talash had the most wet weight of root and khomein , capsule , eyj and shekofa had the least . the wet weight of sticks in sayad , WA , sadri , jegari , Akhtar , E9 and shekofa was the most and in Naz , khomein , capsule and eyj the least . Akhtar had the most and Naz , WA , khomein and capsule , eyj and shekofa had the least dried weight of roots . the most pigmentation in tension were in sayad and Naz and the least in khomein and capsule and eyj . the most height of bushes and wet and dried weight of sticks and roots related to Naz , sayad , WA , Akhtar and E9 and the least were khomein , capsule and eyj . controlling attendance in inoculation of feet of bushes controlling attendance in infecting suspension spore inoculation,infected attendance with the method of inoculation of feet bushes and infected attendance were infecting . According to tables amounts of (chlorophyll , cartonoied , total chlorophyll , wet root and dried and wet sticks) were evaluated and shown .

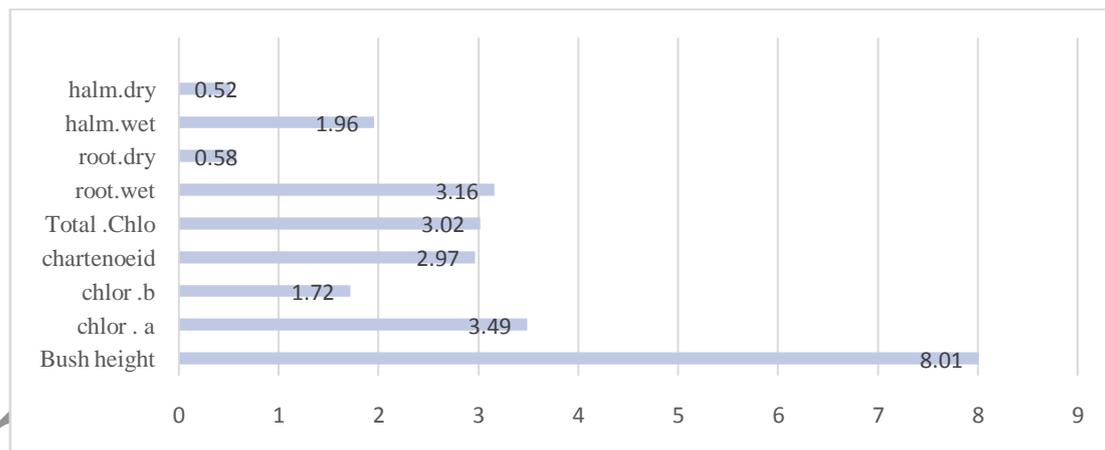
Graph1. Plant foot insemination methods



Graph2. Plant insemination methods in control



Graph3: method of inoculation with suspension of infecting spore



Graph 4 : method of inoculation with suspension of controlling groups



Evaluation of bean varieties reaction to the fungus *Fusarium oxysporum f.sp. phaseoli* using symptoms and severity of disease

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In this method for Evaluation of the resistance the severity of disease symptoms appear to be determined by scale and giving numbers from 1 to 9. So the types of Naz , sayad , WA , sadri were resistance and jegari , Akhtar and E9 semi – resistance and khomein , capsul , eyj , shekofa and talash were evaluated as sensitive . (table 6)

Table6: Compare the severity of the disease in different bean varieties to *Fusarium yellows*

| Scale | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---------|---|---|---|---|---|---|---|---|---|
| Sample | | | | | | | | | |
| Naz | 3 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sayyad | 2 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| WA | 2 | 3 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| Sadri | 0 | 3 | 3 | 2 | 1 | 0 | 0 | 0 | 0 |
| Jegari | 1 | 0 | 0 | 3 | 2 | 1 | 2 | 0 | 0 |
| Akhtar | 0 | 0 | 2 | 1 | 2 | 1 | 3 | 0 | 0 |
| E9 | 0 | 0 | 0 | 2 | 3 | 1 | 2 | 1 | 0 |
| Talash | 0 | 0 | 1 | 0 | 2 | 1 | 0 | 3 | 2 |
| Shokofa | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 3 | 2 |
| Ege | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 1 | 3 |
| Capsul | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 4 |
| khomein | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 4 |

Evaluate different bean varieties reaction to the fungus f.sp. phaseoli *F. oxysporum* using proprietary markers SCAR

In table 7, the result of reaction of different types of bean to SU20 is shown . As it is seen , Akhtar , Naz, sadri, sayad, E9, WA with SU20 had 750 bp band and resistance gene of A55 but talash , shekofa , jegari , eyj , khomein and capsul hadn't a band with SU20 . Although Akhtar , sadri and E9 were semi – sensitive due to intensity of disease and evaluating of characteristics , had band with SU20 . Although jegari, according to disease intensity and evaluation of characteristics was able but it didn't have a band. (as shown in Figure)

Table7: reaction of different types of bean to SU20

| sampale | talash | shokofa | jegari | ahktar | naz | aej | khomein | e9 | capsoli | sadri | sayad | wa |
|---------|--------|---------|--------|--------|-----|-----|---------|----|---------|-------|-------|----|
| a55 | - | - | - | + | + | - | - | + | - | + | + | + |

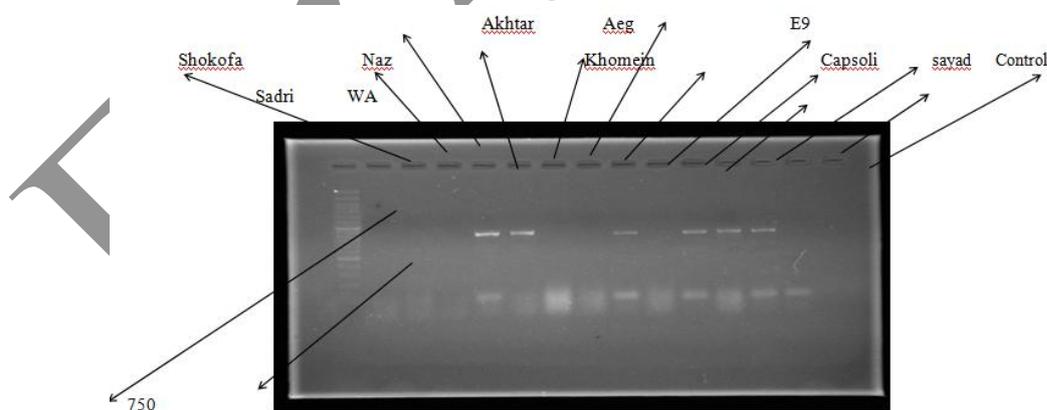


Figure1. Specific band SU20 marker (750bp SCAR marker for A55 gene) In cultivars From 1 to 12 beans.

Estimates of heritability for resistance to other pathogens in common bean are frequent in the literature [36]. The result of previous studies, shows the resistance to *Fusarium languor* is different in various types of beans and model of resistance in some types are due to prevalent gene and in some types became of quantities models of some genes. [37, 38, 39,40] . In similar researches, ten locally and international cultivars of beans in 50 biomarkers as the infected vase and 50 vases are planted as check-were and then assess the reactions different cultivars to fungus *Fusarium oxysporum* and bean yield of were obtained accordingly Naz cultivar had the highest resistance and the highest product

performance. low product performance and high sensitivity showed a cultivars Suhair [41] . Cultivars resistance Naz, in present study with the results of research by saremi et al (2011) is equal . In similar researches, 66 reactions Line bean to yellow pathogen *Fusarium* in greenhouse experiments to a Brazilian strain of the FOP was used That results showed 40 lines were resistant, 6 lines whit average resistance and 20 lines are sensitive [42] . In this study, six cultivars that the band have created with pairs SU20 measured terms of severity and characteristics that are resistant or tolerant and in fact are resistant to A55 gene .

ACKNOWLEDGEMENTS

The first author wishes to express sincere thanks to Shiraz branch, Islamic Azad University, Islamic Azad University for providing the funds. We wish to thank the Chairman, Department of Agriculture , Shiraz branch, Islamic Azad University for extending the laboratory facilities to carry out the research work .

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Early View