ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Biochemical Indicators Related to Grafting Compatibility in Grapevine.

Mohamed Ahmed Fayek[1], Ahmed Abdelhady Rashedy*[1], Rania Ahmed Mahmoud[2], and AMR Ebrahim Mohamed Ali[1].

[1]Pomology department, faculty of agriculture, cairo university, Giza, Egypt, * corresponding author:,12613.
[2] Deciduous fruit research department, horticulture research institute, Agriculture Research Center, Egypt.

ABSTRACT

This study was carried out during two seasons 2015 and 2016 to determine the compatibility between five grapevine cultivars (*Vitis vinifera*) namely; Flame Seedless, Superior, Early Sweet, Crimson seedless beside Thomson and four rootstocks namely, Freedom (*Vitis champini* x *1613C*), Salt Creek (*Vitis champini*), Paulsen (*Vitis berlandieri* X *Vitis rupestris*) beside 110 Richter (*Vitis berlandieri* x *Vitis rupestris*). The grafted plants followed through two years. The results showed that Paulsen rootstock gave the highest grafting success percentages with all studied cultivars. This accompanied with the lowest total phenol compounds and peroxidase activity at above, below and at graft union zone. Moreover, Paulsen rootstock possessed the highest similarity representing 0.857 similarity compared to the other studied rootstocks. While Freedom and Salt Creek rootstocks achieved the lowest grafting success percentage which accompanied with the highest total phenols and peroxidase activity. Compatibility deterioration of Early sweet cultivar grafted onto Freedom rootstock after two years may be due to increasing total phenols, peroxidase activity. Moreover, the above graft zone showed the appearance of a unique band with low Rf value of 0.14. This study indicated that total phenols, peroxidase activity and isoperoxidase pattern could be used as prediction tools for grape graft incompatibility.

Keywords: Grapevine, incompatibility, peroxidase activity, phenols, isperoxidase.

***Corresponding author**
Email id: Ahmed.Rasheedy@agr.cu.edu.eg

INTRODUCTION

Grapes (*Vitis vinifera* L.) considers one of the most popular commercial fruit crops of the world. In Egypt, table grapes are cultivated in an area of over 192934 feddans produced 1596169 ton [1].

However, biotic and abiotic stresses restricted grape production. Selection and using a resistant rootstock against disease, drought and salinity with high grafting compatibility have great importance in many viticultural countries [2-4]

Compatibility level between rootstocks and scion cultivars affect on grafting success [5-6]. The highest grafting success achieved by 1103 P (88,89%) while the lowest recorded with 41 B (61,11%) and SO4 (51,11%) rootstocks[7]. Also, highest sprouting ratio remarked in 41B with a percentage of 47.92%. While, lowest sprouting ratio occurred in 1616C with 27.08% [8-9].

The differences of phenolic compounds in grafting union, as well as peroxidase amount might serve as biochemical markers of the rootstock/scion incompatibility. Biochemically similar scions and rootstocks could improve graft compatibility [10-11]. Since, the high phenol concentrations were obtained in less compatible combinations [12-13]. Many phenolic compounds like gallic acid, caffeic acid, ferulic acid, sinapic acid, catechin and epicatechin are potentially suitable as markers of graft incompatibility of *vitis vinifera* [14]. The less compatibility combinations (Syrah383/110R and Syrah383/SO4) exhibit higher content of gallic acid [15].

Incompatibility degree is related with high peroxidase activity in the rootstock [16-17]. The peroxidase activity could be used as a parameter in early detection of graft incompatibility [18]. Since, increasing in peroxidase in incompatible grafting was recorded on plum and peach [19].

More recently, the compatibility levels can be determined by the band similarities which suggested for forecasting graft incompatibility of *vitis vinifera* [20]. Isozyme analysis of scions and rootstocks could be used to predict incompatibility before grafting in different cultivars of Chinese chestnut [21]. When peroxidase isozyme phenotypes of rootstock and scion matched, grafting resulted in a compatible union and the restoration of vascular continuity [22]. Isozyme analysis of scions and rootstocks succeed in prediction of compatibility of many plants such as red oak [23] and sweet cherry rootstocks [18].The present investigation was outlined for prediction of grape incompatibility through determination of phenols, peroxidase activity and isoperoxidase pattern in some grafting combination.

MATERIAL AND METHODS

The present study was carried out during two seasons 2015 and 2016 at the Pomology department, Faculty of Agriculture, Cairo University and Biotechnology laboratory of the horticulture of research institute, ministry of Agriculture. The aim of this study is determination of the compatibility between five European grape cultivars (*vitis vinifera*) namely; Flame Seedless, Superior, Early Sweet, Crimson seedless beside Thomson seedless and four rootstocks namely, Freedom (*V. champinii x 1613C*), Salt Creek (*Vitis champini*), Paulsen (*V. berlandieri X V. rupestris*), 110 Richter (*V. berlandieri x V. rupestris*) in a twenty grafting combinations.

Grafting and compatibility constant:

In 2015 and 2016 cuttings of scions and rootstocks were prepared at the beginning of January and stored at 4°C and 70 to 80% RH conditions for one month. Just before bench grafting the cuttings of rootstocks were prepared with 3 disbudded nodes, while the scions pruned to one node. Both cuttings were treated with fungicide (1g/L Rizolex) then grafted using a tongue grafting method. Finally, the scion and grafting zone were tightly wrapped and waxed. The grafted cuttings, 45 in three replicates for each combination, were placed in plastic boxes in layers mixed with a mixture of peat and saw dust 1:4 v/v and covered with polyethylene sheet. The grafts material incubated at 27°C and 80 to 90% relative humidity conditions for one month [24]. Early in March, the grafts material were planted in shade-net greenhouse, each of the grafts were planted in a black plastic bag size 30 filled with sand and compost (4:1) and irrigated with tap water. At the end of October graft survival percentage was calculated as follow (survival grafts number/total number of grafts) x 100.

Determination of compatibility constant was obtained at the second season 2016. Twenty one of each successful graft combination representing three replicates were transplanted in 15th January, each plant in a black plastic bag size 45 filled with the same medium and irrigated with tap water. The percentage of successful grafts was counted at the end of the second season (1st November 2016). Chemical analyses: the bark at 4cm above, below the graft union and the graft zone were taken by a sharp knife for determination of total phenols in all graft combination using Folin ciocalteu method [13]. peroxidase activity [25] was determined only in the lowest grafting success (Crimson and Superior on Salt Creek rootstock) compared to the highest grafting success (Crimson and Superior on Paulsen rootstock) at the first season (after nine months of grafting) Besides peroxidase activity was determined in the lowest compatibility constant combination Early sweet on Freedom rootstock and the highest compatibility constant combination Superior on Freedom rootstock were determined at the second season (after nineteen months of grafting).

For determination of isoperoxidase the isoenzyme extraction was prepared following the procedure of [26]. This was achived at the season 2016.

Bark samples of nonbudded rootstocks and scions as well as in the grafts of Superior on Freedom and Early sweet on Freedom were crushed with liquid nitrogen using extraction buffer containing 50mM this-HCL buffer, ph 7.5, 5%Glycerol and 14mM mercaptoethenol. After centrifuged for 15 min at 14000rpm, supernatant was used as enzyme source and kept under -35° C until used.

Native polyactylomide gel electrophoresis (PAGE) was performed according to [27]. Bengaline was used as hydrogen donor to detect peroxidase [28], the incubation solution contained H2O2 so that isoenzyme band formation could be observed directly during the period of enzyme incubation with substrate and donor [29] method was used to calculate the relative distance (Rf value)of bands ; Rf = 1.0, the distance to the fastest band and Rf =0.0 the starting point of the running.

STATISTICAL ANALYSIS

This experiment contains interaction between Four rootstocks and Five scion grapevine cultivars, including 20 treatments each one divided into three replicates. Significant differences among treatments means were separated using LSD at 0.05 using M-Stat-C (ver. 2.10) according to [30].

RESULTS AND DISCUSSION

Grafting success and compatibility constant:

The presented data in tables (1) show graft survival percentage of Five grape scion cultivars grafted onto four different rootstocks after nine months of grafting at the two season of study (2015 and 2016). It is clear that, Paulson rootstock achieved the highest significant grafting success percentage (90.00 and 83.01%) through the first and second seasons compared to the other rootstocks. Meanwhile, Richter and Freedom rootstock gave the lower grafting success (82.82 and 82.45 %) respectively at the first season besides Freedom and Salt creek at the second season (70.74 and 69.20%).

With respect to the effect of cultivars on grafting success, Crimson seedless cultivars recorded the highest significant grafting success (94.23 and 78.5 %) while Flame seedless gave the lowest grafting success (74.08 and 72.42%) and Early sweet at the second season (71.06%).

Concerning to the interaction effect (rootstocks X cultivars) the data showed that Flame seedless grafted on Salt creek and Crimson seedless and Thompson seedless cultivar grafted on Paulson rootstock recorded the highest grafting success (100 %) through the first season. Meanwhile, Flame seedless grafted on Freedom rootstock gave the lowest grafting success (54.02%, 64.07%) in the first and second season, respectively. Also grafting success was low with grafting Flame seedless and Early sweet on Freedom rootstock (76.92 and 66.63 %) in the two seasons. Data of the second season (2016) for determination of compatibility constant after nineteen months of grafting (in table2) revealed that all rootstocks recorded the highest significant graft survival percentage except for Freedom rootstock which gave the lowest value (83.33%). With respect to the difference between the cultivars, graft of only Early sweet cultivar recorded the lowest graft survival percentage (80.83%) after nineteen months of grafting compared to the other cultivars.

ISSN: 0975-8585

Table 1: Plant survival percentage of First and second seasons (2015 and 2016) for Five grapevine scion cultivars grafted onto four rootstocks.

Cultivars(B) / Rootstocks(A)	Flame seedless		Superior		Early Sweet		Crimson seedless		Thompson		Mean	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Freedom	54.02 f	64.07 jk	88.45 b	71.77 ghi	76.92 d	66.63 ijk	96.15 a	74.33 fgh	96.15 a	76.90 d-g	82.43 C	70.74 C
Salt creek	100.0 a	76.90 d-g	82.05 cd	61.50 k	84.61 bc	71.77 ghi	84.61 bc	66.63 ijk	82.05 cd	69.20 hij	86.66 B	69.20 C
Paulsen	65.38 e	73.05 gh	88.45 b	88.45 ab	96.15 a	79.21cdef	100.0 a	92.30 a	100.0 a	82.03 cd	90.00 A	83.01 A
Richter	76.92 d	75.67 efg	80.76 cd	84.60 bc	80.76 cd	66.63 ijk	96.15 a	80.75 cde	79.48 cd	66.63 ijk	82.82 C	74.86 B
Mean	74.08 D	72.42 C	84.93 C	76.58 AB	84.61 C	71.06 C	94.23 A	78.50 A	89.42 B	73.69 BC		

New L.S.D.at (5%) A 2.403, B 2.687, AXB 5.374 for first season, , A 2.695, B 3.013, and AXB 6.026 for second season.

Table 2: compatibility constant after nineteen months of grafting at the second season (2016) for Five grapevine scion cultivars onto four rootstocks.

Cultivars(B) / Rootstocks(A)	Flame	Superior	Early Sweet	Crimson	Thompson	Mean
Freedom	93.33 a	100.0 a	43.33 b	86.67 a	93.33 a	83.33 B
Salt creek	100.0 a	100.0 a	86.67 a	100.0 a	100.0 a	97.33 A
Paulsen	100.0 a	100.0 a	93.33 a	100.0 a	100.0 a	98.67 A
Richter	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 A
Mean	98.33 A	100.0 A	80.83 B	96.67 A	98.33 A	

New L.S.D. (5%) A 8.041, B 8.990, AXB 17.98

Table3: Total phenols (mg/g F.W.) at above, below and grafting union after one year for Superior and Crimson cultivars grafted onto Salt creek and Paulson rootstocks (season 2016).

Cultivars(B) / Rootstocks(A)	Superior seedless			Crimson seedless			Mean		
	Above	Union	below	Above	Union	below	above	union	below
Salt creek	216.03 b	213.03 b	170.7 b	260.47 a	262.18a	161.8 b	238.25**	237.61**	166.24**
Paulsen	180.13 c	187.40 c	181.0 a	181.84 c	186.97c	181.4 a	180.98	187.18	181.20
Mean	198.08 **	200.21**	175.86 ^{ns}	221.15	224.57	171.585			

New L.S.D. (5%) (AXB) 0.5171 (AXB) 0.2447 and (AXB) 0.4238 for above, at grafting union and below it,respectively.
**Significant at P ≤ 0.001. ns - non significant.

Concerning to the interaction effect (rootstocks X cultivars) it showed that all combinations recorded the highest significant graft survival percentage (86.67 to 100%) except that Early sweet cultivars grafted on Freedom rootstock which recorded the lowest significant plant survival percentage (43.33%).

Total phenols:

Data presented in table (3) show total phenols content at above, below graft zone after nine months of grafting of Superior seedless and Crimson seedless cultivars onto Salt Creek and Paulson rootstocks.

Generally, grafts on Salt creek rootstock recorded the highest significant total phenol contents at above and at graft zone compared to Paulson rootstock. However the figure was reversed at below grafting zone.

As for the cultivars effect on total phenols content only superior seedless cultivar recorded the lowest significant values at above and union zone compared to the corresponding value of Crimson seedless. With respect to the interaction, grafting of crimson seedless onto salt Creek rootstock recorded the highest significant phenols content at the scion and graft zone compared to the other combination (Superior seedless /Salt creek). Meanwhile, grafting of Crimson seedless onto Paulsen rootstock recorded the lowest values at above and the graft zone.

Table (4) presented total phenol contents at above, below and graft zone of high alive grafting combination (Superior seedless / Freedom) and low alive (Early sweet / Freedom) after nineteen months of grafting. Data revealed a noticeable decrease in total phenols at above and at union zone in high alive grafting combination compared with the analogous values in low alive grafting combination total phenols content at below grafting zone was not affected by the scion type.

Table4: Total phenols (mg/g F.W.) at above, below and grafting union after nineteen months of grafting of Early sweet and Superior scion onto Freedom rootstock after nineteen months of grafting (season 2016).

Grafts Combination	Above	Union	below
Superior /Freedom	208.763	207.910	204.063
Early Sweet /Freedom	259.620**	224.150 ^{ns}	203.637 ^{ns}

** Significant at P ≤ 0.001. ns - non significant.

Peroxidase activity:

Data presented in table (5) show that Peroxidase activity at above, below and graft zone after nine months of grafting, high successful grafts (Superior and Crimson on Paulson rootstock) and low successful grafts the same scion on Salt creek rootstock. Generally, peroxidase activities were increase above grafting union than at graft zone or and below it.

Table5: Peroxidase activity at above, below and grafting zone after nine months of grafting for Superior and Crimson cultivars grafted onto Salt creek and Paulson rootstocks (season 2016).

Cultivars(B) / Rootstocks(A)	Superior			Crimson seedless			Mean		
	Above	Union	Below	Above	Union	Below	above	union	below
Salt creek	16.67a	14.10a	10.69a	10.81c	10.44c	8.820c	13.742**	12.272**	9.757**
Paulsen	15.06b	11.51b	9.440b	7.947d	9.630d	7.137d	11.505	10.575	8.288
Mean	15.867**	12.807**	10.067 **	9.380	10.037	7.978			

New L.S.D. (5%) (AXB) 0.5171 (AXB) 0.2447 and (AXB) 0.4238 for above, at grafting union and below it , respectively. ** Significant at P ≤ 0.001. ns - non significant.

Concerning to the rootstock effect on peroxidase activity, Salt creek gave the higher values compared to Paulson rootstock. With respect to the effect of cultivars on peroxidase activity, Superior seedless cultivar recorded higher values compared to Crimson seedless cultivar. As for the interaction effect (rootstock X cultivar), the highest peroxidase activity at above, below and graft zone was recorded by grafting either scion under study on Salt creek rootstock compared to with grafts on Paulson rootstock.

Table (6) shows the Peroxidase activity at above, below and grafting union of high alive grafts after nineteen months (Superior seedless / Freedom) and low alive grafts (Early sweet / Freedom). Data revealed an increase in combination of (Early sweet / Freedom) compared to combination of (Superior seedless /Freedom) which recorded the lowest values at all position.

Table6: Peroxidase activity at above, below and graft zone of Early sweet and Superior scion grafted onto Freedom rootstock after nineteen months of grafting (season 2016).

Grafts	above	union	below
Early Sweet / Freedom	16.730**	15.887 ns	15.072 ns
Superior Seedless/ Freedom	11.187	15.150	14.853

**Significant at P ≤ 0.001. ns - non significant

Isoperoxidase patterns: in Figure (1) show the isoperoxidase patterns of nonbudded rootstocks and scions (season 2015). No allozymic polymorphism could be detected between nonbuded scions, where they showed four identical bands. While profiles of isoperoxidase for nonbudded rootstocks showed mismatch patterns either between each other or nonbudded scions with different degrees, where none of rootstocks were identical in relation to others or any of the scions. However, Paulsen nonbudded rootstock possessed the highest similarity representing 0.857 similarity (Figure 2). Meanwhile Salt creek rootstock showed the highest number of isoperoxidases (**5**). Freedom is the only rootstock contains the band with the low Rf value of 0.08 as in scions.

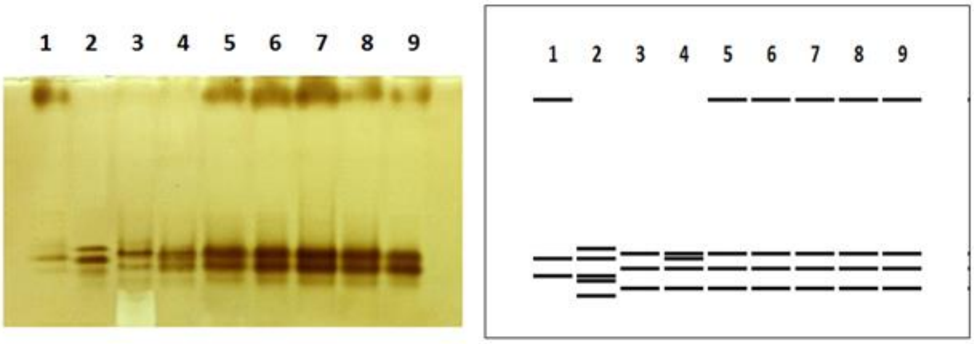

Figure 1.Isoperoxidase patterns of nonbudded rootstocks and scions: Rootstocks; Freedom (1), Salt creeck (2), Paulsen (3), Richtir (4) - Scions; Flame (5), Superior seedless (6), Early sweet (7), Crimson seedless (8), Thompson seedless (9).

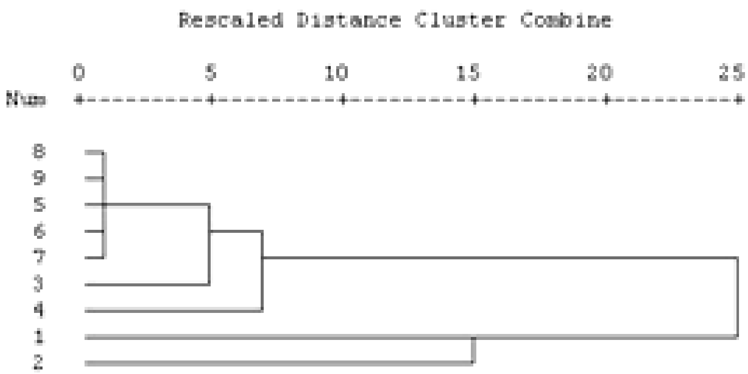

Figure 2.A dendrogram showing the genetic distance amongnonbudded rootstocks and scions: Rootstocks; Freedom (1), Salt creeck (2), Paulsen (3), Richtir (4) - Scions; Flame seedless (5), Superior seedless (6), Early sweet (7), Crimson seedless (8), Thompson seedless (9).

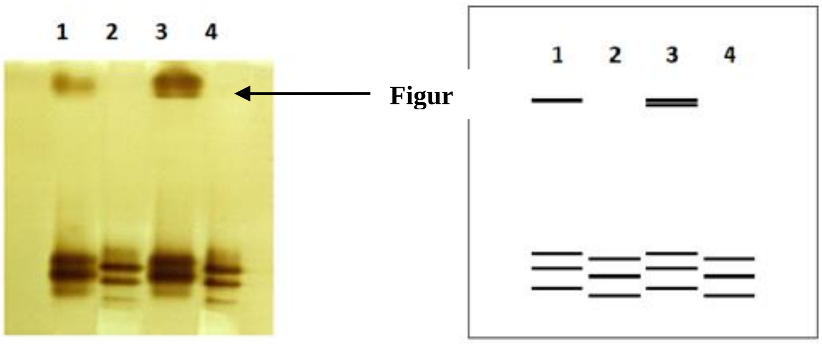

Figure 3.Isoperoxidase patterns of above and below the grafting zone of budded plants; 1: Superior seedless /Freedom (above), 2: Superior seedless /Freedom (below), 3: Early sweet/Freedom (above), 4: Early sweet/Freedom (below).

The isoperoxidase patterns at above and below graft zone of high compatible grafts (Superior / Freedom) and low compatible grafts (Early sweet / Freedom) after nineteen month of grafting (season 2016) are presented in Figure 3.

The above graft zone sample of low compatible graft (Early sweet/Freedom) showed the appearance of a unique band with low Rf value of 0.14. on the opposite, the below graft zone samples of Freedom rootstock in both high compatible (Superior seedless /Freedom) and low compatible combinations revealed identical isoperoxidase patterns.

The present study revealed that the endogenous factors could related to compatibility in grapevine. Reduction of total phenols and peroxidase activity either above or at graft zone consider as indicator for compatibility between scions and rootstocks. Since, the best compatible combination was Crimson seedless grafted onto Paulson rootstock which recorded the lowest total phenols and peroxidase activity. While, the combination of Early sweet grafted onto Freedom which have low compatibility recorded the highest total phenols and peroxidase activity. These results were in agreement with [13] and [31] as they found that high total phenol concentrations were obtained in less compatible combinations than in compatible combinations. Phenol compounds may be caused poor callus formation through affecting cell division, development and differentiation at the graft union [15]. Also, phenols may be caused dysfunctions in the growth of xylem and phloem, which may manifest during the first year after grafting and after several years later [32] and caused necrotic tissues of grafting union [33-34].

Previous researches on other genera showed that, peroxidase isozyme analysis of rootstock and scion before grafting could be used to predict intraspecific compatibility or incompatibility. In our study the highest isoperoxidase similarity was recorded in the Paulson rootstock which had the highest compatibility combination on the test scions was obtained in the lowest similarity Salt creek rootstock which gave low compatible with the tested scions. Moreover, after nineteen months of grafting the above graft zone of low compatible graft (Early sweet / Freedom) showed the appearance of a unique band with low Rf value. These results go in line with [35] on Chinese chestnut; [23] on red oak and [22] on pear.

Dissimilarities in isoperoxidase composition between rootstock and scion might result in abnormal lignification and lack of vascular connections at the graft union, resulting in an incompatible combination [35]. However, that different isoperoxidases are involved in production of structurally different lignins which perhaps have different bonding (with cell wall carbohydrates) characteristics [23].

Rather interesting patterns of appearance or disappearance of isoperoxidases may be brought about due to altered gene expression (de novo expression or suppression). These changes may be triggered by a signal produced by the contact between the two graft partners [22]. Specific peroxidase isozymes could be responsible for compatible graft unions through their role in lignin biosynthesis, resulting in a stronger graft union [22].

CONCLUSION

1. The present investigation suggest that total phenols content and peroxidase activity at above of union grafting and graft zone can be used as a biochemical markers to detect early graft incompatibility in

grapevine. According to the results, these biochemical markers are increasing in graft incompatibility combination.

2. Isoperoxidase patterns of nonbudded scions and rootstocks can be used also as a tool to predict early graft incompatibility. According to the results, dissimilarities in isoperoxidase composition between rootstock and scion are find with graft incompatibility combination.

REFERENCE

[1] Agriculture Directorates of Governorates. Economic Affairs Sector Ministry of Agriculture, Egypt 2014; Pp 313-314.
[2] Reynolds AG, Wardle DA. Hortechnology 2001; 11(3):419-427.
[3] Moretti G. Vignevini 2005; 32(11):68-95.
[4] Cookson SJ, Moreno MJC, Hevin C, Mendome LZN, Delrot S, Trossat-Magnin C, Ollat N. Journal of Experimental Botany 2013; 64(10): 2997–3008.
[5] Çelik H, Odabas F. Turkish Journal of Agriculture and Forestry 1998; 22(3), 281-290.
[6] Verma SK, Singh SK, Patel VB, Singh KM. The Journal Of rural and Agriculural Research 2010; 10(1):1-3.
[7] Kamiloğlu O, Guler EA. Journal of Agricultural and Natural Sciences 2014; 1: 1005-1010.
[8] Cakr A, Karaca N, Sdfar M, Baral C, Soylemezoglu G. Journal of Agricultural Sciences 2013; 23(3):229-235.
[9] Somkuwar RG, Taware PB, Bhange MA, Sharma J, Khan I. International Journal of Fruit Science 2015; 15:251–266.
[10] Darikova JA, Savva YV, Vaganov EA, Grachev AM, Kuznetsova GV. (2011). Journal Siberian Federal University2011; 1(4): 54-63.
[11] Gainza F, Opazo I, Muñoz C. Chilean Journal of Agricultural Research 2015, 75: 28-34.
[12] Errea P, Garay L. Mari JA. Physiologia Plantarum 2001; 112: 135-141.
[13] Mngomba SA, Du toit ES, Akinnifesi FK. ScientiaHorticulturae 2008; 117(3), 212-218.
[14] Canas S, Assuncão M, Brazão J, Zanol G, Eiras-Dias J. Journal Phytochemica Analysis 2015; 26:1–7.
[15] Assunco M, Canas S, Cruz S, Brazão J, Zanol GC, Eiras-Dias JE. Scientia Horticulturae 2016; 207:140-145.
[16] Rodrigues AC, Machadb LB, Diniz AC, Fachinello JC, Fortes GRL. Revista Brasileira de Fruticultura 2001; 23(2):359-364.
[17] Telles CA, Biasi LA, Neto URM, Deschamps C. Ciência e Agrotecnologia, Lavras 2009; 33(1):86-91.
[18] Guclu SF, Koyuncu FA. NotulaeBotanicae Horti Agrobotanici Cluj-Napoca 2012; 40(1):243-246.
[19] Zarrouk O, Testillano PS, Risueño MC, Moreno MÁ, Gogorcena Y. Journal of the American Society for Horticultural Science 2010; 135(1): 9-17.
[20] Gokbayrak Z, Söylemezoğlu G, Akkurt M, Celik H. ScientiaHorticulturae 2007; 113: 343-352.
[21] Gulen H, Arora R, Kuden A, Krebs SL, Postman Journal of the American Society for Horticultural Science 2002; 1027(2): 152-157.
[22] Santamour FSJR, Mcardle AJ, Jaynes RA. Cambial isoperoxidase patterns in Castanea.Journal of Environmental Horticulture 1986; 4: 14-16.
[23] Santamour FSJR.. Journal of Environmental Horticulture 1988b; 6: 87-93.
[24] Paunovic SM, Miletic R, Mitronic M, Jankovic D. Journal of Fruit and Ornamental Plant Research 2011; 19(2):5-14.
[25] Fernandez-Garcia N, Crvajal M, Olmos E. Annals of Botany 2004; 93: 53 – 60.
[26] Wender JF, weeden NF. Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS. Isozymes in Plant Biology: advance in plant sciences series. Washington. 1989, p.5-45.
[27] Lamme UK. Nature 1970; 227: 680-685.
[28] Laberge DE, Kruger JE, Meredith WOS. Canadian Journal of Plant Science 1973. 53: 705-713.
[29] Manganazis AG, Alston FH. Theoretical and Applied Genetics 1992; 83: 392-399.
[30] Snedecor GW, Cochran WG. Statistical methods.8. ed. Iowa State University Press, 1989. 503p.
[31] Stino RG, Ghoneim IE, Marwad IA, Fadi TR. Journal of Horticulture science and Ornamental plants 2011; 3(1):86-90.
[32] Feucht W. Acta Horticulturae 1992; 314: 331-338.
[33] Salvatierra G M, Gemma H, Lwahori S. Journal of the Japanese Society for Horticultural Science 1999; 68 (4):724-733.
[34] Çölgeçen H, Azimi M. Jordan Journal of Agricultural Sciences 2015; 11(3):705-712.
[35] Santamour FSJR. Journal of Environmental Horticulture 1988a; 6: 33-39.