THE EFFECTS OF ROOT INTERVENTION ON STAND ESTABLISHMENT RATE OF GRAFTED WATERMELON (*CITRULLUS AEDULIS L*) SEEDLINGS UNDER SALINE CONDITIONS

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Abstract

A commercial watermelon (*Citrullus aedulis L*) variety was grafted onto a commercial variety rootstock (*Cucurbita maxima x C. moschata*). Two different grafting methods; splice grafting (SG) and root pruned splice grafting (RPSG) were simultaneously applied. End of nursery period, a sufficient number of 14 day old seedlings of each grafting method were transplanted in large pots filled with a mixture of peat compost and vermiculite (3:1). The plants were split in three equal groups and in the following 14 days each group was periodically irrigated with equal amounts of respectively tap water or saline water (50 mM NaCl and 100 mM NaCL). The relative growth rate (RGR) and its components; net assimilation rate (NAR) and leaf area ratio (LAR), root relative growth rate (RRGR), stem elongation rate (SER) and leaf expansion relative rate (LER) were computed for each experimental plot. Additionally to that, equal weights of fresh roots from seedlings of both grafting methods were washed out carefully with tap water and immersed into equal quantities of methanol. Several diluted solutions (10% vol/vol, 15% vol/vol and 20% vol/vol) were prepared from each root extracts, parallel with control solutions (0.01, 0.05 and 0.1 ppm) of artificial cytokinins, and used as rooting medium for 20 day old cuttings of tepary bean (*Phaseoulus acutifolius*). The average number of root nodes and lateral roots as well as the average length of lateral roots of tepary bean cuttings was used to indicate the difference between RPSG and SG seedlings regarding the nature and quantity of growth hormones extracted from the fresh rootstock roots. A significantly higher relative growth rate was found for root pruned splice grafted seedlings after transplanting, mostly due to higher net assimilation rate, as well as a higher root relative growth rate indicating a faster stand establishment rate for RPSG seedlings. Generally speaking, the relative growth rate of transplanted seedlings was drastically reduced due to the increase of nutrient solution salinity. However, significantly higher values were recorded for root pruned splice grafted seedlings compared to splice grafted ones. The same was true regarding root relative growth rate, stem elongation rate and leaf expansion rate. A higher concentration of cytokinins, was indicated by a higher rooting index of *Phaseoulus acutifolius*, from the extracts of RPSG seedlings and to that is attributed their significantly higher stand establishment rate.

Key words: splice grafting, root pruned splice grafting, relative growth rate, root relative growth rate, rooting index, growth hormones

1. Introduction

Intensive farming practices with limited crop rotations can lead to a buildup of soil-borne pathogens and other deleterious factors, such as increased salinity [10]. Salinity and drought conditions can greatly reduce stand establishment, thus reducing yield in high-value vegetable cropping systems. Considering that, the ultimate goal of the nursery is to produce a quality transplant with the ability to withstand transplanting. As long as, root vigor is crucial for healthy seedlings and fast stand establishment is a common request for the commercial production of vegetable transplants [5], extensive efforts have been made to develop cultural practices that stimulate rapid root development, in order to withstand post-transplantation stresses in the field [9].

Seedling establishment in any field environment depends on adequate development of a taproot, associated laterals, and basal roots for dicot species, or adventitious roots and associated lateral roots for most monocot species [13]. The capacity of a containerized transplant to overcome transplant shock and become established in a field environment following transplanting depends on the capacity of seedlings to withstand root disturbance, the water and nutrient uptake capacity of the roots, and the capacity of the preexisting roots to rapidly regenerate new lateral, basal, or adventitious roots [13].

Grafted vegetable transplants represent a significant component of vegetable industries throughout the world [1]. Though the main purpose, still remains the control of soil-borne disease, recently the purpose of grafting has been expanded to obtain increased heat and cold tolerance, to enhance water and nutrient uptake, and to improve plant growth and marketability [12].

Over the last few years, progress has been made in understanding the physiological, biochemical, and hormonal bases for root development and root response to stress conditions. However, the mechanism(s) by which plant hormones are involved in root responses to environmental stresses are not well-understood. Numerous studies have demonstrated that grafting has a significant contribution to scion performance under diverse cultivation conditions, but only few of them have attempted to study the link between the horticultural benefit and hormonal relationship [1]. Increased understanding is important to improve the management of transplants in a nursery, and following their transfer to the field [9]. This paper presents some of our findings regarding the effects of root modifications on hormonal activity of root system and stand establishment rate of grafted watermelon seedlings.

2. Materials and Methods

A commercial watermelon (*Citrullus aedulis* L) variety (Elisa F_1) was grafted onto a commercial rootstocks (*Cucurbita maxima* x *C. moschata*) cultivar (Nimbus F1). Graded rootstock and scion seeds were sown in foam trays filled with peat substrate. Fourteen days after sowing, two different grafting methods; splice grafting (SG) and root pruning splice grafting (RPSG) were applied. Two weeks after grafting, an equal number of each grafting methods were transplanted in to 200 cm^3 plastic pots filled with vermiculite. The plants of each group were split in three equal subgroups and irrigated several times with equal amounts of the same nutrient solution (N 100 mg L^{-1}, P 25 mg L^{-1}, K 150 mg L^{-1} and other necessary elements but differing by each other by the amount of NaCl added in the nutrient solution (0, 50 and 100 mM).

Prior to transplanting and 14 day after transplanting, 10 plants of each experimental plot were randomly selected. Root, stem and leaf dry matter was weighted, and leaf area of plants was measured successively for each harvested plant. Based on the primary data, the relative growth rate (RGR), and its components; net assimilation rate (NAR), leaf area ratio (LAR), were computed according to Hunt et.al, (2002) and Hoffmann and Poorter (2002). Meantime, stem elongation rate (SER) and leaf expansion rate (LER) of each experimental unit were calculated as the slope of linear regression of natural logarithms (*ln*) of respectively stem length and leaf area on time.

Additionally to that, equal amounts of fresh roots of seedlings from both grafting methods were washed out carefully with tap water and immersed into equal quantities of methanol. Few days later, methanol solutions were filtered and concentrated by evaporation, and brought to the same volume by addition of distillated water. From that stock solution, several diluted solutions (10% vol/vol, 15% vol/vol and 20% vol/vol) were prepared and rooting index of 20 day old cuttings of tepary bean (*Phaseoulus acutifolius*), were used to indicate the presence of growth hormones extracted from fresh rootstock's roots. Parallel with that, a similar experiment was conducted with synthetic cytokinines at known concentrations (0.01, 0.05 and 0.1 ppm). The rooting index of *Phaseoulus acutifolius* at known cytokinines concentration was used as a reference for the presence and of the differences in cytokinines concentration between the roots extracts of splice grafted and root pruned splice grafted watermelon seedlings.

Factorial analyses of variances were conducted by MSTAT C for each experimental parameter, and respective means were separated by least significant differences test (LSD) at 5 % significance level.

3. Results and Discussion

There are evidences that the growth rate of grafted seedlings was reduced by the pruning of rootstock roots [3]. Due to that, the first day of transplanting (Table 1), the plant dry matter of root pruned grafted seedlings (RPSG) was significantly smaller compared to splice grafted seedlings (SG). The same was true for root dry matter. Started with almost no roots, RPSG seedlings had a quick increase of dry weight of the root system throughout the whole nursery period, but still in the first day of transplanting their dry weight was significantly smaller compared to SG seedlings (Table 1). Meantime, till that moment, no significant difference was found between RPSG and SG seedlings regarding leaf area per plant.

Obviously, RPSG seedlings grew much faster in terms of relative growth rate during the transplanting period. The respective relative growth rate (RGR) of RPSG seedlings was significantly higher compared to SG ones (Table 2). Consequently, despite of heavier and larger seedlings provided by SG method in the end of nursery period, 14 day after transplanting RPSG seedlings recorded a significantly higher dry matter, versus SG seedlings (Table 1).

Table 1. Mean values of plant dry weight (W), leaf area per plant (LA), root weight (RW), and root-shoot allometry of grafted watermelon seedlings under no salinity conditions (0 mM NaCl), one and fourteen days after transplanting (DAT), according to grafting method.

Parameters	Grafting method	Days after transplanting (DAT)	
		1	14
Plant dry matter (W), g pl^{-1}	RPSG	0.57 b*	2.85 a
	SG	0.86 a	2.00 b
Root dry matter (RW), g $g^{-1}pl^{-1}$	RPSG	0.093 b	0.54 a
	SG	0.122 a	0.47 b
Leaf area (LA), cm^2 pl^{-1}	RPSG	189	295 a
	SG	182	275 b

* Different letters within the same column indicate significant difference at P<0.05

Table 2. Mean values of plant relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), root relative growth rate (RRGR), stem elongation rate (SRE) and leaf expansion rate (LER) of grafted watermelon seedlings under different salinity conditions (0, 50, 100 mM NaCl) during the establishment period, according to grafting method.

Grafting method	NaCl (mM)	RGR g $g^{-1}day^{-1}$	NAR g $m^{-2}day^{-1}$	LAR m^2g^{-1}	RRGR g $g^{-1}d^{-1}$	SER cm $cm^{-1}d^{-1}$	LER $cm^{-2}cm^{-2}d^{-1}$
RPSG	0	0.141a*	9.076 a	0.010	0.172 a	0.124 a	0.052 a
	50	0.134 b	8.880 a	0.009	0.162 b	0.128 a	0.039 b
	100	0.125 c	8.066 b	0.009	0.129 c	0.122 a	0.031 c
SG	0	0.073 d	4.991 c	0.013	0.130 c	0.113 b	0.045 a
	50	0.067 e	4.870 c	0.011	0.084 d	0.101 c	0.030 c
	100	0.061 f	4.263 d	0.012	0.084 d	0.101 c	0.028 c

* Different letters within the same column indicate significant difference at P<0.05

The same was true for root relative growth rate (RRGR), which can be used as an indicator of the stand establishment rate of young transplants. Hence (Table 2), we can conclude that RPSG seedlings own enhanced capabilities to withstand the transplanting process. The superior stand establishment capabilities were also reflected to significantly higher stem elongation rates (SER) and leaf expansion rates (LER) of RPSG seedlings versus common grafted seedlings during the transplanting period (Table 2).

Statistically significant differences were found between RPSG and SG seedlings for net assimilation rate (NAR), but no significant differences were found for LAR. Consequently, we can assume that the differences found during the establishment period between RPSG and commonly grafted seedlings regarding RGR, were mostly related to differences did exist regarding the efficiency of their respective photosynthetic activity (NAR).

The addition of NaCl in post transplanting nutrient solutions has significantly reduced the stand establishment rate (in terms of root relative growth rate up to 14 day after transplanting, RRGR) and the overall plant growth rate of transplanted seedlings (RGR). Table 2 shows a very clear and distinguished trend of a significant reduction of all growth parameters due to the increase of solution salinity form 0 to 100 mM. This is a clear evidence of the negative effects the salinity plays to the development of new root system and the stand establishment rate of transplanted plants. As Bernstein and Kafkafi (2002) explain, roots exposed to a salt stress lose their turgor and respond in an immediate cessation of elongation. A significant reduction of root growth due to enhanced salinity was true for both types of seedlings

(RPSG and SG), but it is important to note that still, mean RGR values of RPSG seedlings remained significantly higher compared with common SG seedlings. Practically (Table 2), mean RRGR values of RPSG seedlings in high salinity conditions (100 mM NaCl) were at the same statistical group with mean RRGR values of SG seedlings under normal conditions (0 mM NaCl). Obviously, root pruned seedlings (RPSG) holds improved abilities which enables them to better react under saline conditions and ensures a higher relative growth rate.

The dissimilar hormonal activity of root pruned and non-pruned grafted plants could be a possible explanation of these facts. As it is largely admitted, the root tips are major sites of CK synthesis. From the root cap, the CK is transported upward through plasmodesmata, which provide symplastic continuity in the meristematic and elongation zones, and from the differentiation zone through vessels of the xylem by the transpiration stream, mainly to developing shoot organs with high transpiration rates [2].

Root pruning removes the root tips, and in this way their apical dominance within the roots, allowing for the formation of more side roots and actively growing root tips, where extracts of cytokines were detected in high concentrations [6]. Lee and Oda, 2003, has also noted that plants with vigorous root system, produce more cytokines and the yield increase induced by a vigorous rootstock is closely associated with the amount of cytokines in the ascending xylem sap. The same is confirmed by Sakakibara, 2006, who addresses to cytokines a crucial role in various processes in plant growth and development, including the control of shoot/root balance.

CK and IAA have antagonistic roles in root development; auxin promotes the formation of lateral roots and adventitious roots, whereas CK application in physiological concentrations inhibits root formation and reverses the IAA effect [2, 15]. These conclusions fit very well with our experimental results. The increase of synthetic cytokinins concentration was followed by a significant reduction in number of lateral roots and root length of *Phaseoulus acutifolius* cuttings (Table 3). Meantime, the bioassay test indicated a higher amount of plant hormones extracted by root pruned grafted seedlings (Table 3). Since the total number of lateral roots, total length of roots and average length of individual roots of Phaseolus cuttings was declined versus the gradual increase of concentration of diluted solutions from root extracts of grafted seedlings, it might concluded there was a gradual increase of cytokinins. As long as number of roots and average length of individual roots of RPSG seedlings were smaller compared to SG seedlings, it seems that there was a higher amount of cytokinins produced by RPSG seedlings.

Table 3. Number of lateral roots and total and average length of lateral roots of *Phaseoulus acutifolius* cuttings in different concentration of synthetic cytokinins and root extracts of root pruned splice grafted (RPSG) and splice grafted (SG) watermelon seedlings.

	No. of lateral roots	Total length of lateral roots (mm)	Average length of lateral roots (mm)
CK 0.1 ppm	63	69.6	1.10
CK 0.5 ppm	30	17.0	0.57
CK 1.0 ppm	15	4.2	0.28
RPSG 10%	26	14.6	0.56
RPSG 15%	24	11.2	0.46
RPSG 20%	23	9.2	0.40
SG 10%	30	14.8	0.49
SG 15%	30	12.5	0.41
SG 20%	24	10.1	0.42

CKs have opposing roles in shoots and roots. While in roots they are negative regulators of growth and development, in young shoot organs the CKs positively regulate development and promote shoot growth [3]. This was clearly demonstrated by the differences found regarding the stem growth rate (SER) and leaf expansion rate (LER). Both, SER and LER, of RPSG seedlings were significantly higher compared to SG seedlings (Table 2). Considering that roots are the major source of cytokinins in scions, the higher growth rate of root pruned grafted seedlings (RPSG) can be logically linked with the higher amount of cytokinins produced by a more vigor root system. For the same reasons RPSG seedlings demonstrated a higher stand establishment rate compared to SG seedlings.

4. Conclusions

Root pruned grafted seedlings (RPSG) demonstrated a significantly higher stand establishment rate compared to commonly splice grafted seedlings (SG) due to enhanced capabilities to withstand the transplanting process. The addition of NaCl in post transplanting nutrient solutions has significantly reduced the stand establishment rate of transplanted seedlings, though again RPSG demonstrates a very distinguished advantage.

The dissimilar hormonal activity could be a possible explanation of enhanced establishment capabilities of RPSG seedlings. Considering that roots are the major source of cytokinins, the higher stand establishment rate of RPSG seedlings can be logically linked with the higher amount of cytokinins produced by a younger and more vigor root system.

5. References.

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