

Grapevine Scion-Rootstock Combinations of Palestinian Local Cultivars and Rootstocks Resistant to Grape Phylloxera *Daktulosphaira vitifoliae* (Fitch) [Phylloxeridae: Homoptera]

Rezq B. Salimia and Abdul-Jalil S. Hamdan *

ABSTRACT

Grapevine-rootstock experiment was carried out in 2006 at Hebron University, Palestine. The experiment included a set of clonal-scions of five local cultivars (*Vitis vinifera* L.) namely: Shami, Beitoni, Darawishi, Masri, and Hamadani-Faransi, grafted on to five rootstocks resistant to grape phylloxera namely: Richter-110, Paulsen-1103, Ruggeri-140, B41, and 216/3, in addition to un-grafted vines as control treatment. The grafting was done using tongue grafting indoor technique.

All grafted plants showed excellent percentage of grafting success after ten weeks in the incubation room with almost 100% success. Positive correlations were found between callus development and graft success in the nursery. After five months in the nursery, Shami and Masri cultivars demonstrated high compatibility while Beitoni and Hamdani-Faransi cultivars showed intermediate level of compatibility, but Darawishi cultivar showed poor compatibility with all tested rootstocks.

Keywords: Grapevines, *Vitis vinifera*, Rootstocks, Scions, Compatibility, *Daktulosphaira vitifoliae*.

1. INTRODUCTION

Viticulture in Palestine goes back at least to 4000 years ago. Due to the unique geographical and ecological environment suitable for growing high quality of table grape, its plantation and production still limited to the southern part of West-Bank especially in Hebron area (MOA, 1998, 1999). The area planted to grapevines during 2005/2006 was 7178 ha (PCBS, 2007). In addition, grapevine ranks the second among fruit crops grown in Palestine in terms of the planted area and economic returns.

Phylloxera *Daktulosphaira vitifoliae* (Fitch) [Phylloxeridae: Homoptera] is one of the main problems facing grapevine production growers in Palestine making the vine production very difficult, resulting in severe

damage drastically affecting the yield world-wide. (Omer *et al.*, 1999, Al-Mommany and Al-Antary, 2008). Using resistant rootstocks against this pest is one of the important tactic to reduce the effect of this pest in major viticultural countries world-wide (Mustafa-Al-Antary and Al-Mommany, 1990, Troncoso *et al.*, 1999, Omer *et al.*, 1999).

Due to numerous available rootstocks and the difficulty in predicting the interaction of scion and rootstocks at short as well as long term, make it difficult to choose the suitable rootstock (Cus, 2004, Loreti and Massai, 2006). Indeed, different levels of compatibility existing between resistant grapevine rootstocks and *Vitis vinifera* cultivars (Celik *et al.*, 2003, Zink and Schropp, 2004, Todic *et al.*, 2005), but some grafting resulted as incompatibility. However, two types of graft incompatibility were classified: the so-called translocated and localized incompatibility (Errea, 1998, Read and Sanjun, 2003, Gokbayrak *et al.*, 2007). The first is associated with symptoms that showed leaf aging, alterations in the riduculer system as well as degradation

* Department of Plant Production and Protection, Faculty of Agriculture, Hebron University, Hebron, Palestine. Received on 18/9/2008 and Accepted for Publication on 12/1/2009.

of the sieve tubes and companion cells of the phloem of the grafts (Herrero, 1968), while, in the second type malformation is associated at the graft union resulted in mechanical weakness and subsequent breakdown of that union (Errea, 1998). However, if incompatibility between rootstocks and local cultivars resulted in death of the trees (Errea, 1998) and therefore, catastrophic losses of grapevine farms. Early and accurate prediction of graft incompatibility in nursery has great importance because incompatible combinations may be avoided and compatible once could be selected (Petkou *et al.*, 2004).

The main goal of this work was to test the levels of compatibility-incompatibility "scions x rootstocks combinations" between five Palestinian local table-grapevine cultivars (*Vitis vinifera*) and five grapevine rootstocks resistant to phylloxera insect in nursery.

2. MATERIALS AND METHODS

Planting Material:

a. Rootstocks: Cuttings of 30-40 cm in length with 3 nodes of the following grapevine rootstocks known to be resistant to phylloxera namely: Richter-110, Paulsen-1103, Ruggeri-140, B 41, and 216/3 were obtained from the Holly Land Nursery officially licensed grapevine rootstock production in Palestine at Sa'er-Hebron.

Cuttings were tested against grapevine leaf roll associated viruses, *Fanleaf*, and Corkey-Bark using ELISA technique (Al-Moudallal *et al.*, 1984, Boscia *et al.*, 1992). The tests were conducted at the biotechnology laboratory of Bethlehem University.

The nodes were blinded using sharp sterilize knife. For rooting enhancement, 2cm vertical cuts were done at the bases of each cutting.

The cuttings were soaked for 30 minutes in a disinfectant aqueous solution containing 2 gm/L Bavestin (Carbendazim 50% W.P.), 2 gm/L Merpan (Captan 50% W.P.), 2 ml/L Roger (Dimethoate 40% E.C.), and 1 ml/L Confidor (Imidapride 35% E.C.). Then, the cuttings were left to dry and placed into wood-meal that was treated also with the disinfectant solution mentioned previously above.

b- Scions: Twelve to Fifteen cm long healthy hardwood cuttings (node + internode) of five Palestinian local table-grapevine cultivars including: Shami, Beitoni, Darawishi, Masri, and Hamadani-Faranse were obtained

from Al-Arroub Agricultural Experimental Station. The cuttings were disinfected as mentioned before.

Grafting: Grafting was performed during February 2006 using tongue grafting indoor technique (Todic *et al.*, 2005), and for comparison, another set of cuttings were grown on their own-root as check. Grafted areas were covered (rolled and tied) with special plastic parafilm and dipped for 10 seconds into hot grafting-wax (Paraffin wax 5%). Then, the lower parts of the rootstocks were dipped for 5 seconds into 4000ppm Indole-3-Butyric Acid.

Each scion/rootstocks combination and own-rooted vines "check plants" was replicated 20 times.

Incubation Room: All grafted plants as well as own-rooted vines were placed in plastic boxes filled with the treated wood-meal and incubated for 10 weeks in an incubation room under 22-25°C, 95% RH and 80% darkness (Todic *et al.*, 2005), then transferred to the nursery for acclimatization.

Nursery: Successful grafted seedlings were transferred into 2 liter black plastic bags filled with a sand and compost (1:1), and watered with the disinfectant aqueous solution. All scions were pruned to two nodes for hardiness, and then transferred to the nursery and distributed into completely randomized design (CRD), kept for the whole summer before transplanting into the open field for further investigation. During this period, all transplants were maintained under nursery conditions. The transplants were watered daily and treated against insect pests and diseases when needed.

Measurements: To determine the levels of compatibility-incompatibility of the different scion/rootstock combinations (Celik, 2000, Celik *et al.*, 2003), the following parameters were studied :

- Grade of callus development at the grafting union; a scale of 1 to 4 was used where: 1=no callus, 2=low, 3=intermediate, and 4=high callus formation on graft union surface.
- Grade of rooting system development; a scale of 1 to 4 was used where: 1=no roots, 2=low, 3=medium, and 4=high).
- Days required to scion bud-burst.
- Percentage (%) of successfully grafted grapevine where plants develop new shoots after ten weeks of incubation.

- Percentage (%) of survival grafted plants moved to nursery, where plants had a vegetative growth and the graft union was lignified after five-months.
- Average length of the main shoot after five-months in nursery (cm).

Data analysis: Data were statistically analyzed using the one-way analysis of variance ANOVA and means were separated using the Tukey's pairwise comparisons at a significance of $p \leq 0.05$ using the MINITAB package system.

3. RESULTS

As a result of this study, different levels of graft-compatibility were recorded between rootstock/scion combinations. The cultivars Shami and Masri had significantly greater callus development resulting in a greater root development among all tested rootstock compared to Beitoni, Darawishi, and Hamadani-Faranse cultivars (Table 1, 2).

All own-rooted vines had good rooting with no significant difference in between. Interestingly, own-rooted Shami and Masri vines had similar amounts of root development in comparison to other combinations (Table 2).

All grafted plants as well as own-rooted vines showed higher percentage almost 100% of grafting after ten weeks of incubation. After five months in the nursery and as a primary index of compatibility, all tested rootstocks showed high graft-compatibility with Shami and Masri cultivars (Table 3). In contrast, a significant incompatibility existed with Beitoni, Darawishi, and Hamadani-Faranse cultivars except the combination of Darawishi/B41 with compatibility of 90%. The combinations of Hamadani-Faranse with Ruggeri-140 and B41 rootstocks revealed respectively very poor compatibility (Table 3). Own-rooted vines resulted in a high successful rate of 95% with Shami and Darawishi, 90% with Beitoni, and 80% with Masri cultivar. However, Hamadani-Faranse cultivar showed lower success of 55% (Table 3).

Buds of all tested grafted plants opened within 33 to 48 days after grafting (Table 4). Bud-break of own-rooted vines developed after 61, 62, 63, 66, and 67 days with Darawishi, Masri, Shami, Hamadani-Faranse, and Beitoni

cultivars, respectively (Table 4).

Variation in shoot lengths resulted between all grafted and own-rooted vines. Some rootstocks tend to lengthen the shoot length while others not. For all tested rootstocks, Masri cultivar showed significantly longer shoot length (Table 5) than Darawishi and Hamadani-Faranse cultivars. Own-rooted Shami and Darawishi vines had significantly longer shoots compared to their grafted scions (Table 5).

4. DISCUSSION

Few researches had been devoted to study the interaction between grapevine rootstocks and cultivars, particularly at the early stages of graft establishment in nursery. Effects of graft incompatibility have often been described in late stages of development such as grape-yield (Lipe and Perry, 1988, McCarthy and Cirami, 1990, Ezzahouani and Williams, 1995, Main *et al.*, 2002, McKenry *et al.*, 2004); berry size (Kubota *et al.*, 1993, Koblet *et al.*, 1994); content of sugar, organic acids, amino acids and anthocyanin (Kubota *et al.*, 1993, Jackson and Lombard, 1993) and wine quality (Walker *et al.*, 1998, Gawel *et al.*, 2000, Reynolds and Wardle, 2001, Ollat *et al.*, 2003).

Differential effects have been reported between rootstocks and grafted scion (Howell, 1987, Celik *et al.*, 2003, Zink and Schropp, 2004, Todic *et al.*, 2005). In our study, 80-100% of compatible grafts with Shami and Masri cultivars among all tested rootstocks and the 90% of compatible grafts obtained from the combinations of Darawishi/B41 might be due to the following reasons (Table 3): (1) high proliferation rate (Celik, 2000, Todic *et al.*, 2005), (2) great rooting development of rootstocks (Neves *et al.*, 1998, Celik, 2000), (3) vascular connection across the graft union (Moore, 1984), and (4) free exchange of water and nutrients between both genotypes (Wang and Kollmann, 1996). Once the rootstocks and scions are in contact, the cambial region capable of meristematic activity produces parenchymatic cells and callus tissue that fills the space between the two components (Errea *et al.*, 1994, Wang and Kollmann, 1996, Hartmann *et al.*, 1997). Several researchers (Yeoman *et al.*, 1978, Yeoman, 1984) considered this step as essential for the development of vascular

connections and suggested that the primary plant processes involved in the incompatibility mechanisms occurred at the point of cell-to-cell contact. The recognition system could be a protein released from the plasma lemma forming a complex with catalytic activity resulting in a successful grafting (Errea, 1998, Gokbayrak *et al.*, 2007).

Significant high grade of callus development had been reported on all grafted Shami and Masri cultivars (Table 1). It might be concluded that callus development is needed for good compatibility. Our findings agreed with the results of Celik (2000) who reported that the grade of callus formation at the grafting union was the main factor for good compatibility between stock and scion. The rootstock genotype might increase the free proline content and/or prevent IAA conversion to the scion, which will promote rooting and thereby resulting in the grafting success (Chanana and Singh, 1974, Durand and Nitsch, 1977, Bautista and El-injerto, 1985, Celik *et al.*, 1992).

Total graft losses of Hamadani-Faranse/Ruggeri-140 and B41, respectively, and the significant poor to medium compatibility of cultivars Beitoni, Darawishi and Hamadani-Faranse among all examined rootstocks could be due to the unfavourable environmental conditions in the nursery and/or the graft-incompatibility of rootstocks/cultivar combinations (Celik, 2000). The environmental conditions to which grafts were subjected such as water vapor saturated atmosphere, low light intensity and heterotrophical nutrition may induced lower plant resistance, resulting in specific morphological and physiological changes (Debergh and Zimmerman, 1991, Sebastian *et al.*, 1996, 1997). Nevertheless, throughout the whole study the same environmental conditions did not result in losses in other tested cultivars such as Shami and Masri, therefore, it looks like that graft-incompatibility was likely to cause the losses.

The primary causes of graft incompatibility still unknown, however, biochemical causes such as phenolic compounds (Feucht and Treutter, 1991), antioxidant enzymes (Deloire and Hebant, 1982, Quesada and Macheix, 1984, Pina and Errea, 2005, Gokbayrak *et al.*, 2007), and anatomical ones (Errea, 1998) were suggested to be responsible for graft incompatibility. Indeed, abnormal cambium division in the graft union usually

resulted in spherical vascular complexes without connection to the vascular system of the stock (Wang and Kollmann, 1996). According to Sachs and Cohen (1982) these complexes might lead to a circular flow of auxin. Subsequently, the older vascular elements become dis-functional resulting in decreasing the translocation rate from scion into stock (Wang and Kollmann, 1996). The lack of the newly formed cambium, lack of differentiation of the new tissues to the imperfect lignifications (Errea, 1998), lack of vascular continuity might cause the breakdown of the grafts at the grafting union either at early or late stages of graft establishment.

In addition, the presence of phenolic compounds also appears to be involved in all those events. It had been shown that a continuous stress situation, caused by the lack of adaptation of the two genotypes forming the graft, may trigger a series of steps resulting in abnormalities to the normal development of the graft union. Changes affecting translocation of phenols may be implicated in cellular damage and alter the complete cambium system around the graft union (Yuri *et al.*, 1990, Feucht and Treutter, 1991). Moreover, in the adaptation mechanism between the two genotypes, a strong accumulation of phenols can lead to the degradation of some of them, then oxidized to quinones. Their polymerization could be toxic to a number of metabolic reactions, such as inhibition of the lignin synthesis in the grafting union (Errea, 1998).

Although the specific role of enzymes and their effects on incompatibility is still unclear, involvement of certain enzymes such as peroxidase, catalase, acid phosphatase or leucine aminopeptidase during the first stages of graft formation may exist (Deloire and Hebant, 1982, Quesada and Macheix, 1984, Pina and Errea, 2005, Gokbayrak *et al.*, 2007). A single or in combination of many of these factors might be the reason for failure of grafts establishment.

In spite of the poor to medium rooting and callus development of grafts of Darawishi, Beitoni, and Hamadani-Faranse cultivars, their own-rooted once showed high percentage of successful grafting with 95%, 90%, and 55%, respectively. The present results agreed with the results of several workers (Manuel, 1948, Bouquet, 1980, and Sarooshi, *et al.*, 1982), who tested a number of phylloxera-resistant rootstocks and found that local cultivar perform best on their own roots.

Buds of grafted plants opened within 33 to 48 days (Table 4), whereas, own-rooted vines opened 19-28 days later. Differences in bud bursting between grafted and un-grafted might be possibly due to high concentration of plant growth regulators (PGRs) especially cytokinins and gibberellins, resulted from "blinding" rootstock nodes. These PGRs probably moved down to the base resulted in initiating root premordium formation and thus earlier bud burst (Hackett *et al.*, 1997, Lund *et al.*, 1997).

Significance rootstock genotypes dependent differed in shoot lengths of Masri cultivar and there were variable results with other cultivars observed among grafted grapevine (Table 5). In fact, rootstock might have a substantial influence on the vegetative growth, gas-exchange and on the water status of the scion (Paranychianakis *et al.*, 2004, Soar *et al.*, 2006). However, there is no convincing mechanistic explanation for this phenomenon.

Rootstock genotype affected vegetative growth via root distribution (Williams and Smith, 1991, Smart *et al.*, 2006); vine hormonal status (Nikolaou *et al.*, 2003, Soar *et al.*, 2006); water and nutrient-uptake efficiencies (Pulgar *et al.*, 2000, Bavaresco *et al.*, 2003, Alvarenga *et al.*, 2004, Wolpert *et al.*, 2005); and associated differences in root hydraulic conductivity (Bavaresco and Lovisolo, 2000, Cohen and Naor, 2002, Atkinson *et al.*, 2003).

The scion vigor had been reported to be affected by stomatal conductance (Cochard *et al.*, 2000, Hubbard *et al.*, 2001, Meinzer 2002, Sperry *et al.*, 2002), as well as changes in hydraulic conductance of both the roots and grafting union (Bavaresco and Lovisolo, 2000, Cohen and Naor, 2002, Clearwater *et al.*, 2004, Sampaio, 2007). In fact, stomata plays a role in regulating water loss from plants, but still not clear how this regulation is achieved

(Sampaio, 2007). The stomata is thought to respond to hydraulic and chemical signals in a way that integrates the hydraulic conductance of the soil to leaf pathway, thus maintaining constant water potential and maximizing photosynthesis while minimizing the risk of hydraulic failure through xylem cavitation (Sperry, 2000).

The above-mentioned factors might have direct and indirect effects on leaf gas-exchange and plant water status and consequently on vegetative growth. However, additional effects of other physiological processes also could play a role in explaining the significances in shoot lengths of grafted Masri cultivar.

In conclusion, Shami and Masri cultivars presented a good compatibility with all examined grapevine-rootstocks used in this study, should be strongly considered as suitable local cultivars selection for the evaluated rootstocks. In contrast, and due to their poor performance and low success, combinations of Beitoni, Darawishi, and Hamadani-Faranse cultivars except the combination of Darawishi/B41 are not recommended. Further field investigations are needed in order to continue observation on compatibility and effects on yield and quality.

ACKNOWLEDGMENTS

The authors gratefully thank the Palestinian Ministry of Higher Education and AED organization for their financial support of this project. They are also thankful to Prof. Dr. Jens Wunsche (Fruit Sciences, Department of Special Crop Cultivation and Crop Physiology, University of Hohenheim, Germany), for his helpful comments on this manuscript.

Table 1. Grade means of callus development on graft union surfaces in different scion/rootstock combinations after ten weeks of graft establishment.

Local cultivars	Grade of grapevine rootstocks ± SE				
	Richter 110	Paulsen 1103	Ruggeri-140	B41	216/3
Beitoni	2.0 ^a ±0.1	2.0 ^a ±0.07	1.25 ^c ±0.10	1.8 ^b ±0.09	2.05 ^a ±0.11
Darawishi	1.95 ^b ±0.09	1.25 ^c ±0.10	1.75 ^b ±0.14	2.5 ^a ±0.14	1.15 ^c ±0.08
Hamadani-Faranse	1.85 ^b ±0.11	1.2 ^c ±0.09	1.1 ^c ±0.07	2.4 ^a ±0.13	1.45 ^c ±0.11
Masri	3.6 ^b ±0.15	3.9 ^a ±0.07	3.95 ^a ±0.05	3.85 ^{ab} ±0.08	3.85 ^{ab} ±0.08
Shami	3.80 ^a ±0.12	3.75 ^a ±0.12	3.70 ^a ±0.13	3.80 ^a ±0.09	3.95 ^a ±0.05

Means within rows followed by different letters are significantly different at $p \leq 0.05$.

Table 2. Grade means of root development of own-rooted as well as grafted plants after ten weeks of graft establishment.

Scions (local cultivars)	Grade of own roots (control) ± SE	Grade of rootstocks ± SE				
		Richter 110	Paulsen 1103	Ruggeri- 140	B41	216/3
Beitoni	3.8 ^a ±.12	1.2 ^c ±.09	1.45 ^c ±.11	1.35 ^c ±.11	1.3 ^c ±.10	1.95 ^b ±.13
Darawishi	3.85 ^a ±.08	1.7 ^b ±.10	1.4 ^c ±.11	1.35 ^c ±.15	1.75 ^b ±.12	1.2 ^c ±.09
Hamadani-Faranse	3.1 ^a ±.14	1.55 ^c ±.11	1.3 ^{cd} ±.10	1.2 ^d ±.09	2.1 ^b ±.10	1.55 ^c ±.11
Masri	3.65 ^a ±.11	3.8 ^a ±.09	3.95 ^a ±.05	3.9 ^a ±.07	3.75 ^a ±.10	3.85 ^a ±.11
Shami	3.65 ^a ±.15	3.85 ^a ±.08	3.85 ^a ±.11	3.65 ^a ±.13	3.7 ^a ±.10	3.65 ^a ±.11

Means within rows followed by different letters are significantly different at $p \leq 0.05$.

Table 3. Successful percentage of own-rooted as well as grafted plants after five months in nursery.

Local cultivars	% of own roots (control)	% of grapevine rootstocks ± SE				
		Richter- 110	Paulsen- 1103	Ruggeri- 140	B41	216/3
Beitoni	90 ^a ±6.88	45 ^b ±11.4	55 ^b ±11.4	45 ^b ±11.4	35 ^b ±10.9	50 ^b ±11.5
Darawishi	95 ^a ±5.0	30 ^b ±10.5	20 ^{bc} ±9.18	30 ^b ±10.5	90 ^a ±6.88	5.0 ^c ±5.0
Hamadani-Faranse	55 ^a ±11.4	45 ^a ±11.4	60 ^a ±11.2	00 ^b ±00	5.0 ^b ±5.0	40 ^{ab} ±11.2
Masri	80 ^b ±9.18	100 ^a ±00	100 ^a ±00	95 ^{ab} ±5.0	95 ^{ab} ±5.0	80 ^b ±9.18
Shami	95 ^a ±5.0	100 ^a ±00	90 ^a ±6.88	85 ^a ±8.19	90 ^a ±6.88	90 ^a ±6.88

Means within rows followed by different letters are significantly different at $p \leq 0.05$.

Table 4. Mean number of days required for buds burst of own-rooted as well as grafted plants.

Local cultivars	Own roots (control) in days ± SE	Grapevine rootstocks (days) ± SE				
		Richter-110	Paulsen- 1103	Ruggeri- 140	B41	216/3
Beitoni	66.65 ^a ±0.25	38.5 ^c ±0.21	35.55 ^c ±0.22	36.55 ^c ±0.44	47.65 ^b ±0.15	40.4 ^c ±0.15
Darawishi	61 ^a ±0.27	47.35 ^b ±0.18	47.95 ^b ±0.33	39.35 ^c ±0.42	33.4 ^d ±0.18	40.65 ^c ±0.18
Hamadani-Faranse	66.5 ^a ±0.17	47.55 ^b ±0.22	47.7 ^b ±0.27	47.8 ^b ±0.18	47.35 ^b ±0.19	47.5 ^b ±0.23
Masri	62.5 ^a ±0.22	33.55 ^c ±0.17	33.55 ^c ±0.20	39.2 ^b ±0.43	33.5 ^c ±0.18	34.25 ^c ±0.12
Shami	62.70 ^a ±0.22	33.35 ^b ±0.17	33.55 ^b ±0.22	34.35 ^b ±0.41	33.65 ^b ±0.28	34.45 ^b ±0.18

Means within rows followed by different letters are significantly different at $p \leq 0.05$.

Table 5. Means of shoot lengths (cm) of own-rooted as well as grafted plants after five months in nursery.

Local cultivars	Shoot lengths of own roots (control) ± S.E	Shoot lengths of grapevine rootstocks (cm) ± S.E				
		Richter-110	Paulsen-1103	Ruggeri-140	B41	216/3
Beitoni	59.72 ^b ±5.95	62.11 ^b ±6.63	60.82 ^b ±3.89	101.33 ^a ±6.11	74.86 ^{ab} ±3.46	69.7 ^b ±2.53
Darawishi	41.37 ^a ±3.53	28.33 ^{ab} ±3.16	32.75 ^{ab} ±3.2	26.17 ^b ±2.02	26.22 ^b ±1.32	35 ^{ab} ±0.0
Hamadani-Faranse	67.16 ^b ±4.54	26.11 ^c ±3.23	30.25 ^c ±1.75	00 ^d ±00	7.0 ^d ±7.0	82.5 ^a ±2.93
Masri	133.13 ^a ±11.5	116.6 ^{ab} ±5.22	104.35 ^b ±4.23	108.68 ^b ±3.6	112 ^{ab} ±3.79	117.5 ^{ab} ±4.25
Shami	96.42 ^a ±3.83	66.85 ^b ±4.9	76.00 ^b ±2.8	67.53 ^b ±2.97	67.61 ^b ±2.64	74.22 ^b ±2.34

Means within rows followed by different letters are significantly different at $p \leq 0.05$.

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