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Short Communication

Peroxidase and polyphenol oxidase activity on the yield of grafted and ungrafted cucumber plants

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The first experiment was carried out under green house and involved nine treatments: 'Tsuyoi' cucumber, 'Shelper' squash and 'Green-stripped cushaw squash' ungrafted plants and 'Tsuyoi' cucumber plants grafted onto 'Shelper' squash and 'Green-stripped cushaw squash (lower, mid and upper region of the recommended and non-recommended rootstock, respectively). After grafting, plant tissue samples were collected 1, 4, 7, 10 and 13 days after grafting for analysis of peroxidase (EC 1.11.1.7) and polyphenol oxidase (EC 1.10.3.1) activity. In the second experiment, yield and number of marketable fruits were evaluated. The differences in peroxidase activity at the rootstock region and in polyphenol activity at the region between the scion and the rootstock seem to be determining factors for a successful grafting process, increasing the yield and the number of marketable fruits.

Key words: *Cucumis sativus*, antioxidant enzymes, yield, Japanese cucumber.

INTRODUCTION

In Brazil, some vegetable producers have intensively cultivated cucumber plants in protected environments since the 1980s. Although this activity has contributed to increasing production and quality, it has resulted in problems related to incidence of diseases caused by soilborne fungi and infestation of nematodes, as well as higher salinity levels in the soil, which are impractical for some cultures and lead farmers to use new production systems, adopting as a short-term solution in some cases grafting onto resistant materials (Da Hora, 2006). Currently, a large part of Japanese cucumber cultivation in protected environment already uses cucumber grafted onto squash (Cardoso, 2010).

The grafting activity has widely expanded, improving the yield by increasing the capacity of nutrient uptake by the soil, preventing infection by soilborne pathogens and increasing the tolerance to low temperatures, salinity and soaking of the soil (Martinez-Balesta et al., 2010).

The incompatibility mechanism is not yet fully understood and there are a large number of studies aimed at understanding the mechanism of grafting development. Most reports, however, are related to biochemical responses and peroxidase activity, which occur at the initial phase in response to grafting, as well as to the consequences of these events for future responses associated with the plant development (Pina and Errea, 2005).

Peroxidase activity and phenol levels are important for the scion and the rootstock joining and may thus influence the stress response of incompatibility in the grafting process (Rodrigues et al., 2002). According to Santamour (1992), to allow the vascular system to work in the grafting region, peroxidases must be similar in both the scion and the rootstock, so that lignin is produced. In plants with similar peroxidases, incompatibility is rare. Incompatibility between the scion and the rootstock

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Table 1. Activity of the enzyme peroxidase in samples from ungrafted 'Tsuyoi' cucumber, 'Shelper' squash, Green-stripped cushaw squash and grafted plants in samples collected from three different regions of the stem, at 1, 4, 7, 10 and 13 days after grafting (nmol min^{-1} purpurogallin mg $protein^{-1}$).

Treatment	Days				
	1	4	7	10	13
	nmol min^{-1} purpurogallin mg $protein^{-1}$				
Cucumber hybrid Tsuyoi ungrafted	7.076^{c}	6.498^{c}	1.461^{b}	2.662^{b}	1.171^{d}
Squash hybrid Shelper ungrafted	5.543^{c}	5.883^{c}	4.424^{b}	3.818^{b}	4.249^{c}
Green-stripped cushaw squash ungrafted	3.563^{c}	6.889^{c}	3.365^{b}	7.630^{b}	3.091^{c}
Region below the grafting (Tsuyoi onto Shelper)	20.435^{b}	31.813^{b}	28.665^{a}	17.650^{a}	1.500^{d}
Grafting region(Tsuyoi onto Shelper)	43.297^{a}	40.773^{a}	37.433^{a}	19.430^{a}	15.293^{a}
Region above the bud (Tsuyoi onto Shelper)	13.991^{b}	6.225^{c}	5.116^{b}	5.787^{b}	2.193^{d}
Region below the grafting (Tsuyoi onto GSCS)	4.340^{c}	5.599^{c}	2.784^{b}	4.870^{b}	4.457^{c}
Grafting region (Tsuyoi onto GSCS)	42.616^{a}	31.152^{b}	25.054^{a}	23.002^{a}	22.980^{a}
Region below the grafting (Tsuyoi onto GSCS)	4.968^{c}	2.881^{c}	2.226^{b}	3.697^{b}	3.198^{c}
VC (%)	34.18	41.29	66.67	40.87	17.24
F value	26.6*	21.8*	11.7*	15.4*	182.0*

*Means followed by same letter in the same column do not differ significantly from each other according to Scott-Knott test at 5% probability. Collections for enzymatic analyzes: Samples for enzymatic analyzes were collected from the grafting region; from 1 cm above the grafting region; and from 1 cm below the grafting region for grafted plants. For ungrafted plants, samples were collected from heights similar to those of grafted plants. Such plant material was frozen in liquid nitrogen and stored at -80°C. Enzyme extract was obtained according to the method described by Ekler et al. (1993) and the activity of peroxidase (POD - EC 1.11.1.7) was determined according to the methodology described by Teisseire and Guy (2000). Experimental design was completely randomized with nine treatments and four replicates of five plants. Treatments consisted of the following stem samples: 1. Cucumber hybrid Tsuyoi – ungrafted; 2. Squash hybrid Shelper – ungrafted; 3. Green-stripped cushaw squash (GSCS) – ungrafted; 4. Region below the grafting (Tsuyoi grafted onto 'Shelper'); 5. Grafting region (Tsuyoi grafted onto 'Shelper'); 6. Region above the bud (Tsuyoi grafted onto 'Shelper'); 7. Region below the grafting (Tsuyoi grafted onto GSCS); 8. Grafting region (Tsuyoi grafted onto GSCS); 9. Region below the grafting (Tsuyoi grafted onto GSCS).

may manifest in future responses, as noted by Pina and Errea (2005), consuming research time due to the large number of tested rootstocks. Therefore, the aim of this study was to verify whether the enzymatic difference between the scion and the rootstock, before and after the healing process, can be used as a rapid mechanism to verify incompatibility, reducing thus experimentation time in the field.

RESULTS AND DISCUSSION

For ungrafted plants, peroxidase activity was not greater than 8,000 nmol purpurogallin min^{-1} µg $protein^{-1}$ (Table 1) during the assessment period; it was five-fold lower compared to the grafting region on the day following the process. Some authors have suggested that the different peroxidase activity between the scion and the rootstock could be the limiting factor for compatibility. This hypothesis, however, cannot be accepted in this experiment since there was no statistical difference among ungrafted plants until the tenth day of analysis.

Nevertheless, peroxidase seems to have more influence after grafting, ranging in activity between the rootstocks used in this experiment. After grafting, the site of contact between the scion and the rootstock showed high activity of this enzyme, probably due to healing, typical of injured tissues that are lignified. However, increased POD activity at this site can be caused by the stress of grafting, since this enzyme is considered a stress marker, which could only be elucidated with specific analysis of lignin peroxidase.

POD activity was low below the grafting joining region (1 cm) throughout the experimental period, regardless of the used rootstock, maintaining thus the activity equal to that before the grafting. However, below the grafting joining region (1 cm), POD activity was high in the recommended rootstock; this activity was similar to that at the grafting joining region, not statistically differing, until the tenth day of analysis, which is the period for the grafting establishment. The non-recommended rootstock had low POD activity in the lower region compared to the grafting joining region; this activity was similar to that in ungrafted plants.

At the grafting region, initially, there was no statistical difference for POD activity between the used rootstocks. However, this activity decreased during the establishment of grafts, so that the amplitude was approximately 17,550 nmol purpurogallin min^{-1} mg $protein^{-1}$ in cucumber plants grafted onto the non-recommended rootstock and 5,900 nmol purpurogallin min^{-1} mg $protein^{-1}$ in those grafted onto the recommended rootstock. This lower amplitude of peroxidase activity for the recommended rootstock is probably due to hydrogen peroxide supply below the

Table 2. Activity of the enzyme polyphenol oxidase in samples from ungrafted 'Tsuyoi' cucumber, 'Shelper' squash, Green-stripped cushaw squash and grafted plants in samples collected from three different regions of the stem, at 1, 4, 7, 10 and 13 days after grafting (nmol of purpurogallin min^{-1} µg of $protein^{-1}$).

Treatment	Days				
	1	4	7	10	13
	nmol purpurogallin min^{-1} µg $protein^{-1}$				
Cucumber Hybrid Tsuyoi ungrafted	3.658[b]	2.517[a]	3.343[a]	1.562[b]	1.250[c]
Squash hybrid Shelper ungrafted	4.058[b]	3.238[a]	1.155[b]	2.392[b]	3.447[a]
Green-stripped cushaw squash ungrafted	2.290[c]	2.080[b]	1.788[b]	3.717[a]	2.089[b]
Region below the grafting (Tsuyoi onto Shelper)	3.820[b]	3.178[a]	1.756[b]	3.776[a]	1.792[b]
Grafting region (Tsuyoi in Shelper)	1.506[c]	1.347[b]	1.200[b]	710[b]	1.341[c]
Region above the bud (Tsuyoi onto Shelper)	5.105[a]	2.937[a]	3.749[a]	1.792[b]	2.058[b]
Region below the grafting (Tsuyoi onto GSCS)	2.717[c]	1.659[b]	1.814[b]	3.445[a]	2.461[b]
Grafting region (Tsuyoi onto GSCS)	2.248[c]	2.554[a]	2.181[b]	1.329[b]	2.048[b]
Region below the grafting (Tsuyoi onto GSCS)	2.691[c]	2.213[b]	1.907[b]	4.248[a]	2.091[b]
VC (%)	20.68	23.74	36.77	29.34	19.63
F value	12.0*	5.1*	5.3*	11.6*	10.1*

*Means followed by same letter in the same column do not differ significantly from each other according to Scott-Knott test at 5% probability. Collections for enzymatic analyzes: Samples for enzymatic analyzes were collected from the grafting region; from 1 cm above the grafting region; and from 1 cm below the grafting region for grafted plants. For ungrafted plants, samples were collected from heights similar to those of grafted plants. Such plant material was frozen in liquid nitrogen and stored at -80°C. Enzyme extract was obtained according to the method described by Ekler et al. (1993) and polyphenol oxidase activity (POL - EC 1.10.3.1) was determined based on the method described by Kar and Mishra (1976). Treatments consisted of the following stem samples: 1. Cucumber hybrid Tsuyoi – ungrafted; 2. Squash hybrid Shelper – ungrafted; 3. Green-stripped cushaw squash (GSCS) – ungrafted; 4. Region below the grafting (Tsuyoi grafted onto 'Shelper'); 5. Grafting region (Tsuyoi grafted onto 'Shelper'); 6. Region above the bud (Tsuyoi grafted onto 'Shelper'); 7. Region below the grafting (Tsuyoi grafted onto GSCS); 8. Grafting region (Tsuyoi grafted onto GSCS); 9. Region below the grafting (Tsuyoi grafted onto GSCS)

grafting region, which showed high peroxidase activity, providing a better connection of vessels.

The high POD activity at the grafting joining region in 'Green-stripped cushaw squash' on the day after grafting may be attributed more to the stress caused to that region than to the lignification process itself, since it did not persist during the days after collection, as shown for the recommended rootstock. This study suggests that the involvement of this enzyme in the different enzymatic activity between the rootstock and the scion is not before grafting but after grafting, that is, the smaller the difference in activity between the rootstock and the grafting region, the better the connection of vessels; however, this fact can only be confirmed by verifying the plant anatomy.

Peroxidases have been studied in apricot plants, indicating that compatible cultivars have higher activity of this enzyme than incompatible ones and such incompatibility could be provided by inefficient lignifications at the grafting joining region (Quesada and Macheix, 1984).

Cucumber plants grafted onto 'Shelper' squash had a reduction in POD activity during the evaluation period, which remained high until the seventh day of analysis, when it started deeply decreasing, maintaining a similar activity until the end of the experiment. According to Da Hora (2006), who studied grafting onto cucumber plants, the perfect connection of vessels occurs at 8 days after the procedure.

Therefore, the reduced peroxidase activity in grafting onto 'Shelper' squash around the eighth day after grafting would result from the complete lignification process. Even with such a reduction, the values remained higher compared to those at 1 cm above and below the grafting joining region, probably due to the high concentration of auxin, responsible for the tissue differentiation.

As shown in Table 2, there was statistical difference among the three regions analyzed for grafting onto 'Shelper' squash, so that the grafting joining region showed significantly lower POL activity compared to the lower and the upper region.

Such a lower activity of POL enzyme at the grafting joining region is suggested to be a result of lower levels of phenolic compounds due to the use of this substrate by the enzyme peroxidase in the lignifications process.

The higher activity at the grafting joining region on the day after grafting is a consequence of increased levels of phenolic compounds in regions which have undergone some type of injury, as in the grafting process.

After the reduction in POL activity in plants grafted onto 'Shelper' squash over the experiment, there was an increase on the 13th day, probably due to the levels of polyphenols or monophenols, responsible for the degradation of auxin and the inhibition of this process, respectively.

POL activity was not significantly different among the three regions analyzed for grafting onto Green-stripped cushaw squash, except for the eleventh day after

Table 3. Average number of marketable fruits (fruits per plant) and yield (kg per plant) of 'Tsuyoi' cucumber plants grafted onto 'Shelper' squash or ' Green-stripped cushaw squash' and ungrafted plants, São Manuel-Paraná, Brazil. 2010.

Treatment	Fruits marketable per plant	Yield (kg per plant)
Cucumber 'Tsuyoi' - ungrafted	15.0[b]	2.430[a]
Grafting onto 'Shelper' squash	21.8[a]	3.261[a]
Grafting onto GSCS	12.4[c]	1.980[b]
VC (%)	20.68	23.74
F value	12.0*	5.1*

*Means followed by same letter in the same column do not differ significantly from each other according to Scott-Knott test at 5% probability. Fruits were harvested when their length was between 20 and 22 cm at every two days during 8 weeks. Yield: All fruits harvested during the whole collection period divided by the number of plants; the result was expressed as kg plant^{-1}. Number of marketable fruit: Fruits that remained after the removal of those showing internal length / external length rate inferior to 0.85; the result was expressed as marketable fruits per plant. The experimental design for cucumber production was in randomized blocks, consisting of three treatments: Ungrafted cucumber hybrid Tsuyoi, 'Tsuyoi' cucumber grafted onto 'Shelper' squash, and 'Tsuyoi' cucumber grafted onto Green-stripped cushaw squash, with 10 replicates of 3 plants per plot.

grafting. Enzyme activity in the non-recommended rootstock was different from that in the rootstock recommended for the culture, both for peroxidase and for polyphenol oxidase. Both enzymes may be closely related in the process of compatibility between the rootstock and the scion.

Therefore, higher activity of POD, directly responsible for the process of vessel lignifications, and lower activity of POL, which uses phenolic compounds essential for this process, would provide a more rapid and efficient grafting process, changing thus the transport of nutrients, photoassimilates, plant hormones and consequently plant vigor.

Grafting onto 'Shelper' squash increased the number of marketable fruits and the yield, while plants grafted onto Green-stripped cushaw squash were negatively influenced by these parameters, showing lower yield and fewer marketable fruits, which demonstrates that there may be levels of compatibility between the different rootstocks used in this experiment.

The rootstock 'Shelper' provided 31.2 and 43.2% increase in the number of marketable fruits and 35.5 and 39.5% in the yield, relative to ungrafted plants and cucumber grafted onto Green-stripped cushaw squash, respectively (Table 3). According to Khryanin (2007), cytokinin can induce feminization in various plant species. Zhou et al. (2007) studied cucumber grafted onto *Cucurbita ficifolia* and found that grafted plants had twice the level of cytokinin at a temperature without chilling; when the temperature was reduced, cytokinin level in grafted plants was up to 33-fold higher than that in ungrafted plants. These data support the hypothesis that the rootstock 'Shelper' may have increased cytokinin level, providing a larger number of fruits, as observed in this study, particularly because this experiment was partially performed during the cold period.

According to Ciobotari et al. (2010), incompatibility may have negative consequences on the growth and the development of plants. In studies of grafting in cucumbers, data related to yield are contradictory; depending on the experiment a higher or a lower yield is obtained, which explains the difference between the rootstocks in this experiment. Thus, successful grafting is closely related to the used rootstock.

Conclusion

The difference in enzyme activity between the rootstock and the grafting joining region during the healing process influences the performance of grafted plants and could be used as a rapid mechanism to verify incompatibility.

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