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Research Article

EFFECT OF ROOTSTOCKS ON POLLEN PRODUCTION, VIABILITY AND GERMINATION IN GRAFTED TETRAPLOID AND DIPLOID WATERMELON

İlknur Solmaz[1*], Mohamed Dhamir Kombo[1], Suhayb Hussein[1], Mihriban Namlı[1], Nebahat Sarı[1]

[1]*Cukurova University, Faculty of Agriculture, Department of Horticulture, 01330, Adana, Turkey*

[*]*Corresponding author:*
E-mail: isolmaz@cu.edu.tr

ABSTRACT. The two years study was conducted to investigate the effects of graft combinations on pollen production, pollen germination, pollen viability and normal pollen development in grafted tetraploid and diploid watermelons. Two scions (tetraploid watermelon line 'ST 101' and diploid watermelon line 'WL 92') were grafted onto three rootstocks (*Cucurbita* interspecific hybrid 'Nun-9075'; *Lagenaria siceraria* 'Argentario' and citron watermelon 'PI 296341') and non-grafted ST 101 and WL 92 were used as control. Cultivation was conducted in spring seasons of 2016 and 2017 at horticultural experimental fields; flower and pollen analysis were done at the cytological laboratory of the Department of Horticulture, Cukurova University in Turkey. All graft combinations were grown and received the same management practices and flowers for analysis were collected randomly from every plot. There was a significant difference in pollen germination among graft combinations. In 2016, the highest pollen germination percentages were 94.13% and 89.85% obtained in non-grafted ST 101 and PI 269342/WL 92. In 2017, the highest pollen germination percentage value (79.75%) was obtained in Nun-9075/ST 101. No significant difference was found among graft combinations in percentage of pollen viability and normal pollen development in both years. Although no significant difference was observed in 2016 in number of pollen, the highest value was recorded in Argentario/WL 92 (509719.61 pollen per staminate flower) and the lowest value was obtained in Nun-9075/ST 101 (279494.76 pollen per staminate flower). In 2017, Nun-9075/WL 92 graft combination resulted in the highest number (260682.61 pollen per staminate flower) of pollen compared to other graft combinations. WL 92 diploid scion resulted in a higher number of pollen compared to tetraploid ST 101 scion. This study indicates that grafting increases production and development of normal pollen and improve pollen germination and viability.

Keywords: *Germination percentage1, graft combination2, pollen3, pollen viability4, rootstock5*

INTRODUCTION

Watermelon production increases day by day in both open field cultivation and in protected cultivation such as greenhouses and high tunnels. Production under protected cultivation is carried out two to three times a year [9]. The production during winter influences anther dehiscence which results in poor fruit set due to low temperature and high humidity [1].

Pollen is very important for fertilization and seed formation especially in triploid seed production due to low quality of triploid seeds formed. Good pollen viability and germination are useful in explaining the lack of fertility that determines fruit set and total fruit yield. In the production of triploid fruits, tetraploid female flowers must be pollinated by pollen from diploid plants this is because triploid watermelon plants do not produce sufficient viable pollen to pollinate themselves [11]. Besides triploid watermelon, the quality and quantity of pollen are very important in the production of diploid and tetraploid fruits.

Recently different techniques have been used in watermelon production where some of them have caused low pollen production. Mapping populations developed from crosses between cultivars have been observed to cause diminishing pollen viability, high levels of marker segregation and low fruit set [14, 15]. Also, the performance of pollenizers is species and or variety dependent; some watermelon varieties produce more viable pollen than others. Fiacchino and Walters [4] showed that 'Crimson Sweet' was more effective than 'Fiesta'. Freeman et al. [5] observed triploid plants pollinated by 'Companion' variety yielded less than those pollenized by 'Jenny', 'Patron', 'SP-1' and 'Sidekick'. The pollen quality and quantity in watermelon genotypes are very important in breeding programs because the superior genotypes can be used to increase the success of crosses [6]. Therefore, this study aimed at determining the best rootstock with the highest pollen viability, pollen germination and normal pollen production capacity so that they can be used in diploid crosses and triploid watermelon production.

MATERIALS AND METHODS

A two-year study was conducted in the open field of the Department of Horticulture of Cukurova University in Adana, Turkey during 2016 and 2017.

Plant material

Three rootstocks, 'Nun-9075' the *Cucurbita* interspecific hybrid rootstock (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) which is widely used for grafting watermelons in Turkey, the bottle gourd 'Argentario' (*Lagenaria siceraria* (Mol.) Standl., and citron watermelon 'PI 296341' (*Citrullus lanatus* var. *citroides*), which have high grafting combination ability and high resistance to *Fusarium* were used as rootstocks. Seeds of 'PI 296341' were obtained from the watermelon genetic resources collection of the Department of Horticulture, Cukurova University and other seeds were obtained from the Antalya Tarim Company.

Two watermelon lines, *Citrullus lanatus* var. *lanatus*, were used as scions, the tetraploid 'ST 101' was used as the female parent and the diploid 'WL 92' was used as male parent. All rootstocks were grafted with ST 101 and WL 92 scions, therefore, the following graft combinations were obtained (Table 1).

Table 1. *Graft combinations used in the experiment*

ST 101 (Tetraploid female parent scion)	WL 92 (Diploid male parent scion)
Argentario/ST 101	Argentario/WL 92
Nun-9075/ST 101	Nun-9075/WL 92
PI 296341/ST 101	PI 296341/WL 92
Control - ST 101 (Non grafted)	Control - WL 92 (Non grafted)

Seed sowing, Grafting and Transplanting

Seed sowing, grafting and management practices of the grafted seedlings were conducted at Antalya Tarim Company in Antalya, Turkey. Seeds were sown on 26^{th} January 2016 and grafting was done on 23^{rd} February 2016. Seed sowing and grafting practices for year 2017 were performed on 03^{rd} February 2017 and 22^{nd} February 2017 respectively. Seedlings were transplanted to the open field in Adana on 30^{th} March 2016 and on 07th April 2017. Grafted and control (non-grafted) plants were transplanted at a spacing of 3 m × 0.75 m. In every plot, 16 plants grafted with tetraploid ST 101 female parent scion followed by 4 plants grafted with diploid WL 92 male parent scion were transplanted with four replications in a Latin Square design. Soon after transplanting plants were covered with low plastic tunnels to protect from cold weather and heavy rain; wide open holes on the plastic tunnels were made to allow air exchange and the tunnels were completely removed after 3 weeks. Plants were irrigated with drip irrigation once after every two days for the first two months. Fertilizer was applied through a drip irrigation system at a rate of 15:15:20 kg per 1000 m^2 with pure nutrients as $N:P_2O_5:K_2O$. Monoammonium Phosphate (MAP) and Potassium Nitrate (KNO_3) at a ratio of 18:18:18 and 20:20:20 respectively were used. Weeds, insect pests and diseases were regularly controlled whenever the signs of presence were observed.

To determine pollen production capacity, in vitro pollen viability and pollen germination of the rootstocks, 10 flowers were used in every replication. Five mature flowers before opening when pale yellow color started to develop (approximately one day before anthesis) were selected and closed by using clips in the afternoon for pollen germination and viability. In the morning, the 5 flowers closed by clips were picked from each replicate and other 5 flowers that were not closed by clips but unopened near to anthesis were picked and sent to the laboratory for anther counting and determination of pollen production capacity. In vitro pollen viability, pollen germination and normal pollen production assays were conducted according to Norton [12], Eti and Stosser [3], Ozkan and Eti, [13].

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) to determine the effects of different rootstocks in two different years. A significant difference among means was calculated by using Tukey Multiple Range Test at a significance level of $P \leq 0.05$. Statistical analysis was performed using JMP (v8.00, SAS Institute Inc., NC 27513-2414, USA) statistics software.

RESULTS AND DISCUSSION

Results of pollen viability percentage of the grafted and non grafted watermelon for both diploid (WL 92) and tetraploid (ST 101) scions are presented in Fig.1. There was no significant difference between rootstocks and scions in pollen viability for 2016 and 2017 growing seasons. The percentage pollen viability for 2016 ranged between 99.16 - 100.00% for WL 92 and 99.37 - 100.00% for ST 101. In 2017 the pollen was somehow lower compared to that of 2016, also WL 92 scions resulted in a higher pollen viability percentage value compared to ST 101. The pollen viability percentage value for ST 101 ranged from 81.00 - 87.40% obtained in Argentario and Nun-9075 respectively while that of WL 92 ranged between 95.53 - 99.16%.

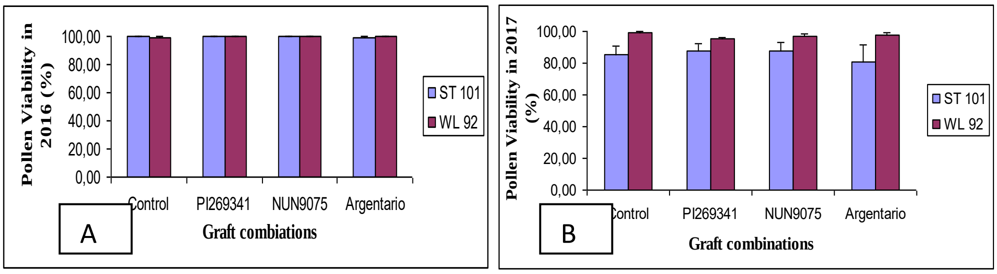

Fig. 1. *Pollen viability percentage of 3 different rootstocks and control for 2016 (A) and 2017 (B) growing seasons*

Significant differences were observed between rootstocks and also between scions in pollen germination percentage (Fig. 2). In 2016 (Fig. 2 A), significant difference was only observed in WL 92 scion with the highest percentage obtained in control (96.13%) and in grafted plants the highest value was obtained in PI 269341 rootstock (89.85%). No significant difference in pollen germination percentage was observed between graft combinations in ST 101, the values ranged between 39.78% - 58.80% obtained in Nun-9075 and PI 269341 rootstocks respectively. In 2017 (Fig. 2 B), the highest pollen germination percentage was obtained in Nun-9075/ST 101 (79.75%) and in Argentario/WL 92 (78.70%). However, Argentario/ST 101 resulted in the lowest pollen germination value (28.60%). The lower pollen germination percentage in ST 101 may be due to the few and immature flowers. The flowers in ST 101 were very few in such away necessitated picking of those available which also were immature. Generally the flower production and pollen content in ST 101 group were very poor compared to WL 92.

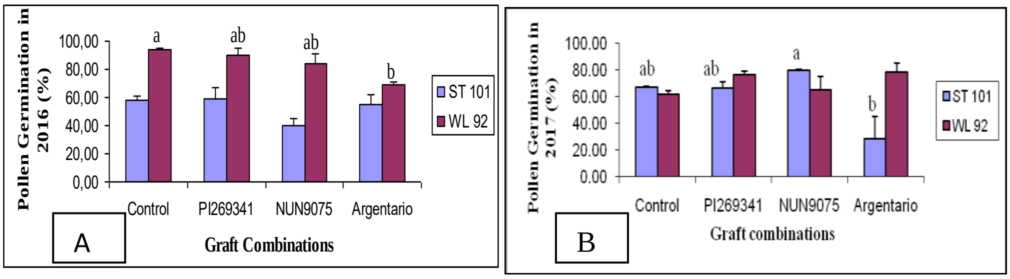

Fig. 2. *Pollen germination percentage of 3 different rootstocks and control for 2016 (A) and 2017 (B) growing seasons*

There was no significant difference between rootstocks in normal pollen development in both scions and years (Fig. 3). However, In ST 101 scion, PI 269341 rootstock resulted in the highest average normal pollen percentage value of 99.34% and the lowest value was obtained in control (97.84%). In WL 92 scion, the highest normal pollen percentage value was obtained in Nun-9075 (99.51%) and the lowest average value was obtained in control (98.22%). The percentage pollen viability and pollen germination obtained in this current study are higher compared to those reported by Kombo and Sari [8].

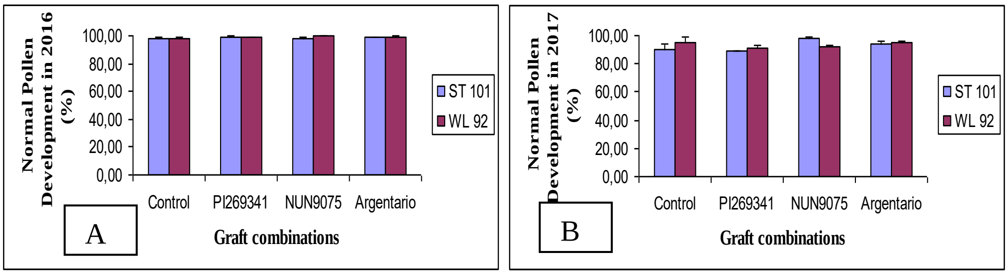

Fig. 3. *Normal pollen development of 3 different rootstocks and control for 2016 (A) and 2017 (B) growing seasons*

There was a significant difference among rootstocks in the number of pollen per anther in both years and the number of pollen per staminate flower in the 2017 season (Table 2). Argentario/WL 92 resulted in the highest average number of pollen per anther in the first year (169906.54), while Nun-9075/WL 92 resulted in highest number of pollen per anther (82863.59) in the second year. The highest number of pollen per staminate flower was found in the first whereby Argentario/WL obtained 509719.61 number of pollen per staminate flower, two times higher than the highest number of pollen found in the second year in Nun-9075/WL (260682.61).

Table 2*. Pollen production in different graft combinations*

Graft Combination	**Number of pollen per anther**		**Number of pollen per flower**	
	2016	**2017**	**2016**	**2017**
Control (ST 101)	90885.857 b	58034.59 ab	326077.22	186320.88 ab
PI 269341/ST 101	112328.37 ab	39194.77 b	368180.60	125423.27 b
Nun-9075/ST 101	91391.95 b	58060.86 ab	279494.76	193496.37 ab
Argentario/ST 101	132431.54 ab	40973.23 b	425512.85	133485.62 ab
Control (WL 92)	101525.87 ab	76834.74 ab	311744.15	239183.02 ab
PI 269341/WL 92	143330.65 ab	76256.38 ab	429991.94	233808.12 ab
Nun-9075/WL 92	122664.29 ab	82863.59 a	402221.63	260682.61 a
Argentario/WL 92	169906.54 a	72027.01 ab	509719.61	221230.85 ab
Prob>f	0.014	0.01	0.06	0.02
LSD 5%	68951.89	40283.77	ns	131687.47

Few flowers and low number of pollen per staminate flower in the second year may be due to the stress caused by heavy rainfall at the beginning of the spring season. Several studies have shown that stress adversely affects pollen production per flower [16]. Virus infection [7], amount and timing of leaf damage [2] and soil fertility [10] affect staminate flower production and pollen production per flower. Thus, less number of flowers and low number of pollen found in this current study also reveals that stress caused by rainfall at flowering affects pollen production.

CONCLUSION

This study indicates that grafting highly increases the production and development of normal pollen and improves pollen germination and viability. 'Nun 9075' and 'Argentario' are the best rootstocks that provide more number of pollen per staminate flower with higher percentage viability, hence, these rootstocks can directly increase fruit yield and quality in triploid watermelon.

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