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Causes of Low Survival in Cucumber (*Cucumis sativus* L.) Plants Grafted onto Pumpkin (*Cucurbita moschata* Duch.) Rootstocks by Horizontal-Cut Grafting at the Center of the Hypocotyl

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Abstract

Factors related to the low survival of cucumber plants grafted onto pumpkin rootstocks using a newly developed grafting robot were determined. Horizontal-cut grafting (HCG), whereby hypocotyls of the scion and rootstock are cut at right angles and the cut surfaces are spliced, is used with a robot. The proportion of surviving grafted plants using HCG were compared with those using slant-cut grafting (SCG). Without cotyledons, proportion of surviving grafted plants was lower using HCG than using SCG, whereas the cut level at the hypocotyls had no effect. The proportion of surviving grafted plants was highest at a splicing pressure of 830g/cm^2. Pressed cut surface areas at more than 5.5 cm^2 increased the proportion of surviving grafted plants compared with 0 cm^2 (HCG) The proportion of surviving grafted plants and plant growth after transplanting increased with increasing area of cotyledons. These results suggest that low survival of cucumber plants grafted by horizontal-cut grafting is attributed to low pressure on the spliced cut surfaces and loss of cotyledons on the rootstock.

Key Words: cotyledon, rootstock, scion

Introduction

Cucumbers are produced using grafted transplants at a rate of 72% of the cultivation area of cucumber plants in Japan (Oda, 1995). Cucumber plants have been grafted onto various species of pumpkin rootstocks (*Cucurbita* spp.) generally by tongue approach grafting or recently using slant-cut grafting (SCG) at the top of the rootstock hypocotyl where a cotyledon remains (Fig.1). These grafting methods, however, are labor-, time- and space-consuming.

Grafting robots have been developed to solve these problems. Kurata (1994) introduced grafting robots developed by some groups: Bio-oriented Technology Research Advancement Institution, Japan Tobacco Inc., Osaka Prefecture University and Techno Grafting Research Inc. (TGR). We developed a grafting robot in cooperation with TGR, and a robot has been released for practical use after confirming the high productivity of grafted plugs in tomatoes (Oda *et al.*, 1995) and eggplants (Oda *et al.*, 1997). In cucumber plants, however, the survival ratio of plants grafted by the robot is too low for practical use.

In the grafting using TGR's robot, horizontal-cut grafting (HCG), whereby the axes of the scion and rootstock are cut at right angles and the cut surfaces are spliced, is applied to hypocotyls of the scion and rootstock. This grafting method is different from the conventional grafting method in terms of 1) different

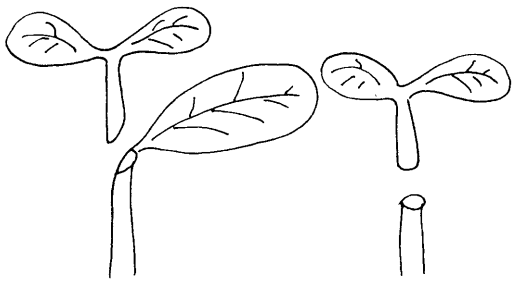

Fig. 1. Slant-cut grafting (SCG, left) and horizontal-cut grafting (HCG, right) at the top and center of the rootstock hypocotyl, respectively.

areas of cut surfaces and pressure on them due to different cutting angles, 2) the cut level at the rootstock hypocotyl and 3) the existence of cotyledons

This study was made to determine the factors strongly affecting the survival of cucumber plants grafted onto pumpkin rootstocks using HCG at the hypocotyl using robotic grafting.

Materials and methods

Germinated seeds of cucumber 'Nankyoku 2-go' (Tokiwa kenkyujo) were sown in a 72-cell plug tray (50mL/cell), and 4 days later pumpkin 'Kongo' seeds (Sakata no Tane, Tokyo) were sown in a plastic pot (35mL). The trays and pots were filled with a medium mixed with Super cell top (Sakata no tane) vermiculite (=2:1,v/v) Nutrient solution containing 4.7 N, 1.3 P, 1.9 K, 2.1 Ca and 0.9 Mg (in me/L) was given to the medium every day Cucumber scions unfolding the first true leaf were grafted onto pumpkin rootstocks having expanded cotyledons The grafted plants were transferred to containers and were acclimatized at 28±3℃ at more than 90% relative humidity under 50μ mol/m^2/s of photosynthetic photon flux (400-700 nm, continuous light) provided by cool-white fluorescent lamps for 10 days and were transferred to a glasshouse

Exp 1 *Effect of grafting methods*

The proportion of surviving grafted plants using HCG at the center of the rootstock hypocotyl (method for robotic grafting) was compared with that by SCG at the top of the hypocotyl where a cotyledon remained (a control) Thirty plants were used for each treatment with six plants in each of five containers

Exp. 2 *Effects of cutting angle and level at the rootstock hypocotyl*

The rootstock was cut at the top or the center of the hypocotyl at cut angles of 30 degrees (SCG) and 90 degrees (HCG) to the longitudinal direction of the hypocotyl In this experiment, all cotyledons were removed to eliminate their effects. Fifteen plants were used for each treatment with five plants in each of three containers

Exp 3 *Effect of the cut surface areas of the scion and rootstock*

Fig. 2 shows how the hypocotyls of the scion and rootstock were cut and cliped The area of cut surface at right angles was 5.7±0.3 (mean± standard error), and the areas cut vertically were 0, 5.5±0.2, 11.4±0.1, 17.9±0.6 and 24.7±0.6 mm^2. By clipping, about 830 g/cm^2 pressure was given only on vertically-cut surfaces using grafting clips having various clipping pressures. Thirtyfive plants were used for each treatment with five plants in each of seven containers

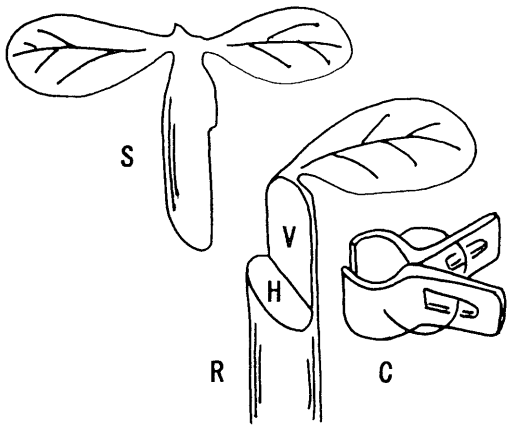

Fig. 2. The method to supply pressures to the cut surfaces of scion and rootstock in experiment 3. Vertical-cut surfaces (V) of scion (S) and rootstock (R) were spliced and pressed with grafting clips (C). (H) horizontal-cut surface.

Exp 4 *Effect of pressure on the cut surfaces of the scion and rootstock*

Scions and rootstocks were grafted at the center of the hypocotyls using SCG and the spliced cut surfaces were given pressures at 0, 249, 830 and 1,414 g/cm^2 with various grafting clips. Fifty-five plants were used for each treatment with five plants in each of eleven containers.

Exp. 5 *Effects of cotyledons remaining on the rootstock*

Scions were grafted using SCG at the top of the rootstock hypocotyl at the remaining 0, 1/4, 1/2 and 1 cotyledon. These plants were compared with plants having two intact cotyledons by cleft grafting at the top of the rootstock hypocotyl. Ninety-five plants were used for each treatment with five plants in each

of nineteen containers.

Thirteen middle-sized grafts were transplanted into pots (500mL) on 7 November, and growth parameters were measured at 0 (at transplanting) and 2 weeks after transplanting to pots. Nutrient solution was supplied to the pots every day.

Results

Fig.3 shows the proportion of surviving grafted cucumber plants having one cotyledon using SCG, a conventional method, and of cucumber plants using HCG without cotyledons, a method for robotic grafting. The proportions were 83% and 30% using SCG with one cotyledon and HCG without cotyledons, respectively. The proportion of surviving grafted plants using HCG without cotyledons was markedly lower than using SCG with one cotyledon.

Fig.4 shows the effect of the cutting angle and level at the rootstock hypocotyl on the proportion of surviving grafted plants without cotyledons. The proportion of surviving plants grafted using HCG was lower than using SCG, while the cutting level had no marked effect.

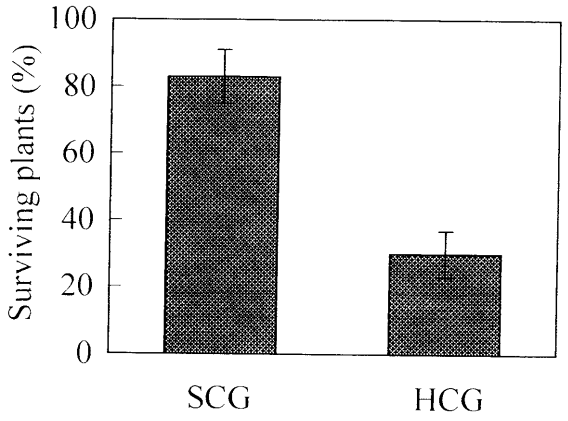

Fig. 3. Proportion of surviving cucumber plants using slant-cut grafting (SCG) with a cotyledon and using horizontal-cut grafting (HCG) without cotyledons. The vertical bar means standard error (n =5).

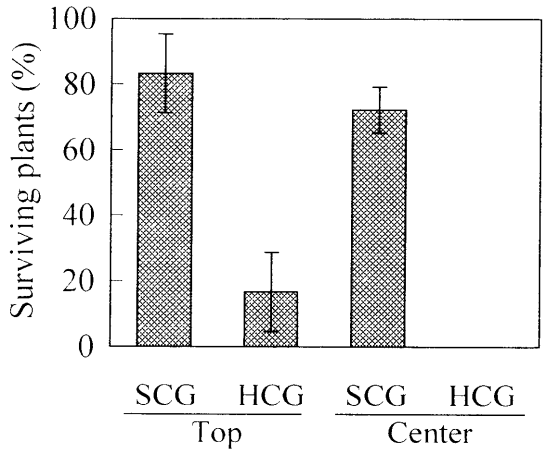

Fig. 4. The proportion of surviving cucumber plants using slant-cut grafting (SCG) and horizontal-cut grafting (HCG) at the top and center of the rootstock hypocotyl, respectively. All cotyledons were cut off. The vertical bar means standard error (n =3).

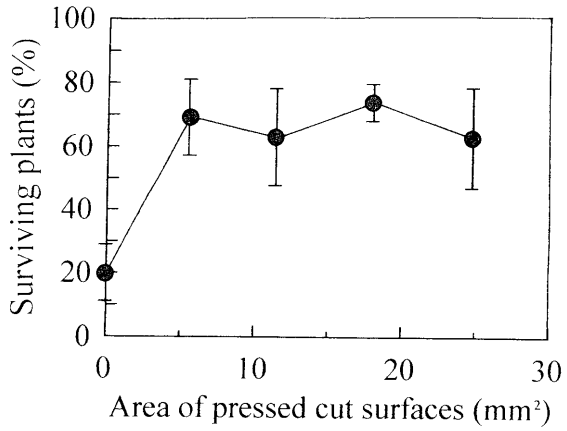

Fig. 5. Proportion of surviving cucumber plants grafted with different areas of pressed cut surface. The vertical bar means standard error (n =7).

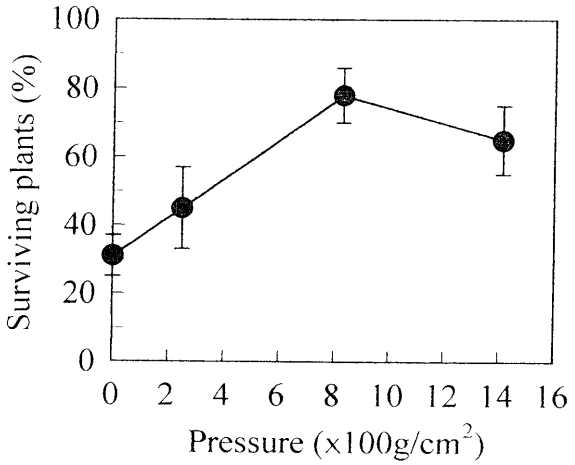

Fig. 6. Proportion of surviving cucumber plants using slant-cut grafting associated with splicing presssure on cut surfaces. The vertical bar means standard error (n =11).

Fig5 shows the effect of pressed cut surface areas of the scion and rootstock on the proportion of surviving grafted plants. When the pressed cut surface was more than 5.5 mm^2, the proportion of surviving grafted plants was constantly high, but was low at 0 mm^2 (HCG)

Fig6 shows the effect of pressure on the cut surfaces of the scion and rootstock The proportion of surviving grafted plants was highest at a splicing pressure of 830 g/cm^2, which was applied using a commercial grafting clip

Fig7 shows the effect of cotyledons remaining on a rootstock on the proportion of surviving grafted plants. The proportion of surviving grafted plants decreased with decreasing number of cotyledons remaining on the rootstock. Table 1 lists the growth parameters of cucumber plants grafted onto rootstocks with various numbers of cotyledons after transplanting. The leaf number of plants with 0-2 cotyledons was the same at transplanting, but was smallest by removing all cotyledons at 2 weeks after transplanting. The stem length at transplanting was smallest with no cotyledons on the rootstock, and at 2 weeks after transplanting the stem length decreased with decreasing area of cotyledon The shoot fresh weight also decreased with decreasing area of cotyledon at 2 weeks after transplanting.

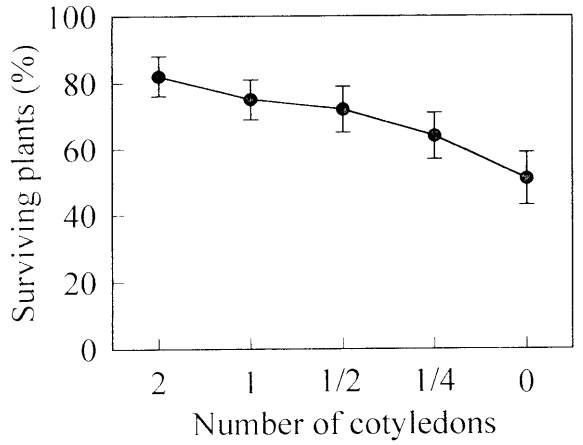

Fig. 7. Proportion of surviving cucumber plants grafted with various numbers of cotyledons on rootstocks. The vertical bar means standard error (n =19).

Table 1. Effects of rootstock cotyledons on the growth of cucumber plants after transplanting

No. of cotyledons on rootstock	No. of leaves		Stem length (cm)		Fresh weight (g/plant)
	0z	2	0	2	2
2 (control)	2 0ay	4 8a	11 5a	27 3a	10.0a
1	2 0a	5 1a	11 6a	26 7ab	7 9b
1/2	2 0a	4 6ab	11.4a	24 1b	6 4c
1/4	2 0a	4 5bc	11 5a	21 4c	5 5d
0	2 0a	4 1c	9.9b	18 4c	4 8d

z Weeks after transplanting Grafting was 12 days before transplanting

y Mean separation within columns by Fisher's LSD at P≦ 0.05

Discussion

As the proportion of surviving grafted plants decreased with increasing difference in hypocotyl diameter between cucumber and pumpkin seedlings (Oda *et al.*, 1993), seedlings with a similar hypocotyl diameter were used in this experiment.

The first experiment was made to confirm that the proportion of surviving cucumber scions grafted using HCG, a robotic method, at the hypocotyl of the pumpkin rootstock is low compared with that using a conventional grafting method, SCG. As a result, we confirmed that the proportion of surviving cucumber scions grafted by the robotic method was low

The HCG and SCG grafting methods differ in: 1) cutting level of rootstock hypocotyl where the distribution of vascular bundles is different at the cutting levels, 2) the cutting angle and 3) the area of rootstock cotyledons In experiment 2, therefore, the third difference was eliminated because of removing the cotyledons. Although the distribution density of vascular bundles is higher at the top of the hypocotyl than at its center (Nakamori, 1968), the cutting level at the rootstock hypocotyl had no or littlel effect on the survival of cucumber plants in experiment 2. In contrast, the cutting angle affected strongly the survival.

Hypocotyls of the scion and rootstock cut at different angles and fixed with a clip resulted in different areas of cut surface and the splicing pressures Therefore, the relationship of cut surface area and the proportion of surviving grafted plants was investigated in experiment 3, where a constant pressure was supplied to the vertically cut surfaces with different splicing area. The proportion of surviving grafted plants was lower only with 0 cm^2 of pressed cut surface, whereas it was higher at more than 5.5 mm^2

of an area commonly used in the conventional grafting method. Therefore, no splicing pressure on the cut surfaces might be the main cause of the low proportion of surviving grafted plants using HCG. To confirm this hypothesis, the single effect of splicing pressure on the survival ratio was investigated in experiment 4. The proportion of surviving grafted plants was maximum at 830g/cm^2 using the pressure of a commercial clip. An appropriate pressure that does not destroy the cells at the cut surfaces might be effective to keep the moisture and to contact closely the cut surfaces.

In experiment 5, the number of cotyledons is in proportion to the area of cotyledons. A decreasing area of the cotyledons remaining on the rootstock led to the low proportion of surviving grafted plants, accompanying vitrification of the rootstock hypocotyl. Plant growth after planting was also inhibited more strongly with a smaller area of cotyledon of the rootstock, which may be due to the lack of reserve substances or photosynthates, or both, supplied by the cotyledons In our previous study, the low proportion of surviving cucumber scions grafted onto pumpkin rootstocks using HCG at the hypocotyl was ascribed to the removal of cotyledons and the fewer number of vascular bundles in contact with the cut surfaces of the scion and rootstock (Oda *et al.*, 1994).

Consequently, we conclude that the loss of splicing pressure on the cut surfaces and the loss of cotyledons on the rootstock cause the low survival of cucumber plants grafted onto pumpkin rootstocks using HCG at the center of the hypocotyls. Cotyledons of pumpkin rootstock are necessary for the survival of cucumber scions through supplying reserve substances or photosynthate, or both.

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