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Effect of the Shank Length of *Pyrus betulaefolia* Rootstock on the Photosynthesis and the Sodium and Chloride Distribution of Japanese Pear Saplings under Saline Conditions

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Abstract

In this study, 'Kousui' pear (*Pyrus pyrifolia* Nakai) was grafted onto *P. betulaefolia* (Bunge), a salt-tolerant rootstock for the Japanese pear, using different shank lengths. Following this, the salt tolerance of the plants was compared. Potted one-year-old plants were subjected to 30 mM or 60 mM NaCl solutions for seven weeks. Shoot growth, photosynthesis, and Na and Cl concentrations and distributions were assessed. Plants with longer shanks had a higher photosynthetic rate than those with shorter shanks; however, comparisons of leaf Na and Cl contents showed only slight differences when exposed to the 30 mM NaCl solution. For plants exposed to the 60 mM NaCl solution, those with longer shanks showed lower Na and Cl concentrations in the leaves than plants with shorter shanks. Moreover, those with longer shank lengths maintained structural root growth. Thus, plants with longer shanks restricted the transport of Na and Cl to their leaves by maintaining structural root growth and accumulating Na and Cl in the structural root. Grafting the scion onto a long shank rootstock can improve the salt tolerance of a young pear tree. Consequently, this practice may be useful for Japanese pear cultivation in regions where the plants would otherwise suffer salt stress.

Salt tolerance is an important factor in horticultural plant production in coastal, arid, and/or semi-arid areas, as horticultural plants, especially deciduous fruit trees, are generally sensitive to salt stress (2). Recently, the number of regions with salinity problems has increased in conjunction with the ongoing worldwide climatic changes and due to improper agricultural practices (7).

Pyrus species are also classified as salt sensitive. Myers et al. (15) reported that high concentrations of salt in irrigation water have influenced European pear (*P. communis* L.) production in southeastern Australia. In the semi-arid region in northwestern China, high levels of alkali and salt in soil have caused problems in Chinese pear (*P. bretschneider* Rehd.) production (12, 20). To date, there are no reports concerning salinity problems with Japanese pear production; however, such problems may occur in the near future, because the cultivation area for Japanese pears has extended beyond East Asia (1).

The salt tolerance of fruit trees can be improved through the use of salt tolerant rootstocks. In general, salt tolerant rootstocks have a number of salt exclusion or salt isolation systems, although the availability is different in each rootstock species (18). In a previous study, the authors compared the salt tolerance of five Japanese pear rootstocks. It was found that *P. betulaefolia* had the highest salt tolerance among the five (13). Moreover, the findings suggested that *P. betulaefolia* restricts the translocation of Na and Cl from roots to shoots and prevents over accumulation

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of these ions in leaves. The inhibition of Na and Cl translocation to leaves may occur in the structural roots of *P. betulaefolia*. Moreover, the salt tolerance of *P. betulaefolia* was maintained even when a scion was grafted (14). However, further investigation is necessary to determine the most effective means of grafting to use this rootstock under salt-stressed conditions. Quince (*Cydonia oblonga* Mill.) is widely used as a European pear rootstock because it can control tree vigor depending on the shank length used (19). For Japanese pear, the effects of rootstock shank length has not been studied, as tree vigor is controlled by bending the branches on a horizontal trellis (9). However, *P. betulaefolia* may have same salt tolerance mechanisms in the structural root; thus, there is a possibility that shank length also influences the salt tolerance of this rootstock.

In the present experiment, the salt tolerance levels of long and short shank plants were compared using 'Kousui' pear grafted on *P. betulaefolia* seedlings. The photosynthetic rate, Na and Cl distribution, and root growth were compared.

Materials and Methods

Plant materials and treatments. One-year-old 'Kousui' pear grafted onto *P. betulaefolia* (Bunge) was used in the experiment. In April of 2003, 'Kousui' was grafted onto carefully selected uniform *P. betulaefolia* seedlings at 2 cm (shorter shank) or 15 cm (longer shank) above the soil level and grown in the orchard of Tottori University in Tottori, Japan. In December of 2003, the plants were transplanted into 20 L terracotta pots filled with medium-textured decomposed granite soil (pH 6.2) and were cultivated in a greenhouse under natural conditions until the saline water treatment began. The trunk was pruned 50 cm above the ground, and two new upper shoots developing from the trunk were retained for the experiment. The plants were irrigated with 500 mL of 30 mM or 60 mM NaCl solution once a day for 49 d from late July of 2004. During the experiment, the daytime temperature in the greenhouse was 30 ± 5 ºC and the temperature at night was maintained at 20 ºC. Relative humidity ranged from 60 to 80% and the day length was approximately 13 h. Control plants were irrigated with water alone once a day for the same period.

Measurement of shoot growth. The shoot lengths from the shoot base to the shoot tip of two shoots per plant were measured during the experimental period.

Measurement of mineral content. Plants were destructively harvested and divided into leaves, branches, trunk (scion), structural roots (shank and roots of which the diameter was greater than 5 mm), and fine roots at the end of the treatment time. Fresh weight was recorded and the plants were washed with distilled water. After oven drying at 78ºC for 72 h, the dry weight was measured and the samples were lightly ground into powder. To determine the Na contents, powdered samples were digested using the H_2SO_4–H_2O_2 Kjeldahl digestion method (16). One milliliter of H_2SO_4 was added to 50 mg of each powdered sample in a test tube. This was left for 12 h and then heated to 180ºC and maintained at this temperature for 3 h. During the heating process, 0.9 mL of H_2O_2 was added to digest the samples completely. Samples were diluted with 50 mL of distilled water, and this solution was used for the Na analyses. The Na concentrations were determined using an atomic absorption spectrophotometer (170–30, Hitachi, Tokyo, Japan). The Cl content was determined using the spectrophotometric mercury thiocyanate-iron method of Iwasaki et al. (8). Fifty milligrams of powdered sample and 10 mL of 0.1 N acetic acid were put into a plastic tube, shaken for 1 h, and then left stationary for 12 h. Supernatants were then centrifuged (5 min at 12,000 rpm), and 0.1 mL amounts of each sample solution were mixed with 0.2 mL 13.2 mM mercuric thiocyanate solution, 0.1 mL 0.5 M ferric nitrate solution, and distilled water. Absorbance of ferric thiocyanate was measured using a spectrophotometer at 460 nm (U-2000, Hitachi, Tokyo, Japan).

Photosynthetic activity. Photosynthetic activity, stomatal conductance, and evaporation rates were measured with a portable open-gas exchange system (LCA-4, ADC, Hoddesdon, England) in a greenhouse. Measurements were performed from 10:00 to 12:00 h on clear days, using the third or fourth youngest expanded leaves. Leaf temperature was 30 ± 3ºC, and the concentration of CO_2 within the cuvette was 380 ± 10 ppm. Each leaf was exposed to photosynthetic photon flux density (PPFD) at a level of at least 1200 μmol m^{-2} s^{-1}.

Experimental design and statistic analysis. The factorial experiment (two shank length × three salinity levels) utilized a randomized block design with five blocks represented by five adjacent rows. The data were subjected to two-way ANOVA using JMP IN software (SAS Institute, Cary, NC). Significant differences between the shank lengths were determined using a t-test. Unless otherwise stated, differences were considered statistically significant when p<0.05.

Results and Discussion

Vegetative growth responses. Pyrus betulaefolia is one of the most salt-tolerant wild pear species (13). This tolerance is maintained when it is used as a rootstock for Japanese pear (14). In the present experiment, the shoot growth of a 'Kousui' pear grafted onto *P. betulaefolia* was reduced by the saline water treatment (Table 1). However, no defoliation

Table 1. Effect of NaCl treatments on shoot growth (cm) (49 d after onset of treatment) of 'Kousui' Japanese pear on *P. betulaefolia* rootstock with 2 or 15 cm shank lengths.

Shank length	NaCl treatment (mM)		
	0	30	60
Short (2 cm)	142.4	112.4	103.8
Long (15 cm)	143.8	106.5	100.4
t-test	ns[z]	ns	ns
ANOVA			
Shank length (A)		ns[y]	
NaCl (B)		***	
A × B		ns	

[z] ns indicates non-significant differences between shank length by t-test (*n*=5)

[y] ns, *** indicate non-significant and significant differences at *P*<0.01, respectively, by ANOVA (*n*=5)

Table 2. Effect of NaCl treatments on fresh weight (g) of leaves, branch, trunk, structural roots and fine roots (49 d after onset of treatment) of 'Kousui' Japanese pear on *P. betulaefolia* rootstock with 2 or 15 cm shank lengths.

Shank length	Leaves			Branches			Trunk			Structural roots			Fine roots		
	NaCl treatment (mM)														
	0	30	60	0	30	60	0	30	60	0	30	60	0	30	60
Short (2 cm)	127.0	91.2	81.9	164.2	98.1	95.7	45.8	29.5	32.1	106.7	69.0	63.7	87.1	50.5	46.5
Long (15 cm)	121.3	90.0	85.2	147.1	103.7	91.7	32.9	30.1	29.1	108.8	104.4	101.9	65.5	48.5	36.8
t-test	ns[z]	ns	ns	ns	ns	ns	**	ns	ns	ns	**	***	**	ns	ns
ANOVA															
Shank length (A)		ns[y]			ns			ns			***			**	
NaCl (B)		***			***			*			**			***	
A×B		ns			ns			ns			*			ns	

[z] ns, **, *** indicate non-significant and significant differences between shank length at *P*<0.05 or 0.01, respectively, by t-test (*n*=5).

[y] ns, *, **, *** indicate non-significant and significant differences at *P*<0.01, 0.05 or 0.01, respectively by ANOVA (*n*=5).

Table 3. Effect of NaCl treatments on shoot:root ratio (49 d after onset of treatment) of 'Kousui' Japanese pear on *P. betulaefolia* rootstock with 2 or 15 cm shank lengths.

Shank length	Shoot: root ratio NaCl treatment (mM) 0	30	60
Short (2 cm)	1.76	1.87	1.92
Long (15 cm)	1.73	1.47	1.50
t-test	ns[z]	**	***
ANOVA			
Shank length (A)		***[y]	
NaCl (B)		ns	
A × B		**	

[z] ns indicates non-significant differences between shank length by t-test (n=5)

[y] ns, *** indicate non-significant and significant differences at P<0.01, respectively, by ANOVA (n=5)

or injury symptoms were noted in leaves exposed to as much as 60 mM of NaCl. Okubo et al. (17) reported that 'Kousui' grafted onto *P. pyrifolia* and *P. calleryana* (Dcne.) (they reported it as *P. betulaefolia* strain Blue) showed defoliation. The *P. pyrifolia* scion also reportedly presented a dark-brownish leaf scorch when the plants were treated with a 50 mM NaCl solution. These data suggest that 'Kousui' pear grafted onto *P. betulaefolia* showed relatively high salt tolerance regardless of the shank length.

The fresh weight of each plant organ decreased as a result of the saline water treatment for both longer and shorter plant shank lengths, with the exception of the weight of the structural root. The weight of the structural roots for shorter plant shanks was reduced by the saline water treatment. However, the weight of the structural roots for longer plant shanks was in no case reduced by the treatment and was significantly higher compared to that of plants with shorter shanks (Table 2). In the control samples, the shoot-to-root ratios of plants with short and long shanks were similar. However, when 30 or 60 mM of saline water was used, this ratio for plants with shorter shanks was significantly higher compared to that for plants with longer shanks (Table 3). This indicates the inhibition of structural root growth (Table 2). That is, plants with a longer shank maintained structural root growth under NaCl stress, although the growth of the shoot decreased (Table 2).

NaCl distribution in the plants. Regardless of the shank length, the Cl concentration in each plant organ increased after a saline water treatment; however, the effects differed depending on the shank length and the plant organ (Table 4). The effect of shank length on the Cl concentration was observed in leaves, branches, and trunk (Table 4). Among these three organs, leaves accumulated the highest concentration of Cl after the saline water treatment, and the leaves of plants with shorter shanks showed a three-fold increase in the Cl concentration compared to plants with longer shanks after the 60 mM NaCl treatment. Plants with shorter shanks also showed significantly higher Cl concentrations in the branches and trunk than plants with longer shanks when treated with the 60 mM NaCl solution. However, these concentration differences were smaller than those of the leaves. Shank length did not affect the Cl concentration of the structural roots or the fine roots (Table 4). The fine root showed the highest Cl concentration among the plant organs.

The Na concentration in each plant organ also increased after the saline water treatment in plants with both short and long shank lengths. An effect of the shank length on the Na concentration was observed in leaves and branches (Table 5). In these organs, plants with shorter shanks showed significantly higher Na concentration than plants with longer shanks when treated with the 60 mM NaCl solution. No shank-length effect related to the Na concentration was observed in the trunk, structural roots or fine roots (Table 5). The fine roots showed the highest Na concentration among the organs; however, there was no significant difference in the Na concentrations between the trunk and the structural roots for the saline solution treatment.

When plants were treated with a 30 mM NaCl solution, the total Na and Cl contents in

Table 4. Effect of NaCl treatments on Cl concentration (mmol kg^{-1} DW) in leaves, branch, trunk, structural root and fine root (49 d after onset of treatment) of 'Kousui' Japanese pear on *P. betulaefolia* rootstock with 2 or 15 cm shank lengths.

Shank length	Leaves			Branches			Trunk			Structural roots			Fine roots		
	NaCl treatment (mM)														
	0	30	60	0	30	60	0	30	60	0	30	60	0	30	60
Short (2 cm)	24.3	89.9	257.8	7.3	25.6	74.9	2.2	16.0	34.6	7.9	86.9	78.8	169.8	506.0	453.4
Long (15 cm)	23.3	54.4	76.9	5.6	12.8	29.1	3.0	12.2	19.4	4.9	69.1	88.0	148.5	422.1	491.0
t-test	ns[z]	ns	***	ns	ns	*	ns	ns	**	ns	ns	ns	ns	**	ns
ANOVA															
Shank length (A)		***[y]			**			**			ns			ns	
NaCl (B)		***			***			***			***			***	
A×B		***			*			*			ns			ns	

[z] ns, *, ** , *** indicate non-significant and significant differences between shank length at P<0.05 or 0.01, respectively, by t-test (n=5).
[y] ns, *, **, *** indicate non-significant and significant differences at P<0.01, 0.05 or 0.01, respectively by ANOVA (n=5).

the plants and the content in each plant organ did not differ between plants with shorter and the longer shanks (Fig. 1). However, the total Na and Cl contents in plants with shorter shanks were higher than those with longer shanks under 60 mM of NaCl stress. The Na and Cl levels contained in the leaves and the branches of the plants with shorter shanks were higher relative to those observed in plants with longer shanks. On the other hand, the Na and Cl levels contained in structural roots with shorter shanks were lower than those of plants with longer shanks (Fig. 1).

It is well-known that the Na and Cl absorption rates and/or transport rates to leaves differ depending on the rootstock species in *Citrus* and *Vitis* (6, 18). In a previous report, the authors confirmed that *P. betulaefolia* restricted Na and Cl transport to leaves (13, 14). However, no known report shows that different rootstock shank lengths affect the amount of Na and Cl transported to leaves. In the present experiment, total Na and Cl content in the leaves and branches of plants with a long shank were significantly lower than in plants with a short shank, as the former reduced the transport of Na and Cl to the leaves (Fig. 1). Plants with a long shank accumulated more Na and Cl in the structural root (Fig. 1), although the concentration there did not differ from that of plants with a short shank (Tables 4 and 5). In plants with a short shank, the growth of the structural root was reduced by the NaCl treatment, whereas this reduction did not occur in plants with a long shank (Table 2). Therefore, the shoot-to-root ratio of plants with a shorter shank was increased due to the NaCl treatment (Table 3). That is, plants with a longer shank maintained structural root growth under the NaCl stress, although the shoot growth was decreased (Table 2). As a result, plants with a longer shank were able to store a large amount of Na and Cl in the roots (Fig. 1), although there was no concentration difference compared to plants with shorter shanks (Tables 4 and 5).

Boland et al. (3) reported that apricot and pear accumulate Na in the heartwood of the trunk at the initial stages of salt absorption. Subsequently, when the absorption capacity of the heartwood is exceeded, Na is transported to the sapwood and upper part of the plants. In the present experiment, the Na concentration in the leaves was the lowest among the plant organs. Moreover, the concentration of Na became higher in organs that were closer to the fine root (Table 5). Therefore, maintaining the growth of the structural root might be advantageous in that it suppresses Na transport to the leaves. It was reported that Cl might be transported to leaves through the evaporation stream (11). This hypothesis was confirmed in the present experiment as the Cl concentration of leaves was higher than in other plant organs, except for the fine roots, and because the concentrations in the branches and trunks were much lower compared to those of the other plant organs (Table 4). As a result, the Cl content in the leaves was highest among the aboveground organs, regardless of the shank length and the applied NaCl concentration (Fig. 1). The structural roots

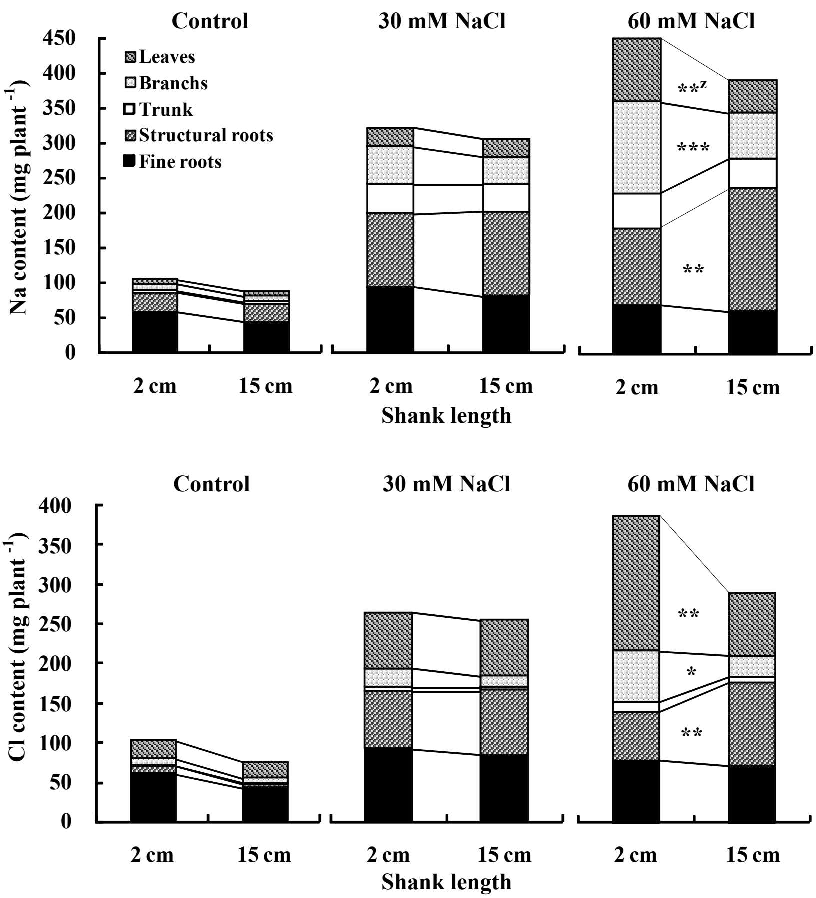

Figure 1. Effect of NaCl treatments on Na and Cl content of each plant organ (49 d after onset of treatment) of 'Kousui' Japanese pear on *P. betulaefolia* rootstock with 2 or 15 cm shank lengths. Symbols *, **, *** indicate significant differences between shank length at P<0.1, 0.05 or 0.01, respectively, by t-test (n=5).

Table 5. Effect of NaCl treatments on Na concentration (mmol kg^{-1} DW) in leaves, branch, trunk, structural root and fine root (49 d after onset of treatment) of 'Kousui' Japanese pear on *P. betulaefolia* rootstock with 2 or 15 cm shank lengths.

Shank length	Leaves			Branches			Trunk			Structural roots			Fine roots		
	NaCl treatment (mM)														
	0	30	60	0	30	60	0	30	60	0	30	60	0	30	60
Short (2 cm)	7.7	34.2	135.6	7.4	56.8	149.3	5.5	129.5	134.1	23.2	125.7	140.9	167.4	511.3	417.0
Long (15 cm)	8.0	33.4	63.1	7.1	41.3	75.2	8.1	118.1	132.0	21.9	99.9	145.9	153.2	403.3	391.6
t-test	ns[z]	ns	***	ns	ns	***	ns	ns	ns	ns	ns	ns	ns	**	ns
ANOVA															
Shank length (A)		***[y]			***			ns			ns			ns	
NaCl (B)		***			***			***			***			***	
A×B		***			**			ns			ns			ns	

[z] ns, **, *** indicate non-significant and significant differences between shank length at $P<0.05$ or 0.01, respectively, by t-test ($n=5$).
[y] ns, *, **, *** indicate non-significant and significant differences at $P<0.01$, 0.05 or 0.01, respectively by ANOVA ($n=5$).

contained a higher concentration of Cl than the branches and trunk, even when the 30 mM NaCl solution was used (Table 4). Thus, the Cl was first stored in the roots and could then be transported to the leaves through the evaporation stream. Therefore, maintaining the root biomass may be an important means of suppressing both Na and Cl transport to the leaves.

Physiological responses. Regardless of shank length, saline water reduced the photosynthetic rate, transpiration rate, and stomatal conductance. However, when plants were treated with a 30 mM NaCl solution, plants with longer shanks showed significantly higher photosynthetic rates compared to those with shorter shanks (Table 6). This finding indicates that the influence of saline water on plants with longer shanks was less than that on plants with shorter shanks. The mechanism behind this difference is unclear. When the 60 mM NaCl solution was used, a significant difference in the Na and Cl content in the leaves was observed (Tables 4 and 5). James et al. (10) reported that the photosynthetic apparatus in wheat was not affected directly when the leaf Na and Cl contents were greater then 200 and 300 mM, respectively, in the tissue water base. These values are higher than the contents observed in the present experiment. Everard et al. (4) also reported that the photosynthetic rate under salt stress was limited both by stomatal and non-stomatal factors and that the first stage of the limitation was caused by stomatal closure. Stomatal conductance is controlled by stress factors, including the osmotic potential and ABA (5).

When plants were treated with a 30 mM NaCl solution in the present experiment, the value of the stomatal conductance of plants with long shanks was significantly higher than that of plants with short shanks (Table 6). However, the values of the water potential did not differ according to the shank length (data not shown). In contrast, the Na and Cl concentrations in fine roots of plants with short shanks were significantly higher than those in plants with long shanks (Tables 4 and

Table 6. Effect of NaCl treatments on photosynthetic rate, transpiration rate and stomatal conductance (40 d after onset of treatment) of 'Kousui' Japanese pear on *P. betulaefolia* rootstock with 2 or 15 cm shank lengths.

Shank length	Photosynthetic rate (μmol m^{-2} s^{-1})			Transpiration rate (mol m^{-2} s^{-1})			Stomatal conductance (mol m^{-2} s^{-1})		
	NaCl treatment (mM)								
	0	30	60	0	30	60	0	30	60
Short (2 cm)	6.7	1.1	1.4	6.2	2.3	2.3	0.34	0.08	0.09
Long (15 cm)	7.5	4.8	1.7	5.6	4.0	4.0	0.33	0.17	0.09
t-test	ns[z]	***	ns	ns	*	ns	ns	***	ns
ANOVA									
Shank length (A)		***[y]			***			ns	
NaCl (B)		***			***			***	
A×B		***			ns			ns	

[z] ns, **, *** indicate non-significant and significant differences between shank length at *P*<0.01 or 0.01, respectively, by t-test (*n*=5).

[y] ns, *, **, *** indicate non-significant and significant differences at *P*<0.01, respectively by ANOVA (*n*=5).

5). Thus, it is possible that differences in the stress resistance of the root affect the stomatal conductance through some stress signal, such as ABA. This may then cause the difference in the photosynthetic rate. Additional experiments should be done to confirm this possibility. However, the photosynthetic rate may be a good early indicator to determine salt effects before they cause fatal problems such as Na and Cl accumulation in the leaves.

*Conclusion***.** This study demonstrated that the salt tolerance of Japanese pear grafted onto a salt-tolerant rootstock species, *P. betulaefolia,* could be further enhanced by grafting it onto a long shank. Plants with a long shank restricted Na and Cl transport to the leaves by maintaining structural root growth and accumulating Na and Cl in the structural roots.

It was reported that the level of salt tolerance differs depending on the developmental stage of the plant and that the tolerance of a young plant (2-3 years old) is lower than it is at other stages (2). As the growth of a fruit tree during its young period determines the life of the fruit tree, it is very important to reduce stressors during this period as much as possible. A grafting technique in which the scion is grafted onto a long shank rootstock can improve the salt tolerance of young pear trees in practical cultivation. Therefore, this technique may be useful for Japanese pear cultivation in regions where the plants would otherwise experience salt stress.

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IS THE PLUM POX VIRUS RESISTANCE OF ‘HONEY SWEET’ PLUM STABLE IN THE PRESENCE OF OTHER VIRUSES?

The transgenic plum Honey Sweet (*Prunus domestica* L.) is highly resistant to plum pox virus. This resistance is due to post-transcriptional gene silencing. The effect of infection by other viruses on the stability of this type of resistance was recently studied. The transgenic plum trees were graft-inoculated with different combinations of Prunus necrotic ringspot virus, apple chlorotic leaf spot virus, prune dwarf virus and a different strain of plum pox virus. Experiments were performed under greenhouse, nursery and field conditions in Romania and Spain, representing continental and Mediterranean climates, respectively. Virus infections were evaluated by visual monitoring of symptoms, serological assays and molecular assays. The engineered resistance to plum pox virus in the Honey Sweet plum trees was stable and was not suppressed by the presence of the other viruses over a three-year experimental period across all trials. Paraphrased from Zagrai et al. 2008. Journal of Plant Pathology 90(1):63-71.