# PHENOTYPIC AND GENETIC EVALUATION OF SOME APPLE SEEDLINGS ROOTSTOCKS TO WOOLLY APPLE APHID (*Eriosomalanigerum Hausm*) RESISTANCE IN SYRIA.

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## Abstract

Woolly apple aphid is the main important pest which attacks apple trees and causes severe damages. This investigation was conducted to evaluate some apple seedling rootstock genotypes established through apple breeding program in Syria. One year old apple seedlings rootstocks from 5 genotypes (A, B, C, S2, H) selected through apple breeding program were infested with woolly apple aphid (*Eriosomalanigerum*Hausm) to investigate their susceptibility to this pest. The results showed that S2 was the most susceptible genotype, followed by A and C, with infestation rates of 92.2%, 90% and 90%, respectively. On the other hand, H genotype was the least susceptible to woolly apple aphid followed by B (59.4% and 85.4% infestation rate, respectively).For genetic studies, the resistant plants and some of susceptible one from each genotype, in addition to the mother plants were used to detect the tolerant genes to woolly apple aphid by using 8 markers related to suggested tolerant genes, for integral combination with phenotypic selection. As a result, NZsn_O05 and NZSc_E01 markers were more efficient to distinguish resistant plants. Likewise, NZms_EB145764, NZms_EB106753 and NZSc_A01 revealed bands in both tolerant and susceptible plants as monomorphic bands, while, NZsc_C20, NZsc_GS327 and NZsc_O05 (linked to *Er1*) did not reveal any PCR product. Consequently, it is important to select the resistant apple rootstocks for woolly apple aphid at early stage in apple rootstocks breeding program. On the other hand, it is necessary to develop new markers tightly linked to the resistant genes depending on studied plant material.

**Key words:** *Apple, Woolly apple aphid, seedling rootstock, resistant genes.*

## Introduction

Woolly apple aphid (WAA) *Eriosoma lanigerum* (Homoptera: Aphididae) is the most destructive and common pest around the world, as in Syriaespecially it distributes in all apple regions and grouped as the second important pest after codling moth (Al-Matni, 1997; Mansour, 2006). WAA can feed on both roots and vegetative parts of apple trees, root infestation can cause the death of the tree in extreme cases. However, vegetative infestation can be controlled by insecticide treatments, while the root infestation cannot be chemically controlled (Klimstra and Rock, 1985). On the other hand,using chemical insecticide with large doses can increase the residual effect in the fruit, pollute soil and water, destroy natural enemies, increase insect resistance, and cause health risks to the workers (Reganold *et al.,* 2001). Nowadays, breeding programs is aiming to produce resistant rootstocks to biotic stress and to achieve sustainable agriculture objective in reducing the use of chemicals to the lowest limit (Hrotko, 2007). "Northern spy" cultivar was used in the past as an apple rootstock due to its resistance to WAA, then introduced into apple rootstock breeding program as a parent in East Malling institute in cooperating with John Innesinstitute, so they produced the MM series and Merton Immune of resistant apple rootstocks to WAA (Preston, 1955; Webster and

Wertheim, 2003), the MM106 and MM111 still the most common resistant rootstocks throughout the world (Webster *et al.,* 2000).

The success of rootstock breeding program depends on the selection of parents for hybridization (Cummins and Aldwinckle, 1995). Rootstock MM106 is used in many breeding programs, especially for the resistance to WAA, in such programs the infestation with the insect is done at early stage to exclude the susceptible plants (Johnson *et al.,* 2001).

Rootstocks with genetic resistance that usually considered as field immune to the pest are used to prevent infestation of the belowground parts (Bus *et al.*, 2008). Traditional breedingforapple rootstock is time and labor consuming, due to the long juvenile period, so MAS is expected to be a useful tool to identify characters of fruit tree at the seedling stage (Ban *et al.,* 1999). As new multi-allelic markers (SSRs, SNPs) become available for the analysis of apple germplasm the prospect of utilizing them in tandem with phenotypic data on breeding population is becoming a reality (Fazio and Mazzola, 2004).

Our research aimed to evaluate and identifysome of apple rootstock genotypes have the genetic resistance to WAA for rootstock breeding program in Syria depending on phenotypic and genetic evaluation.

## Materials and methods

The present investigation was carried out at the agricultural scientific research center – GCSAR- in Sweida province, which located at 1525m altitudeinthe south of Syria, 20.36 to 21.36atitude and 40.3-40.4 longitude.

### Plant material

One year old seedlings from 5 apple genotypeswere introduced into apple rootstock breeding program in Syria: A, B and C genotypes produced by open pollination, S2 is local apple cultivar(Sukari), and H is a hybridgenotype between the rootstock MM106 and the local apple cultivar Sk (Skarji).

### Phenotypicfor resistance to WAA

Seedlings from each genotype (A: 30, B:55, C:44, S2:24 and H:32 seedlings) were planted in lines, the planting distance was 25 cm between plants and 70 cm between lines, all the agricultural processes (irrigation, fertilization and weeding)were achieved.The infestation was done in late June 2010 by placing shoot pieces with heavily infested WAA colonies in each seedling, the infestation was repeated twice in interval two weeks, the seedlings were not subjected to chemical control all the season.

WAA infestation was assessed 4 months after inoculatingat the first season, and at the end of second season using 6- point scales according to(Bus *et al.,* 2008):

0: No infestation
1: Light infestation consisting of several small, separate colonies
2: Medium infestation and galling with some colonies starting to coalesce
3: Many colonies coalescing and up to 2 shoots completely infested and galled
4: Heavy infestation and galling on 2-5 shoots
5: Heavy infestation and galling on more than 5 shoots

The percentage of infested seedlings in each scale within each genotype was calculated. For phenotypic evaluation seedlings classified as 0 or 1 to be resistant and those scoring 2-5 to be susceptible.

### Genetic evaluation

DNA extraction was achieved using CTAB protocol according to Porebski *et al.,* (1997),by collecting leaves from the resistant plants and some of susceptible ones from each genotype, in addition to the mother plants.

PCR amplification was achieved using 8 markers (Table 1) linked to the resistant genes for woolly apple aphid according to Bus *et al.*, (2008).

The reaction was performed with volume (10 µl) consisted of: 1 µl 10 X buffer + 1 µl dNTPs + 1 µl forward primer + 1 µl reverse primer + 3 µl DNA + 0.1 µl taq + 2.9 µl dH$_2$O. The cycling profile for the markers NZsc_G327, NZsc_O05, NZsc_E01 and NZsc_A01 consisted of an initial denaturation step of 3 min at 94 c, followed by 35 cycles of 30 s at 94C, 30 s at 55 C and 1min at 72C, the amplification process was finished with 5 min at 72C. For the markers NZms_EB145764, NZms_EB106753, NZsn_O05 and NZsc_C20 were used touchdown PCR consisted of an initial denaturation step of 5 min at 94 c, followed by 10 cycles of 30 s at 94C, 30 s at 70 C and 45 s at 72C, the temperature was reduced 1C every cycle, followed by 20 cycles of 30 s at 94C, 30 s at 60 C and  45 s at 72 C, the amplification process was finished with 10 min at 72 C.

Table 1: markers linked to the resistant genes to woolly apple aphid, the sequence of forward and reverseprimers, and the product size (bp).

| Marker name | Marker type | Original RAPD /EST | WAA gene | Forward primer | Reverse primer | Product size (bp) |
|---|---|---|---|---|---|---|
| NZsc_C20 | SCAR | OPC20 | *Er1* | TCTCTAACTCAATAACTCCCAAGAC | ACTTCGCCACCATTATCACTCCTGA | 2,000 |
| NZsc_GS327 | SCAR | GS327 | *Er1* | GCCAAGCTTCAATGTCGGAGTAGAT | CAAGCTTCCCCTAAGGCTATTGCCA | 1,600 |
| NZsc_O05 | SCAR | OPO05 | *Er1* | CCCAGTCACTAACATAATTGGCACA | CCCAGTCACTGGCAAGAGAAATTAC | 1,700 |
| NZsn_O05 | SNP | OPO05 | *Er1* *Er3* | AACGTCATGTCAATAT | CCCAGTCACTGGCAAGAGAAATTAC | 880 |
| NZsc_E01 | SCAR | OPE01 | *Er3* | CCCAAGGTCCGAACACAAATGAGAG | CCCAAGGTCCAAAACTATCCCGAAG | 1,350 |
| NZSc_A01 | SCAR | OPA01 | *Er3* | CAGGCCCTTCAGCAAAGAGGTGTCT | CAGGCCCTTCACTACTAATAAGAAC | 1,250 |
| NZms_EB106753 | SSR | EB106753 | *Er1* *Er3* | TCTGAGGCTCCCAAGTCC | TAGGAGCAGAAGAGGTGACG | 175 |
| NZms_EB145764 | SSR | EB145764 | *Er2* | TTCCAGCGATCCAAAACAAT | GCTCAGGAACACCTCGTTCT | 198 |

The PCR productswere detected by electrophoresis on 1% agarose gel in 1X TBE buffer, stained with ethidium bromide and visualized by UV light and photographed using gel doc. NZms_EB106753 and NZms_EB145764 markers detected by running PCR products on a 8% polyacrylamide gel in 1X TBE buffer.

## Results and discussion

Phenotypic evaluation

The results of seedlings infestation with WAA showed differences between the two seasons of assessment and among studied genotypes. At the first season all seedlings from genotype H were presented in scale 0 and 1 (100% resistant), followed by the genotype S2 which the percentage of resistant seedlings was 91.7% ,while the susceptible seedlings were in scale 3.The percentage of  resistant seedlings in genotypes B, A and C were 90.9, 86.6 and 80%

respectively, and the susceptible seedlings was in the scales 2 and 3 for genotype B, and in the scales 2,3 and 4 in the genotypes A and C (Table 2). At the second season the percentage of resistantand susceptibleseedlings were changed among genotypes. However, the percentage of resistant seedlings in the genotype H still the highest one (40.6 %), and its susceptible seedlings became in the scale 3, while the percentage of resistant seedlings decreased clearly in all other genotypes to 14.6, 10, 10 and 8.3 % in B, A, C and S2, respectively.Likewise, the susceptible seedlings in these genotypes became more excessive than the previous season, especially in genotype C which 32.5 % of seedlings were in the scale 5. These results were in agreement with Fazio and Beers (2010) that the resistant rootstocks did not change, while the infestation increased within the susceptible onesin the second season. The genotype H showed the highest percentage of resistant seedlings due to the main role of the rootstock MM106 as a parent takes its resistance property from Northern spy cultivar which has the resistant gene $Er1$ for WAA (Webster $et$ $al.$, 2000).

Table 2: the percentage of infested seedlings for each scaleamong studied genotypesduring the two seasons.

| genotype | Season of assessment | Percentage of infestation % | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| A | 2010 | 63.3 | 23.3 | 3.3 | 3.3 | 6.7 | 0 |
| | 2011 | 0 | 10 | 0 | 63.3 | 0 | 26.7 |
| B | 2010 | 72.7 | 18.2 | 3.6 | 5.5 | 0 | 0 |
| | 2011 | 7.3 | 7.3 | 0 | 76.4 | 0 | 9 |
| C | 2010 | 47.5 | 32.5 | 10 | 5 | 5 | 0 |
| | 2011 | 0 | 10 | 0 | 57.5 | 0 | 32.5 |
| S2 | 2010 | 79.2 | 12.5 | 0 | 8.3 | 0 | 0 |
| | 2011 | 0 | 8.3 | 0 | 75.5 | 0 | 16.7 |
| H | 2010 | 81.2 | 18.8 | 0 | 0 | 0 | 0 |
| | 2011 | 28.1 | 12.5 | 0 | 59.4 | 0 | 0 |

Genetic evaluation

At the end of the second season the susceptible seedlings were excluded from the applerootstock breeding program and the resistant seedlings were genetically evaluated to insure the presence of considered resistant genes for WAA .The results showed that the marker NZsn_O05 linked to $Er1$ and $Er3$ genes was the most efficient marker, it gave alleles have the predictable size 880 bp according to Bus $et$ $al.$, (2008) in 15 seedlings (Figure 1)5 of them from the genotype H (3 in the scale 0 and 2 in the scale 1), in addition to the rootstock MM106 which used as control for the gene $Er1$, 5 seedlings from genotype B (2 in the scale 0, 2 in the scale 1 and 1 in the scale 5) in addition to the mother plant, the C mother plant and 2 seedlings from genotype S2 (1 in the scale 1 and the other in the scale 5).However, this marker could not distinguish all the resistance seedlings in the genotype H, this result was in agreement with Bus $et$ $al.,$ (2008) which they found that this marker

discriminated 70 plants from 77 ones showed the resistance property. On the other hand, from 15 seedlings were detected just 2 seedlings was susceptible (1 from genotype B and the other from genotype S2), this indicated the possibility of the presence of the gene *Er*3 in this two seedlings because the plants which carrying the gene*Er*1 or *Er*2 have a higher level of resistance than those carrying the gene *Er*3 which show high susceptibility to WAA (Sandanayaka*et al.*, 2003).
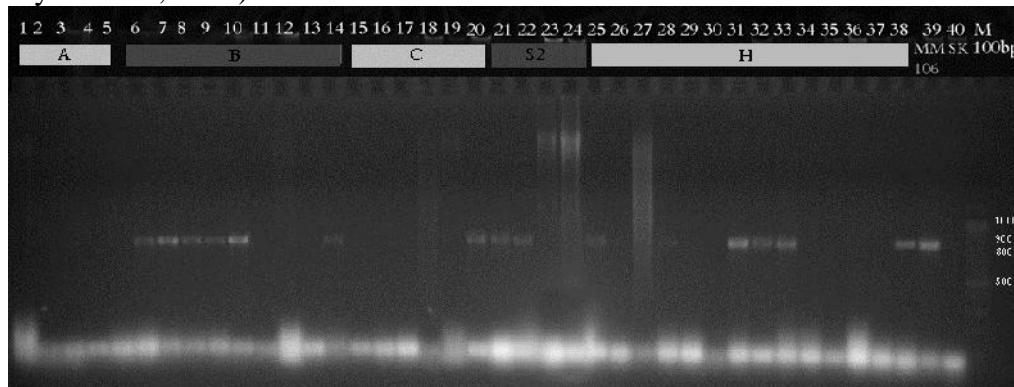


Figure 1: PCR products using the marker NZsn_O05

The marker NZSc_E01 linked to the gene *Er*3 gave three polymorphic alleles, one of them was 1350 bpas the same of predictable size by Bus *et al.,* (2008) which was noticeable in the most studied seedlings (resistant and susceptible ones), while the remaining seedlings have two otheralleles size(700 and 500 bp respectively), most of seedlings were light infestation and susceptible ones except one resistantseedling.Therefore, it was so difficult to identify seedlings which have the gene *Er*3.

The markers NZsc_C20, NZsc_O05 and NZsc_GS327 linked to the resistant gene *Er*1 did not give any PCR products. On the other hand, the markers NZms_EB145764 (linked to the gene *Er*2), NZms_EB106753 (linked to the genes *Er*1 and *Er*3) and NZSc_A01 (linked to the gene *Er*3) gave monomorphic alleles so they were not able to distinguish between resistant and susceptible seedlings. Although, these markers gave the same expected sizeasmentioned by Bus *et al.,* (2008) except NZSc_A01. This is possibly due to the apple species, which were used in primers designing, were different from the studied genotypes origin. On the other hand, these markers were may not tightly linked to the resistant genes.

## Conclusion

As a result the studied genotypes showed high susceptibility to WAA except the genotype H followed by the genotype B.Genetic evaluation of resistant seedlings from all genotypes showedthat the studied markers could not discriminate between all resistant seedlings and susceptible ones except the marker NZsn_O05. Therefore, it is necessary to develop new linked markers to WAA resistant genes depending on studied plant material, through using available techniques such SNPs and SSR. In addition, breeding programs should depend onthe strategy of pyramiding the reresistant genes to give durable resistance to WAA. hybridization caused the presence of two genes or more in the produced progenies.

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