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CROP PRODUCTION

Tolerance of apple rootstocks to short-term waterlogging

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ABSTRACT: *Until few years ago there were limited options of apple rootstocks commercially available for Brazilian growers; although, new series of Geneva® rootstocks introduced recently present desirable features such as vigor control and wider lateral branch angle. On the main apple producing regions of Brazil, intermittent rainfall eventually occurs and waterlogged condition is frequent especially in high clay oxisols; in addition, little is known about the tolerance of rootstocks M.9, Marubakaido/M.9 interstock, G.202, G.213 and G.814 to waterlogging. Thus, the objective of this experiment was to evaluate the tolerance of these rootstocks to short-term waterlogging on root and aerial parameters. Potted 'Maxi Gala' apple plants were kept under 48 hours of waterlogging weekly throughout 19 weeks to be compared with a normal hydric condition control. The evaluated variables were: leaf, stem and root dry matter, number and length of new root emission, and number of leaves, mean leaf size and chlorophyll content. Rootstocks G.202, G.814 and Marubakaido/M.9 interstock presented more tolerance to waterlogging, and the main defense mechanism was the emission of new adventitious roots.*
Key words: *flood, hypoxia, resilience, root system.*

Tolerância de porta-enxertos de macieira ao encharcamento temporário

RESUMO: *Até pouco tempo, haviam poucas opções de porta-enxertos de macieira disponíveis comercialmente para produtores brasileiros. Contudo, com a nova série de porta-enxertos Geneva® introduzidos recentemente, características desejáveis foram apresentadas como, controle de vigor e ângulo de ramo aberto. Nas principais regiões produtoras de maçã no Brasil, chuvas intermitentes ocorrem eventualmente levando muitas áreas ao encharcamento, especialmente em locais com latossolos com elevados teores de argila. Em adição, pouco se sabe sobre a tolerância dos porta-enxertos M.9, Marubakaido com inter-enxerto de M.9, G.202, G.213 e G.814 ao encharcamento. Assim, o objetivo deste experimento foi avaliar a tolerância destes porta-enxertos ao encharcamento temporário através de parâmetros de raiz e parte aérea. Macieiras 'Maxi Gala', cultivadas em vasos, foram mantidas sob encharcamento semanalmente durante 48 horas por 19 semanas, para serem comparadas com o controle em condição hídrica normal. As variáveis analisadas foram: massa seca das folhas, caule e raiz, comprimento e número de raízes novas, número de folhas, tamanho de folha e teor de clorofila. Os porta-enxertos G.202, G.814 e Marubakaido com filtro de M.9 apresentaram maior tolerância ao encharcamento, sendo que o principal mecanismo de defesa para isso foi a maior emissão de raízes adventícias novas.*
Palavras-chave: *alagamento, hipóxia, resiliência, sistema radicular.*

INTRODUCTION

Apple crop in southern Brazil is achieving higher yields along the past years through new technologies that are implemented by growers looking for improving fruit quality and raise revenues (CHAGAS et al., 2012). One of the most promising improvements are the Geneva® series of rootstocks, which were bred to reduce plant vigor and increase lateral branch angle, providing higher plant densities per area and better luminosity within the canopy (ROBINSON et al., 2011). Rootstocks G.202 and G.213 are categorized as dwarfing, equivalently to M.9, but the G.213 is one of the most adapted and stable bearing in southern Brazilian conditions, whereas the rootstock G.202 presented less yield constancy throughout years of experimentation compared to traditional Marubakaido/M.9 interstock rootstock (DENARDI et al., 2015). Rootstock G.814 presents intermediate vigor compared to Marubakaido/M.9 interstock, which is considered semi-vigorous, and both are equivalent in yield of 'Imperial Gala' besides presenting the potential for use in high density orchards (PASA et al., 2016).

It is usual on heavy clay oxisols of the "Campos de Cima da Serra" apple cropping region the occurrence of intermittent rainy periods where specially on low land orchards, the root system remains waterlogged during a couple of days. Waterlogging usually leads to hypoxia and in severe cases to anoxia

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of the root system in plants, and on warmer seasons the depletion on O_2 is faster; after switching the root zone environment to a hypoxic condition, reactive oxygen species are formed, and elevation of H_2O_2 levels, leading to adaptation signals and anaerobic respiration processes; Other plant response to waterlogging is to produce ethylene, which signals various adaptive functions to plant survival, such as increased number of adventitious roots, and formation of aerenchyma in such environment (IRFAN et al., 2010). Different apple genotypes present variable response to hypoxia due to genetic variability. The main responses in apple rootstocks to waterlogging are reduction of new adventitious root growth, leaf senescence and reduced dry weight accumulation in *Malus toringoides*; although, these adverse effects of hypoxia were not observed on *Malus hupehensis* (LI et al., 2010).

The objective of this experiment was to evaluate the effects of short-term waterlogging, of 48 hours per week, in potted apple plants grafted on the commercial available rootstocks: M.9, Marubakaido with M.9 interstock, G.202, G.213 and G.814.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse located at the Embrapa Research Station located at the municipality of Vacaria-RS. Three months prior to the implementation of the experiment, on August 30, 2016 the trees were sorted out and stored in cold chamber until the moment of planting. Concomitantly, the soil to be used for the experiment was ameliorated with limestone and fertilizer. The soil used was an oxisol, which is the predominant soil at the region of the Campos de Cima da Serra. On October 04, 2016 the soil was placed within plastic pots of eight liters and the bare-rooted apple trees were placed individually within each plastic pot without any root pruning, and the pot were filled with soil until its full capacity. Trees consisted of one-year bare-root nursery apple trees of the cultivar 'Maxi Gala' grafted on five different rootstocks: M.9, Marubakaido/M.9 interstock of 30cm, G.202, G.213 and G.814, in a total of ten plants per treatment (rootstock). For planting the trees on the pots, the root system was left intact, as they came from the nursery. After planting, the dormancy was broken by soaking a sponge in a solution of 1.5% of Dormex® plus 4% of mineral oil as adjuvant, and passing through the stems and lateral branches. The experiment begun on December 1, 2016, where five plants of each rootstock was emerged weekly during 48 hours within sealed plastic boxes, where the pots of the emerged plants remained waterlogged until about three centimeters below the top of the soil surface, while the other five plants remained as a control, being irrigated when necessary, and the water was placed in the saucer at every pots.

Variables evaluated were plant height, lateral branch length, both measured weekly until the 19^{th} week on April 13, 2017, which is the moment that the experiment was harvested. Four lateral sprouts of each tree was tagged for evaluation of lateral branch growth rate. These measurements were taken prior to submerging the pots on a weekly basis. At the moment of harvesting, the total number of leaves of each plant was recorded. The chlorophyll content was measured in 40 leaves per plant with a SPAD-502 chlorophyll meter. To measure leaf size, a sample of 20 leaves of each plant was picked up randomly and used in a portable leaf area meter model AM350 from ADC BioScientific®. Root systems were detached from each plant to measure the length and number of new root emission. The criteria used to consider a root as new (not suberized) was the adventitious color, as older roots presented a brownish and suberized aspect. Adventitious roots were measured by ordering them linearly where the number of roots and the final length were recorded. Root mean size was obtained by dividing the final linear length by the number of roots. Plants were sorted out in root systems, stems and leaves, to be dried in a constant drying flux chamber until the samples reached constant weight, to obtain dry matter.

The experiment was conducted in a factorial scheme of five rootstocks and two hydric conditions with five replications. The data were subjected to analysis of covariance ($p \leq 0.05$) where scion's trunk diameter at 13cm of the graft union was used as cofactor, and in case of significance the means were compared through Duncan's multiple range test for rootstocks and T test for the hydric conditions.

RESULTS AND DISCUSSION

There was no significant interaction between rootstock and hydric condition for the variables root, stem and leaf dry matter weight (data not shown). There was significant interaction between hydric conditions and rootstocks for the variables adventitious root number (emissions), linear adventitious roots length and mean root size (Table 1).

In the interaction of hydric conditions within rootstocks for adventitious root number, on normal hydric condition the rootstock G.202 presented more adventitious root number, while in waterlogged condition the rootstock G.213 presented less emission of adventitious roots. On the interaction of rootstocks within hydric conditions, it was observed an increase on the count of adventitious root number, and it was significant elevated by waterlogging in G.202 and M.9 rootstocks (Table 1).

In the interaction of hydric conditions within rootstocks for the variable linear adventitious root length, under normal hydric condition it was significantly elevated in the rootstock G.814, and in waterlogged condition the rootstock M.9 presented longer adventitious roots; in the interaction of hydric condition within rootstocks both M.9 and Marubakaido/M.9 interstock presented significant more linear length of adventitious roots under waterlogged condition. For the variable mean adventitious root length, on the interaction of hydric conditions within rootstocks, mean root emission length was more preeminent in the rootstock G.814 in normal hydric condition, and in waterlogged condition the rootstock G.213 presented longer mean adventitious root emission. In the interaction of rootstocks within hydric conditions, G.814 presented longer mean adventitious root length in normal hydric condition, whereas Marubakaido/M.9 interstock presented longer new root under waterlogging (Table 1).

In opposition to this experiment, BAI et al. (2010) reported an outstanding reduction in adventitious root length of *Malus toringoides* (from dry climate) and a slight reduction on *M. hupehensis* (from wet climate) under hypoxia. For *Prunus* spp. rootstocks, PISTELLI et al. (2012) reported an increment of new adventitious roots oriented to soil surface in response to waterlogging, and it was directly related to stress tolerance response.

In an oxygen deprived root environment, as reactive oxygen species (ROS) are formed and converted to a signal molecule (H_2O_2) by enzymes such as Ascorbate oxidase, Superoxide dismutase, Catalase, etc., to scavenge ROS (BAI et al., 2010) and trigger genetic remediation responses. Root tips are prone to find anoxic conditions as it is a bulky tissue with no vascular system (PISTELLI et al., 2012). At anoxic environment there is an ACC (1-aminocyclopropane-1-carboxilic acid) buildup, which is a precursor of ethylene. Thus, ACC is volatilized to aerenchymas of non-flooded root system at soil surface and reacted with oxygen promoting ethylene through ACC-oxidase. Ethylene buildup endorses the expression of genes that form the protein endotransglycosylase-1 (XET-1) along with auxin related proteins like expansins. This promotes loosening of the cortex cell walls creating aerenchymas. When ethylene accumulates on the aerenchymas near the root nodes, it breaks down the cells epidermis, interacting with auxin and promoting adventitious root formation (IRFAN et al, 2010; PISTELLI et al., 2012). Based on our data of dry matter weight where no difference between hydric conditions was reported for all rootstocks, it is evident a shift of formation of new roots, with numerous emissions to compensate the root tip death from the anoxic submerged roots.

Table 1 - Adventitious root number, linear adventitious root length and mean adventitious root size of 'Maxi Gala' plants in function of five rootstocks (G.202, G.213, G.814, M.9 and Marubakaido/M.9 interstock) and two hydric conditions (normal and waterlogged).

Rootstock	-----Adventitious root number[1]----				Linear adventitious root length (cm)[1]-				----Mean adventitious root length (cm)[1]---			
	----Normal[2]----		----Waterlog----		-----Normal-----		-----Waterlog-----		------Normal------		-------Waterlog------	
G.202	42	a[3]*	63	a	100	ab^{ns}	145	ab	2.7	cd^{ns}	2.4	b
G.213	11	c^{ns}	9	b	52	bc^{ns}	66	b	4.7	ab^{ns}	5.0	a
G.814	39	ab^{ns}	53	a	205	a^{ns}	175	ab	5.2	a*	3.3	b
M.9	25	abc*	65	a	90	bc*	202	a	3.4	bc^{ns}	3.0	b
Mar./M.9[4]	17	bc^{ns}	32	ab	28	c*	111	ab	1.8	d*	3.5	b
CV[5]	-----------------31.79-----------------				-------------------36.97------------------				---------------------18.54---------------------			

[1]Means presented in table as original were transformed by square root (√x) for statistical analysis. * and ^{ns} significant and not significant according to the t test ($p \leq 0.05$) for the comparison between hydric conditions. [2]Plants irrigated when necessary without waterlogging the soil. [3]Means within a column followed by the same letter are not significantly different according to the Duncan's multiple range test ($p \leq 0.05$) for the comparison among rootstocks. [4] Marubakaido with M.9 interstock. [5]C.V.= coefficient of variation.

There was significant interaction between rootstock and hydric conditions for the variable chlorophyll, while no significant interaction was reported for mean leaf size and leaf number (Table 2). Mean leaf size was not different among for the 'Maxi Gala' scion in all rootstocks in normal hydric condition, and in waterlogging 'Maxi Gala' scion on the rootstock G.213 presented more expanded leaves. According to DAVIES et al. (2005), under root stress, the levels of ACC are elevated in the roots and is unloaded to the xylem along with ABA, negatively affecting stomata conductance and leaf expansion. TWORKOSKI et al. (2016) found greater levels of ABA on the scion of 'Gala' apples grafted on M.9 than MM.111 and so lesser carbon assimilation caused by the dwarfing rootstock in drought condition.

Leaf number was superior for 'Maxi Gala' scions on the rootstocks Marubakaido/M.9 interstock in normal and in waterlogged hydric conditions (Table 2). BAI et al. (2013) and LI et al. (2010) reported severe reduction in leaf number in hypoxia of *M. toringoides*, while the specie adapted to wetlands was slightly reduced. In flood susceptible *Prunus* spp. rootstocks, leaves present chlorosis followed by tissue necrosis, which is related with a decrease of mitochondria activity; in addition, sucrose synthesis and accumulation on leaves follows the same pattern as ethylene levels raise (PISTELLI et al., 2012).

For the variable leaf chlorophyll, in the interaction of hydric conditions within rootstocks, under both normal and waterlogging it was achieved the highest level of chlorophyll with 'Maxi Gala' scion on the rootstock G.202; on the interaction of rootstocks within hydric conditions, it was reported significant difference among 'Maxi Gala' scions on the rootstocks G.213, G.814 and M.9 that presented elevated levels under normal hydric condition (Table 2). Waterlogging is strongly related with a decrease in the leaf chlorophyll content along with a decline in carotenoid and chlorophyll levels even in more flood tolerant rootstocks of *Prunus* spp. trees; In flood tolerant rootstocks, leaf gas exchange and elevation of internal CO_2 was not prominent as in flood sensitive the levels raise up, and in flood sensitive rootstocks it can be attributed the low CO_2 fixation not only due to stomata regulation, but due to low chlorophyll level as it is damaged by ROS (PISTELLI et al., 2012; BAI et al., 2013).

For 'Maxi Gala' scion on the rootstock G.202 it was observed the short-term waterlogging effects on the variable plant height from the third week of evaluation, and thenceforth the plants subjected to waterlogging remained growing, but at a reduced rate than the plants under normal hydric condition. For 'Maxi Gala' scion on rootstock G.814, it was observed a growth rate similarly between both hydric conditions. For 'Maxi Gala' scion on the rootstock Marubakaido/M.9 interstock it was observed that the plants on both hydric conditions remained the growth rate similarly until the ninth week of evaluation, and thereafter the plants subjected to short-term waterlogging presented had less growth in height than the plants at normal hydric condition. In the case of 'Maxi Gala' scion on the rootstock G.213, it was observed that since the beginning of the experiment there was a negative effect of short-term waterlogging on the growth in height and accentuated over time throughout the experiment. Plants grafted on M.9 tended to maintain the growth rate similarly in both conditions until the fifth

Table 2 - Mean leaf size, leaf number and chlorophyll of 'Maxi Gala' plants in function of five rootstocks (G.202, G.213, G.814, M.9 and Marubakaido/M.9 interstock) and two hydric conditions (normal and waterlogged).

Rootstock	----------Mean leaf size (cm^2)-------				-------------------Leaf number-----------------				-------Chlorophyll (SPAD)--------			
	----Normal[1]----		-----Waterlog---		--------Normal-------		------Waterlog-----		------Normal----		--Waterlog---	
G.202	20	a[2]^{ns}	19	b	168	ab^{ns}	116	b	40	a^{ns}	40	a
G.213	20	a^{ns}	22	a	149	bc^{ns}	142	b	39	b*	36	c
G.814	20	a^{ns}	20	ab	133	bc^{ns}	154	b	31	c*	28	e
M.9	22	a^{ns}	19	b	105	c^{ns}	125	b	39	b*	37	b
Mar./M.9[3]	20	a^{ns}	19	b	204	a^{ns}	243	a	31	c^{ns}	31	d
CV.	--------------------14.68---------------				----------------------31.70---------------------				-----------------18.38-----------------			

*and ^{ns} significant and not significant according to the t test ($p \leq 0.05$) for the comparison between hydric conditions. [1]Plants irrigated when necessary without waterlogging the soil. [2]Means within a column followed by the same letter are not significantly different according to the Duncan's multiple range test ($p \leq 0.05$) for the comparison among rootstocks. [3]Marubakaido with M.9 interstock. [4]C.V.= coefficient of variation.

week of evaluation, but from that date it was observed a deleterious effect on the waterlogged condition on the growth in rate (Figure 1). It was evident the negative effect of short-term anoxia for M.9.

Lateral branch growth rate was slightly decreased for waterlogging of 'Maxi Gala' scions on the rootstocks G.814 and Marubakaido/M.9 interstock. The scion grafted on M.9 presenting the lowest growth rate throughout the experiment duration. Scion grafted on the rootstock G.213 presented elevated susceptibility with expressive reduction rate of lateral branches. Surprisingly 'Maxi Gala' scion on the rootstock G.202 presented elevated growth rate of lateral branches in the waterlogged hydric condition until the sixteenth week, where it reached the same growth rate of the normal hydric condition. LI et al (2010) reported behavior differences of two *Malus* species, where the originated from wet climates presented more waterlogging tolerance and less growth reduction

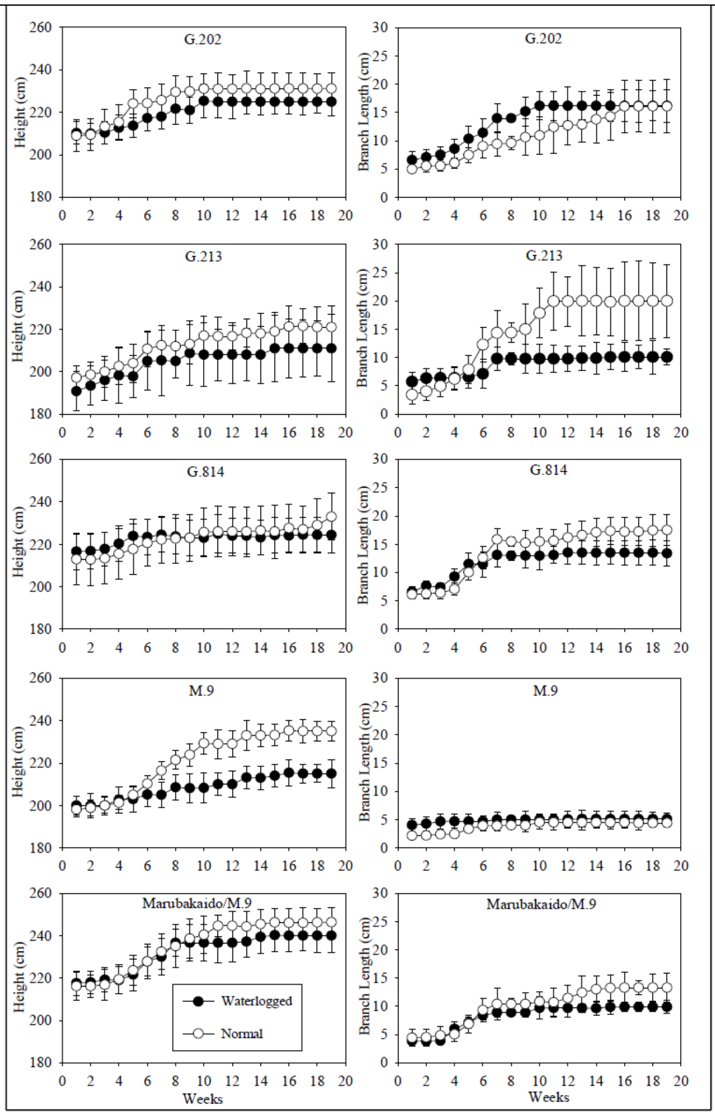

Figure 1 – Plant growth rate of height (left) and lateral branch (right) of 'Maxi Gala' apple plants on five rootstocks throughout 19 weeks on normal and waterlogged hydric condition. Bars represent mean's standard error.

compared to a dry climate originated species due to differentiated ROS scavenging defense systems.

Reduced growth rate is a consequence of diminished leaf stomata aperture due to waterlogging, as it stimulates the production of abscisic acid (ABA) by the roots diminishing gas exchange rate; therefore, as observed in our experiment, no wilting was noticed throughout the time of experiment duration, because the elevated presence of ABA on leaves are related to water maintenance rather than water loss. Although, the concentration of ABA within the xylem sap and its effects on the stomatas are highly dependent on the hydraulic conductivity and the degree of dissolution in the xylem sap that reaches the shoots; in addition, as the stress persists, the levels of cytokinins, which are related to stomata aperture, and are produced in the roots tend to decrease as ABA levels are enhanced, leading to lesser stomata conductance (DAVIES et al., 2005). In this experiment, 'Maxi Gala' scions on the more dwarfing rootstocks presented less growth (represented by plant's height and length of the lateral branches) compared to the more vigorous Marubakaido/M.9 interstock and G.814. This is caused due to the lower hydraulic conductivity of the dwarfing rootstocks caused by different xylem vessels morphology, which is correlated with elevated ABA content in the scion's xylem sap under a stress condition, as the ABA tend to be less dissolved and so more bioactive (TWORKOSKI & FAZIO, 2015).

CONCLUSION

The rootstocks G.202, G.814 and Marubakaido/M.9 interstock are more tolerant to short-term waterlogging. The mechanism to overcome the deleterious effects caused by short-term anoxia is an increment of new adventitious root emission. Even though the rootstock M.9 presented greater new adventitious roots, it was the most susceptible, among the rootstocks evaluated, to short-term waterlogging.

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DECLARATION OF CONFLICTING OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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