5. Koike, H. and T. Ono. 1998. Optimum crop load for Fuji apples in Japan. Compact Fruit Tree 31: 13-16.
6. Koike, H., H. Tamai, T. Ono and H. Komatsu. 1999. Apple growing in Japan. Compact Fruit Tree 32: 18-26.
7. Lakso, A.N., T.L. Robinson and M.C. Goffinet. 1996. Influence of fruit competition on size, and the importance of early thinning. Compact News. December, 1996. 3: 2-4.
8. Looney, N.E., M. Beulah and K. Yokota. 1998. Chemical thinning of Fuji apple. Compact Fruit Tree 31: 55-57.
9. Mantinger, H. 1998. The cultivation of Fuji in South Tyrol and in Italy. Compact Fruit Tree 31: 17-21.
10. McArtney, S., J.W. Palmer, and H.M. Adams. 1996. Crop loading studies with 'Royal Gala' and 'Braeburn' apples; effect of time and level of hand thinning. N. Z. J. Crop and Hort. Sci. 24: 401-407.
11. Sheng, B., W. Wang, and J. Tao. 1998. Fuji adaptability in southern apple economic cultivation districts. Compact Fruit Tree 31: 29-32.
12. Waldner, W. and M. Knoll. 1998. The influence of fruit load on biennial bearing of Fuji. Compact Fruit Tree 31: 25-28.

Journal American Pomological Society 57(4):173-176 2003

The Effect of Soluble Sugars, Total Flavan and Juglone Concentrations in Walnut Scions on Graft Survival

KENAN YILDIZ[1], HÜDAI YILMAZ[1] , AND FIKRI BALTA[1]

Abstract

We examined the effects of soluble sugar, juglone and total flavan concentration of scionwood on graft success in walnut (*J. regia* L). According to simple regression analysis, graft success was linearly related to sugar and juglone concentrations, and quadratically related to flavan concentration. In addition, the best multiple regression model was constructed for graft success according to all possible regression approach. It was found that the best model included linear terms for flavan and sugar, quadratic term for flavan and the interaction term for flavan x sugar. The r square (R^2) for this model was 0.72, meaning that this model explained 72% of variation in graft success. The residual variation (28%) resulted from other factors.

Introduction

Many researchers involved in walnut grafting indicate two main causes of low graft survival percentage. The first is high phenol content of scionwood (5,6,13), and the second is juglone in xylem exudates (1,9,15,16,17). The oxidation of phenols and juglone during grafting may induce a necrotic layer at the graft interface. This layer negatively affects the differentiation of new vascular tissues at the graft union. In addition to these two factors, it has been reported that carbohydrate content of scion is also an important factor affecting graft survival of walnut (10,18).

Previous studies related to this subject were aimed primarily at determination of individual effects of factors that influence graft survival. The objective of this study was to determine individual and cumulative effects of three factors on graft success of walnut by regression analysis.

Materials and Methods

The experiment was carried out under uncontrolled field conditions. Whip and

[1]Yuzuncu Yil University, Faculty of Agriculture, Deparment of Horticulture, 65080 Van, Turkey

tongue grafts were made on April 15. Two-year-old seedlings (*J. regia* L) were used as rootstocks. The scions were collected from twenty different genotypes in Edremid located in the Lake Van Region of Turkey. After grafting, cylindrical plastic pipes were fitted around the graft area and filled with soil to protect the grafting area from cold injury and moisture loss (2).

Sixty grafts were made for each genotype. The experiment was a completely randomized design with three replicates, each replicate having 20 grafts. Graft success was calculated for each replicate as percentage of 20 grafts, so there were 3 observations per genotype. Flavan, juglone, and sugar analyses were made on 5 scions for each replicate. The mean of 5 analyses was used as the replicate value. Data were analyzed by ANOVA, and means of genotype were separated with Duncan's multiple range test. To determine relationships between graft success and flavan, juglone, and sugar, simple and multiple regression analyses were made. In the regression analyses, means values of genotypes were used. Statistical analyses were with SAS (12).

To determine flavan concentrations, phloem tissue of the scion was separated from xylem using a pin with an arrowhead. The sample of phloem tissue was extracted with 80% ethanol and 0.3% ascorbic acid (as an antioxidant) for 72 hours, and then was dried at 40°C. By calculating 1 ml ethanol for each 10 mg sample of phloem tissue, stock solutions were prepared. The stock solutions were diluted with ethanol in ratio of 1/50. After adding 1 ml P-DMACA reagent for each 5 ml of solution, absorbance values were read at 635 nm with a spectrophotometer. A standard curve was constructed using pure catechin. Total flavan concentration was expressed as mg catechin equivalent per g phloem tissue (7, 14).

For juglone analysis, the samples of scion bark (2g) were extracted with 50ml petroleum ether (40-60°C) for 24 hours. After diluting the solutions with petroleum ether to ratio of 1/5, absorbance values were read at 410 nm with a spectrophotometer. A standard curve was constructed using juglone (Sigma, J-500). Juglone concentration was calculated using a standard curve and expressed mg/g of bark (16).

Soluble sugar concentration was measured using the anthron method by spectrophotometer. A sample of dried bark (1g) from the scion was extracted with 50 ml of 80% ethanol, shaken for 2 hours and filtered. Then 10 ml of anthron were added for each 5 ml of solution and kept in boiling water for 15 minutes. After the solution temperature was decreased to 22-24 °C in the ice bag, absorbance values were read at 620 nm with a spectrophotometer. A standard curve was constructed using anhidroglucose. Soluble sugar concentration was calculated using the standard curve and expressed as percentage of bark dry weight (4).

Results and Discussion

The percentage of graft success ranged from 10% to 60% (Table 1). Total flavan concentration was between 0.4 and 0.9 mg/g. The juglone concentration varied between 0.3 and 1.1 mg/g. The soluble sugar concentration was between 12.2% and 22.4%. The differences among genotypes were significant ($p \leq 0.05$) for flavan, juglone, and soluble sugar concentrations of scionwood and graft success (Table 1).

The correlation (0.54) between graft success and soluble sugar was positive and significant (Table 2). The correlation (-0.48) between graft success and juglone was negative and significant ($p<0.05$). On the other hand, the correlation between graft success and total flavan was not significant (Table 2).

In the result of simple regression analsis, soluble sugar concentration was positively and linearly related to graft success (Figure 1). The determination coefficient was 0.30, meaning that soluble sugar explained 30% of variation in graft success. In previous studies, it has been reported that high carbohydrate content of scion is desired for high percentage of graft success in walnut (4, 18). Likewise, Rongting and Pinghai (10,11) reported that carbohydrate concentrations of scion influenced the graft success in walnut.

The juglone concentration was negatively and linearly related to graft success (Figure 2). Many researchers suggested that juglone negatively affects graft success of walnut (9,15,17). In simple regression analysis, juglone concentration explained 23% of variation in graft success.

The linear relationship between flavan and graft success was not significant. The regression analysis confirmed that the response of graft success to flavan concentration is quadratic (Figure 3). Determination coefficient (r square) for this quadratic model was 0.37. According to this model, at first while flavan concentration is increasing, graft success is decreasing. However, above a certain concentration, as flavan concentration is increasing, so is graft success.

It is difficult to explain this kind of relationship with the findings of this study. This is probably due to the fact that some flavan derivatives in total flavan amount are stimulative while others are as inhibitory. Therefore, more worthwhile results can be obtained from the examination of the individual effects of flavan derivatives rather than total flavan. Karadeniz (5) reported that while catechin, constituting 50% of total flavan, had a negative effect on graft success in walnut, other flavan derivatives had a positive effect.

All possible regression models for graft success were produced from linear and quadratic terms of flavan, juglone and sugar, and interaction terms of flavan x juglone, flavan x sugar and juglone x sugar. The best regression model for graft success was determined. This model included linear terms for flavan and sugar, the quadratic term for flavan, and interaction term for flavan x sugar. For graft success (GS), the best multiple regression model was: GS(%)=221-577.9 (flavan mg/g) + 226.2 (flavan mg/g)^2- 4.2 (sugar %)+14.4 (flavan x sugar)

The determination coefficient for the model was 0.72, meaning this model explained 72% of variation in graft success. The remaining variation (28%) could be attributed to other factors affecting graft success.

Table 1. Flavan, juglone, and soluble sugar concentration of the scionwood and graft success

Genotype no	Graft success (%)	Total flavan (mg/g)	Juglone (mg/g)	Soluble sugar (%)
1	60 a[Z]	0.4 c	0.7 cd	22.4 a
2	20 hi	0.6 bc	0.4 fg	16.2 cde
3	53 b	0.6 bc	0.6 de	19.7 abc
4	40 de	0.6 bc	0.8 bc	19.2 abcd
5	13 ij	0.6 bc	0.8 bc	14.8 de
6	15 ij	0.7 ab	0.8 bc	15.2 de
7	30 fg	0.6 bc	0.8 bc	17.8 bcd
8	33 f	0.8 ab	0.6 de	16.4 bcde
9	35 ef	0.6 bc	0.5 ef	20.6 ab
10	55 ab	0.8 ab	0.7 cd	18.2 bcd
11	25 gh	0.7 ab	0.8 bc	16.0 cde
12	57 ab	0.4 c	0.3 g	15.8 cde
13	10 j	0.8 ab	0.7 cd	16.0 cde
14	15 ij	0.7 ab	0.9 b	15.1 de
15	22 h	0.8 ab	1.1 a	17.0 bcd
16	15 ij	0.7 ab	0.8 bc	15.0 de
17	40 de	0.8 ab	0.6 de	17.1 bcd
18	45 cd	0.4 c	0.6 de	12.2 e
19	50 bc	0.9 a	0.4 fg	20.0 abc
20	52 b	0.6 bc	0.7 cd	18.0 bcd

[Z]Means followed by a letter in common are not significantly different at the 0.05 level by Duncan's multiple range test.

Table 2. Correlation coefficients between total flavan, juglone, soluble sugar concentration of scions and graft success.

	Graft success	Total flavan	Juglone	Soluble sugar
Graft success	---	-0.36	-0.48*	0.54*
Total flavan	-0.36	---	0.24	0.03
Juglone	-0.48*	0.24	---	-0.16
Soluble sugar	0.54*	0.03	-0.16	---

*Significant at p≤0.05 (n=20)

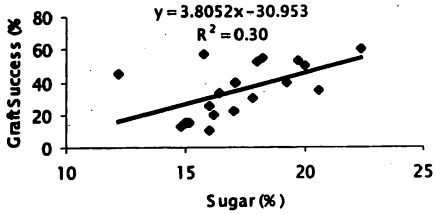

Figure 1. Relationship between sugar concentration and graft success.

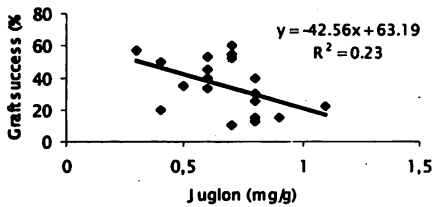

Figure 2. Relationship between juglone concentration and graft success.

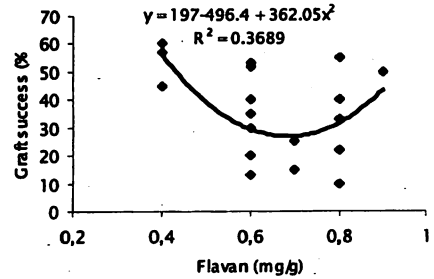

Figure 3. Relationship between flavan concentration and graft success.

Literature Cited

1. Barut, E. 2001. Different whip grafting methods on walnut. Acta Hort. 544:511-503.
2. Barut, E. and A. Eris. 1987. A research on application of fall and growing season grafting and budding on walnuts (in Turkish). Bahçe 16: 3-11.
3. Hartman, W. 1977. Importance of carbohydrate reserves in succesful grafting. Hort. Abst. 47:3379.
4. Kaplankiran, M. 1984. The studies on relationships between growth and hormone, carbohydrate and nutrient element contents of some citrus rootstock. PhD Thesis (in Turkish). Çukurova Univ. Faculty of Agric. Dept. of Hort. Adana Turkey.
5. Karadeniz, T. 1993. The studies on relationships between flavan content and graft success in walnut (*J. regia* L.). PhD Thesis (in Turkish). Yüzüncü Yil Univ. Faculty of Agric. Dept. of Hort. Van, Turkey.
6. Karadeniz, T., F. Balta, S.M. Sen, F.E. Tekintas, and A. Tanrisever. 1995. Relations between some phenolic compounds and graft success in walnut (*J. regia* L.). Acta Hort. 442: 193-196.
7. Karadeniz, T., F. Balta, S.M. Sen, F.E. Tekintas, and A. Tanrisever. 1997. Effects of the flavan contents extracted from wanut (*J. regia* L.) on coleoptile growth, and a comparison of relations between the total flavans and the graft success with these effects. Acta Hort. 442:187-191.
8. Pinghai, D. and X. Rongting. 1993. Effect of phenols on the survival of walnut grafting. Acta Hort. 311:134-139.
9. Prataviera, A.G., A.H. Kuniyuki, and K. Ryugo. 1983. Growth inhibitors in xylem exudates of Persian walnut (*J. regia* L.) and their possible role in graft failure. J. Amer. Soc. Hort. Sci. 108:1043-1045.
10. Rongting, X. and D. Pinghai. 1990. Theory and practice of walnut grafting. Acta Hort. 284: 69-88.
11. Rongting, X and D. Pinghai. 1993. A study on the uniting progress of walnut grafting and the factors affecting. Acta Hort. 311:160-170.
12. SAS 1998. SAS/STAT Software: SAS Inst. Inc. Cary, NC, USA.
13. Sen, S.M., T. Karadeniz, F.Balta, F.E. Tekintas, and A. Tanrisever. 1995. Changing of flavan contents at the some organs of walnut (*J. regia* L) seedling exposed to the controlled grafting condition. Acta Hort. 442: 181-185.
14. Tanrisever, A. 1982. A new method in analysis of condense thanen and their physiological importance. J. of Ege Univ. Faculty of Agric, 19: 27-38
15. Tekintas, F.E. 1988. The investigations on graft formation and grafting problems in the walnut (*J. regia* L). PhD Thesis (in Turkish). Ege Univ. Faculty of Agric., Dept. of Hort. Izmir, Turkey.
16. Tekintas, F.E., A. Tanrisever, and K. Mendilcioglu. 1988. Investigation on the isolation and yearly variation of juglone in the walnut (*J. regia* L). J. of Ege Univ. Faculty of Agric. 25:215-225.
17. Yildiz K. 1997. The effect on graft success of buds taken from different places of the scion in walnut (*J. regia* L) PhD Thesis (in Turkish). Yüzüncü Yil Univ, Faculty of Agric, Dept. of Hort. Van, Turkey.
18. Zachej, S. 1976. Determination of the optimum time for grafting walnuts with the use of heat. Hort. Abst. 46. 10: 9111.