System for Automatic Separation of *Ex Vitro* Micropropagated Sugarcane

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Abstract

A robot system that automatically separates a clump of *ex vitro* micropropagated sugarcane into individual shoots without using a machine vision system was developed and tested. The shoots were planted in a 50-cell plug tray before division. They grew thickly and their roots became entangled with each other in a cell, forming a root ball, therefore the separation was a delicate task. To separate the thickly grown shoots, two types of end-effecters were developed. The first one was a continuous shoots picking mechanism (CSPM). This end-effecter was designed to bring the thickly grown shoots into a line and hold them without damaging them. The second one was a single shoot separator (SSS). This end-effecter was designed to pull off the shoots from the clump one by one. These two end-effecters were installed on two types of robots. As a result, 77.1% of shoots were properly separated into individual shoots by this system and 66.7% of the shoots rooted after division.
[Keywords] micropropagation, sugarcane, shoot, separation, cell plug tray, robot, end-effecter

Introduction

Sugarcane is one of the most important agronomic crops in the world. It is used for producing not only sugar, but also ethyl alcohol and other chemical products. Bagasse can be used as biomass fuel for generating electricity and heat. Recently, a study that aimed to fix carbon dioxide by making charcoal from the bagasse and spreading it on sugarcane fields was reported (Ueno *et al*., 2001).

Only 0.12% of the total world sugarcane production is produced in Japan (FAOSTAT Database, 2000), because only a few areas of Japan such as the southern islands of Kagoshima and Okinawa prefectures are suited for its production. But sugar manufacture is an indispensable industry for the people living in these islands (Matsumoto, 2000). In the last decade, sugarcane harvested area has been decreasing, making it difficult to secure sufficient sugarcane matching the productive capacity of a factory, and so several factories have been shut down. The main reasons for the decrease of harvested area are the aging of farmers, abandonment of farming, young farmers changing to more profitable crops and lack of fully mechanized farming. New technologies, which produce low-cost and high-quality transplants in large quantities, have therefore been developed to increase the production of sugarcane. Micropropagation is one possible solution. Micropropagated sugarcane transplants have been sold and used in practice on Tokunoshima Island in Kagoshima prefecture since 2000. Nansei Togyo Co., Ltd. is playing a central role in commercializing it.

Sugarcane is a gramineous and perennial plant and it propagates vegetatively like potato, sweet potato and strawberry. Usually it is propagated by planting chopped canes in the field. Seed propagation is rarely used except for crossing (Miyazato, 1986). In general, vegetatively propagated plants are easily infected with viruses. Since virus-free transplants can be cultured by shoot tip culture, many kinds of vegetatively propagated plants are produced commercially by micropropagation (Debergh and

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Zimmerman, 1991). In most cases, the produced plants are final products, whereas micropropagated sugarcane is cultured for producing stock cane. Because the micropropagated sugarcane transplant is more expensive (122 yen per transplant) than the conventional stock cane (4 yen per stock cane) and its harvest contains less sugar, it is not suitable for producing sugar. But it shows a high germination rate, high tillering capacity and good growth. These characteristics make it possible to reduce the field area required for producing stock canes by up to 65% and to increase the field area for producing the final product. So the price of produced stock cane can be lowered to 4 yen (Taba *et al*., 1998). It is reported that yields per unit area increase 20% (Anonymous, 2001). So the farmers can understand the significance to introduce the micropropagated sugarcane transplant.

Since the micropropagated sugarcane has such excellent features, its utilization has been steadily extended in Tokunoshima Island. In 2000, 115,200 transplants and in 2001, 150,702 transplants were sold (Anonymous, 2001). But there are still many obstacles for popularization. The principal one is the price of the transplant (Kurtz *et al*., 1991). The basic method of micropropagation has not been beyond the early laboratory level, however shoot tip culture has been used commercially for many years. The production cost is raised too much by the necessities of narrow-mouth vessels such as flasks, delicate operation by human operators, successive transplantations and so on (Vasil, 1991). Micropropagated transplants are acceptable with growers if the final production price is high as with ornamental foliage plants and flowers, but the unit price of raw sugarcane is low, because it is a crop for processing. Hence, innovative techniques are necessary to reduce the price of the micropropagated sugarcane transplant. The major ones are scaling up the micropropagation, developing a new culture container, improving the *in vitro* environment and automating the processes of micropropagation both *in vitro* and *ex vitro* (Kozai, Ting & Aitken-Christie, 1991). Kondo *et al*. have focused on reducing labor cost and automating micropropagation tasks using robots and a machine vision system (1998). At present, robots are used frequently in the semiconductor industry that requires a high degree of cleanness. In the micropropagation industry, the cleanness of air is also most important and as labor cost is rising worldwide, it is logical to substitute human operators by robots in micropropagation related work.

Previous researches on robot systems for *in vitro* micropropagation are as follows. Alper *et al*. developed a system, which cut the clump of multiple shoots of watermelon into certain amount of clusters and then transplanted successively (1994a, b). This system did not need machine vision and was simple. However the method might cut and damage the plantlet. In a robot system that was developed by Fujita and Kinase (1991), the shoots were previously transplanted singly with spacing. Laser beam scanning device recognized the shape of the shoots, the robot cut the shoots at their nodes and then the exsected plantlets were transplanted to a new growth medium. Okamoto *et al*. (1998) developed a robot system, which automates the successive transplantation of the *in vitro* micropropagated sugarcane. The position of a clump of shoots was recognized by the machine vision and an end-effecter divided it at the middle of the clump and transplanted the clumps to a new medium. A demonstration plant was built in Tokunoshima Island. Wang *et al*. (1999) developed another robot system, which automates the same operation. The machine vision detected the position and orientation of each *in vitro* micropropagated sugarcane shoot and two forceps-like end-effecters divided them singly. The shoots had no roots. To facilitate image processing and separation, the clumps of the shoots were cultured between two plates (Schaufler and Walker, 1994).

Very few researches have been done on automation for micropropagated transplants at the greenhouse or *ex vitro* stage, even though 25-40% of cost involves in this stage (Aitken-Christie, 1991). But in the field of horticulture, robot systems have been developed for transplanting bedding plants (Ting *et al*., 1990), for grafting

vegetable seedlings (Suzuki *et al*., 1995) (Nishiura *et al*., 1998) and for processing geranium cuttings (Simonton, 1990). The techniques developed in these researches can be adapted to the automation of *ex vitro* plantlets.

An objective of this research is to separate the rooted *ex vitro* micropropagated sugarcane into individual shoots. The separated shoots are transplanted to a new cell plug tray. Since the shoots extend their stems three-dimensionary, we did not use machine vision for detecting the position, but instead designed and developed new mechanisms.

Materials and Methods

Sugarcane shoots

For the experimental material, NiF8 was used. This cultivar is most popular in Japan. At the sugar manufacturing company, which provides materials, a shoot tip is propagated into up to 2,500 plantlets by successive culture. After multiplication, the plantlets are put into a rooting medium. Once the plantlets have rooted, they are transplanted into a 50-cell plug tray. At this stage, the plantlets are raised in a greenhouse. After 2 months, the plantlets are extracted from the cell plug tray and separated into the individual shoots. The shoots are classified by size and transplanted into a new cell plug tray. In any tissue-cultured plant, it is necessary to separate individually in some stages for further growth. The plantlets are generally separated in this stage, since the density of plantlets *in vitro* influences the production cost, *ex vitro* plantlets are more uniform than at their earlier stages and there is no need for sterility.

Figure 1 shows an image of one plug of shoots before separation. Raised in the cell plug tray, the roots expand in a cell and form a root ball. Figure 2 shows an image of a separated shoot. The total height of the shoots is approximately 300 mm. The length from the base of the stem to the highest blade joint (1) is defined as the stem length. The diameter of the stem at 10 mm from the base of the stem (2) is defined as the stem diameter, because the base of the stem bulges and does not seem to indicate the degree of growth of the shoots.

Figure 1. A plug of shoots

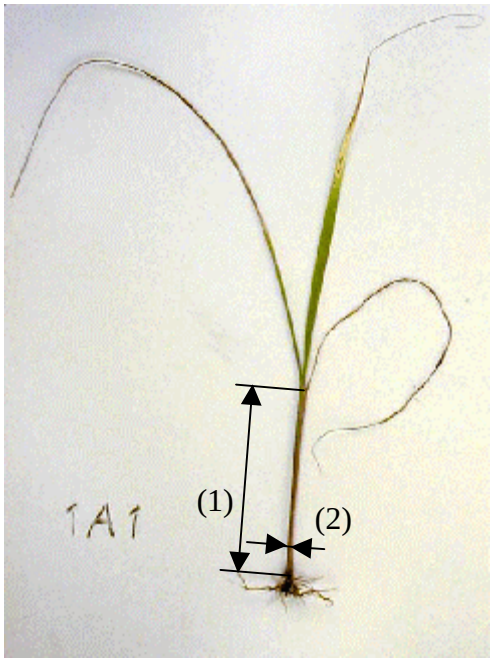

Figure 2. A separated shoot

SCSS (sugarcane separating system)

Figure 3 shows a schematic diagram of the SCSS (sugarcane separating system). This system mainly consisted of a personal computer and two robots. A serial communication board (IBX-4141, Interface Corp.) was installed in an ISA bus slot of the PC to communicate with the robots. A 4-axis stepping motor controller board (PCI-7208, Interface Corp.) was installed in a PCI bus slot. An A/D converter board (PCI-3133, Interface Corp.) was installed in another PCI bus slot. For programming of the PC, C++ (Visual C++ 6.0, Microsoft Corp.) was used.

Two types of robot were used in this system. The first robot was a 4-axis SCARA type robot (SRX-610, SONY Corp.). This robot was in charge of the first half of all processes, picking up the plug of shoots, cutting the root balls, washing off potting compost and feeding the shoots to an intermediate conveyer. The second robot was a 6-axis vertically articulated type robot (RV-E2, Mitsubishi Corp.). This robot was used for the second half of the processes, picking an individual shoot off in cooperation with the intermediate conveyer. Intelligent controllers were connected to each robot and programs in them communicated with the PC via the RS-232C interface to move the manipulators. Figure 4 shows an assembly drawing of the whole system. Figure 5 shows an image of the system.

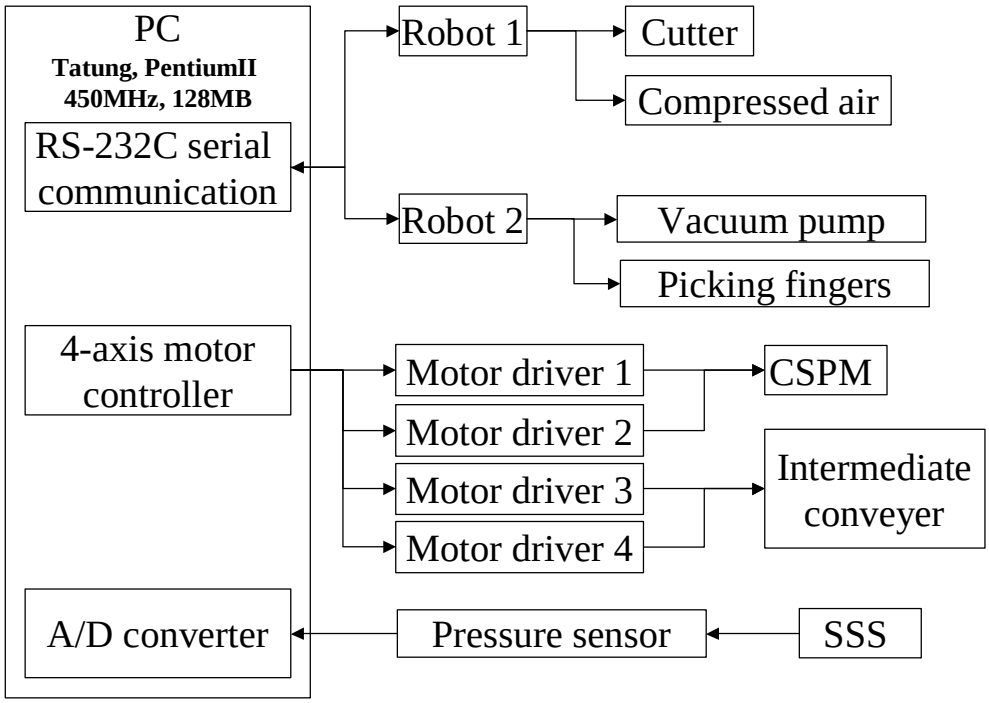

Figure 3. Schematic diagram of sugarcane separating system

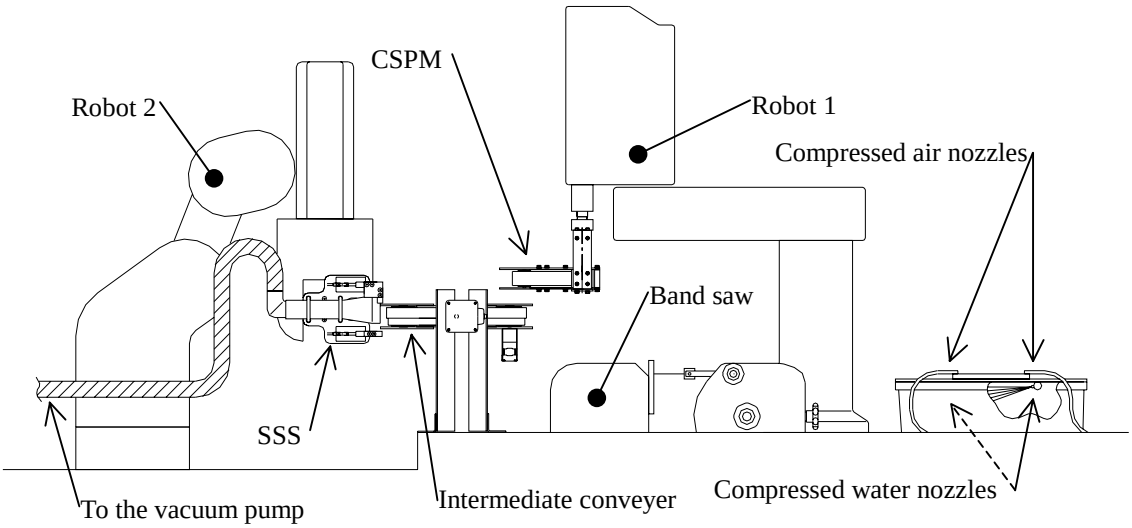

Figure 4. Assembly drawing of sugarcane separating system

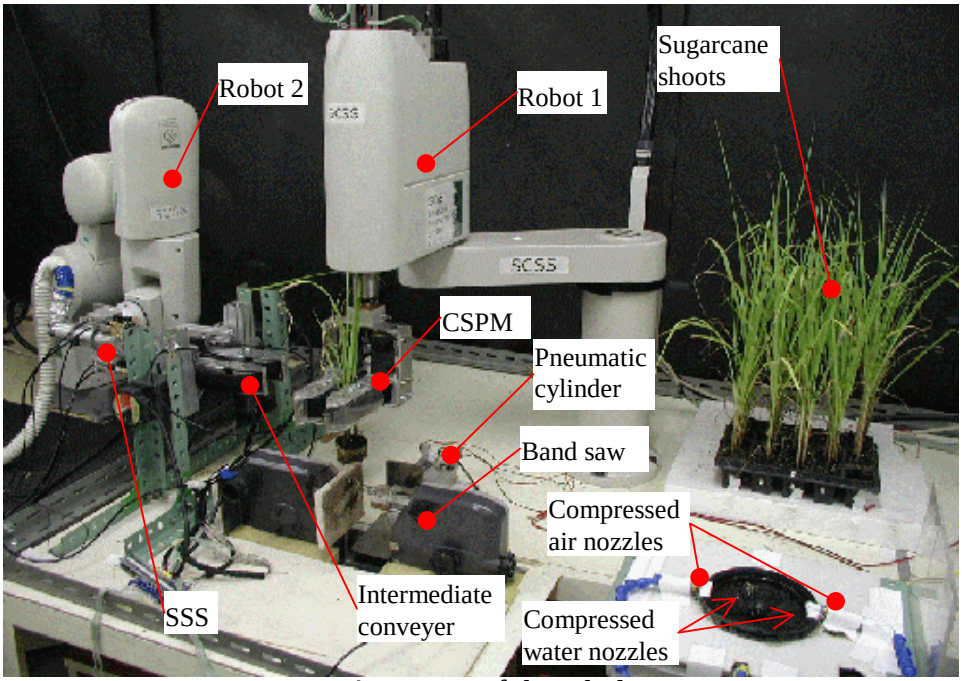

Figure 5. An image of the whole system

Separation procedure

The separation procedure is as follows.

1. Robot 1 picks up the plug of the shoots from the cell plug tray.
2. The band saw cuts the root ball.
3. The compressed water and the compressed air blow off the potting compost and the unwanted thin roots.
4. Robot 1 feeds the shoots to the intermediate conveyer.
5. Robot 2 and the intermediate conveyer separate the shoots cooperatively.

CSPM (Continuous shoot picking mechanism)

The following functions are required for the end-effecter to pick up the shoots from the cell plug tray.

1. Picks up only one plug of shoots at one time
2. Holds the shoots tightly without overlap or damage
3. Let the shoots out from the end-effecter one by one
4. Holds the rest of the shoots

An end-effecter as explained below was developed to fulfill these requirements. The dimensions of each cell were 45 x 45 mm. The shoots grew from nearly the center of each cell. The directions of their elongation were radial but almost vertical. If we use general robot grippers such as a parallel type gripper or an angular type one, the shoots may overlap each other, making separation impossible and in the worst case, the shoot may be damaged. To grip the shoots without overlapping, we developed a CSPM (continuous shoot picking mechanism). The CSPM modeled the movement of human fingertips.

When we grip an object like a baseball bat, our palm is in full contact with the object. When we grip a pencil-like object, we grip it between two fingers. And when we pick and separate long, slender objects like sugarcane shoots, we press the thick part of the thumb against a forefinger and let the object into the Y-shaped gap so formed. Then we grip it by moving the contact point of the fingers (Figure 6). This movement makes it possible to pick up long, slender objects one by one. If we have prior knowledge of the approximate positions of the shoots, by moving the hand forward and repeating this movement, we can pick up scattered shoots in order of distance from the Y-shaped gap between the two fingers without visual information. It is possible to model this movement of the fingers by using a linkage mechanism. However, the fingers can hold only one object at a time. The CSPM was designed to do this movement continuously.

Figure 7 shows an assembly drawing of the CSPM. It consisted of a pair of soft sponge rubber belts and stepping motors. The width of the belt was 20 mm and the thickness was 5 mm, therefore the CSPM could hold the shoots tightly without damage. The stepping motors could control the moving velocity and distance of the belts precisely. The CSPM was attached on the robot 1 as shown in figure 8. When the CSPM picked up the plug of shoots, the CSPM was moving forward and the belts were moving backward synchronously. If the belt velocity is the same as the CSPM velocity, the relative velocity at the shoots becomes 0. Practically the belt velocity must exceed the CSPM to prevent the shoots overlapping between the belts. In the experiment, the belts moved 25% faster than the CSPM. Once the shoots were kept between the belts, their alignment could not be changed. Hence, the CSPM could let out the shoots sequentially after cutting and washing the root ball. The CSPM lifted up the plug of the shoots as shown in Figure 9.

This mechanism is resembled to that of a belt type thresher. The differences between them are first the CSPM moves itself as the belts move, second the velocities of the belts are same and third there is no gap between two belts of the CSPM while the belt type thresher shears crops between gap of two belts, which are moving in different velocities.

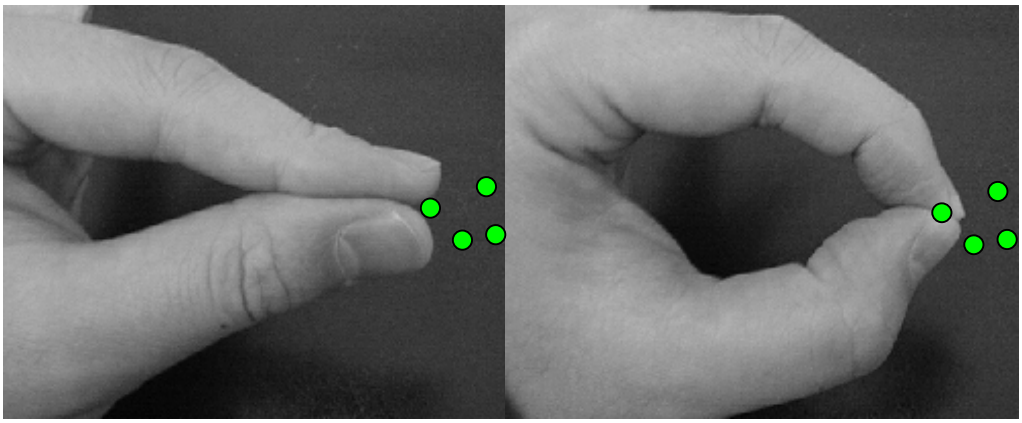

Figure 6. Movement of the human fingers when picking up long, slender objects

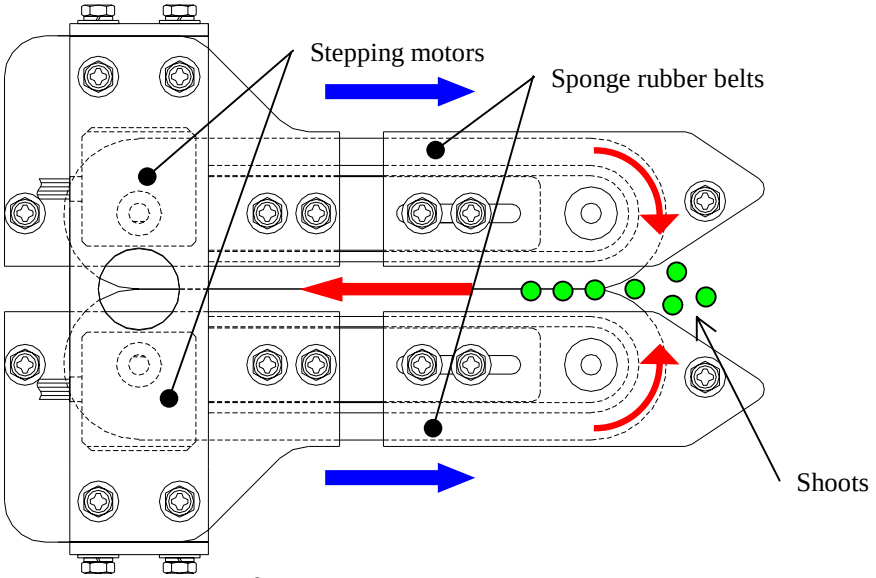

Figure 7. Assembly drawing of the CSPM (continuous shoot picking mechanism)

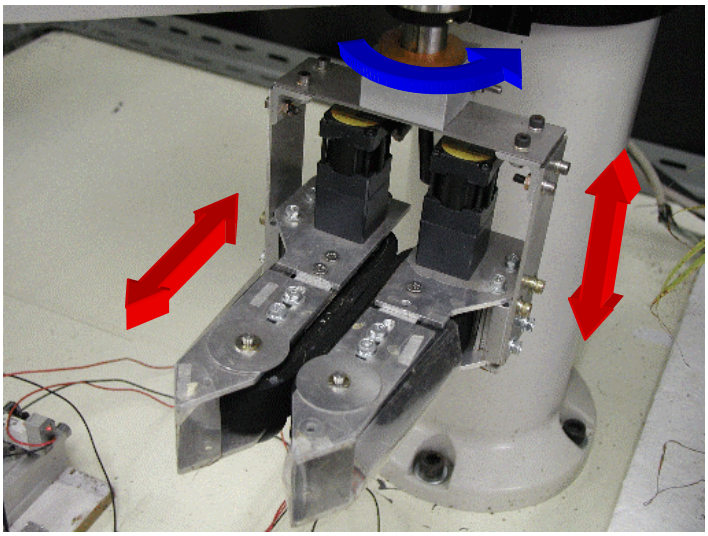

Figure 8. An image of the CSPM

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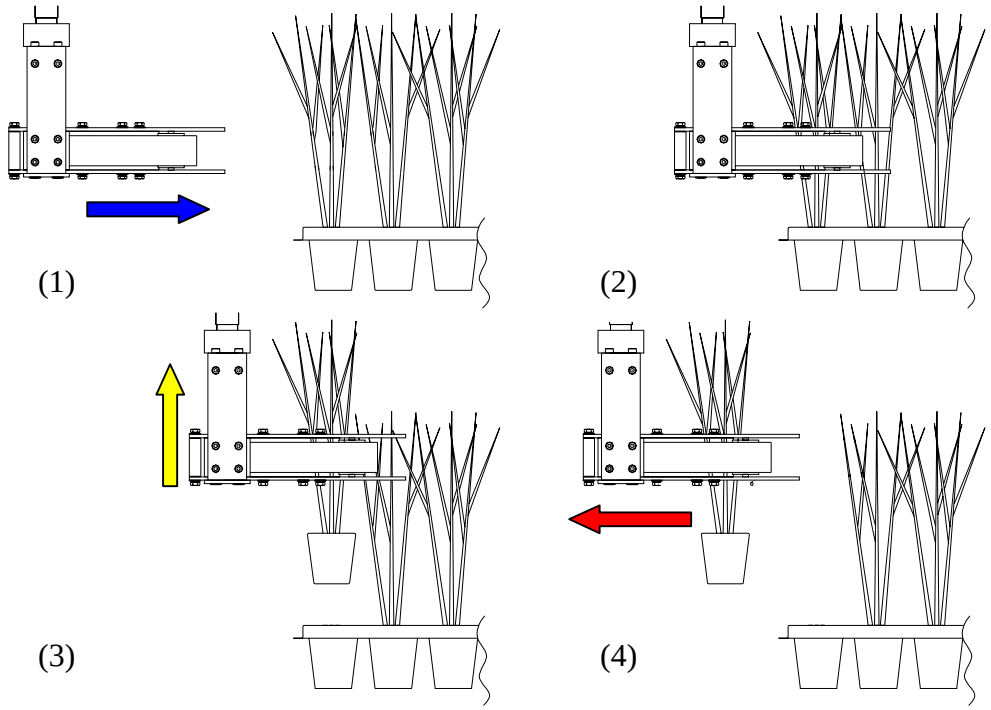

Figure 9. Procedures of lifting up the plug of the shoots

Cutting the root ball and removing the residual potting compost and roots

The sugarcane shoots root in the cell as shown in Figure 1. Their roots spread in the potting compost and become entangled, forming a root ball. The root ball must be cut and the residual potting compost and exsected roots must be removed to prevent breaking their stems during the separation. In the conventional method, the root ball is cut at 20 mm from the bottom by scissors and then the residue is washed off in water by hand. Figure 1 shows a cutting line. In our system, the band saw was used to cut the root ball. A pneumatic cylinder pushed the root ball from behind while the root ball passed through the band saw. The advantage of the band saw to the conventional method are that it is easily automated and it is able to cut fibrous root ball surely. Four nozzles sprayed compressed water onto the root ball to wash off the residue, then the compressed air blew off the water.

Separating multiple shoots into individual shoots

The shoots remain connected together at their roots, even after the root ball has been cut and the residue has been washed off. A separating method was developed as explained below.

1. The CSPM feeds the shoots to the intermediate conveyer.
2. Multiple shoots are brought into a line with no overlap.
3. The end-effecter that is attached to robot 2 waits at the end of the intermediate conveyer.
4. When the front shoot is let out from the end of the intermediate conveyer, the end-effecter sucks a shoot and detects it by drop of air pressure.
5. The end-effecter holds a single shoot by its fingers and the intermediate conveyer holds the rest of the shoots.
6. The end-effecter and the intermediate conveyer move synchronously and execute the separation.
7. Steps 3 to 6 are repeated until no shoots are left.

The CSPM brings the shoots to the intermediate conveyer. Figure 10 shows an

image of the intermediate conveyer. The intermediate conveyer was installed between robot 1 and robot 2 (Figure 4). The basic design of the intermediate conveyer was the same as the CSPM. It consisted of a pair of stepping motors and sponge rubber belts. The intermediate conveyer differed from the CSPM in that it rotated as shown in Figures 13 to 16 and it had pneumatic grippers to hold the shoots. The CSPM and the intermediate conveyer drove the belts in the same way to hand over the shoots. The belt speed of the intermediate conveyer was twice as fast as that of the CSPM to create more spaces between the shoots and to make sure that no shoots were overlapped

The end-effecter that holds a single shoot is called a single shoot separator (SSS). Figure 11 shows an assembly drawing of the SSS and figure 12 shows an image of the SSS. It consisted of an aluminum pipe, pneumatic grippers, an air pressure sensor and a vacuum pump. One end of the pipe was pressed to form a narrow slit to suck only one shoot at a time. The width of the slit was 1 mm, which was suitable for sucking the long, slender shoots. The other end of the pipe was connected to a vacuum pump, which was a household vacuum cleaner in consideration of its large suction volume. The air pressure in a pipe drops at the moment the shoot is sucked by the SSS. The air pressure sensor detects this pressure drop and the PC stops the belt. As a result, only one shoot is sucked by the SSS and the rest of the shoots remain in the intermediate conveyer. As already described, the roots are entangled. So they may be broken if they are pulled in the horizontal direction by force. We learned from experience that the smallest force is needed if the shoot is pulled fanwise. This separation method was adopted so as not to break the shoots. The CSPM and the intermediate conveyer move as explained below.

1. The SSS sucks the front shoot and the air pressure sensor detects it (Figure 13).
2. The SSS and the intermediate conveyer move at the same angular velocity but in opposite directions while keeping the distance between the stem base of the sucked shoot and that of the rest of the shoots constant.
3. The belt of the intermediate conveyer is moved backward to ensure the separation.
4. The fingers of the pneumatic gripper installed above the slit are closed to hold the stem of the single shoot after the SSS rotates 10 degrees.
5. The fingers of the pneumatic gripper installed below the slit are closed to hold the roots of the single shoot, and the rest of the shoots are held by the pneumatic grippers installed on the intermediate conveyer after being rotated 20 degrees from the initial position (Figure 14).
6. The shoot is completely separated after the SSS and the intermediate conveyer rotates 90 degrees (Figure 15, Figure 16).

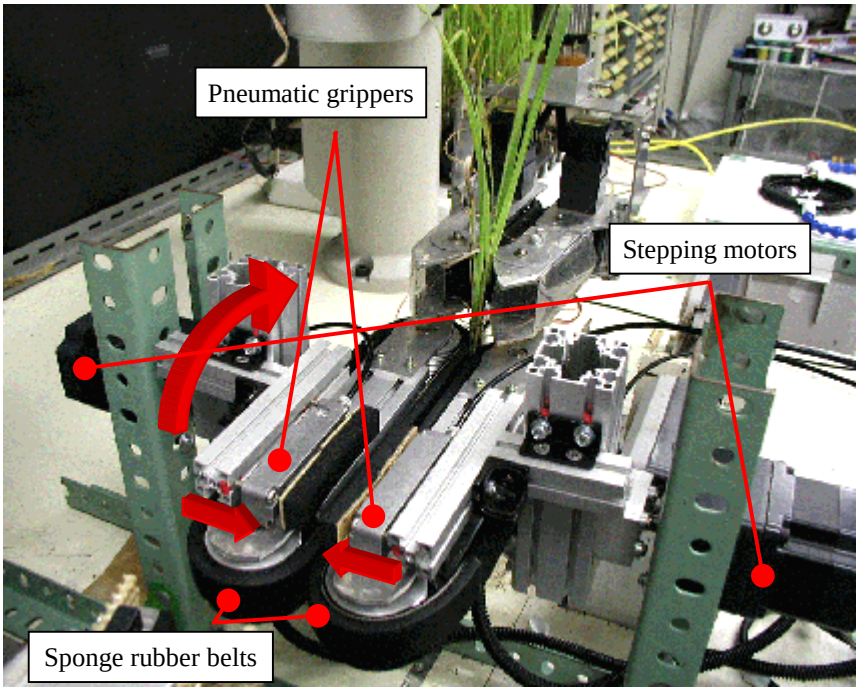

Figure 10. An image of the intermediate conveyer

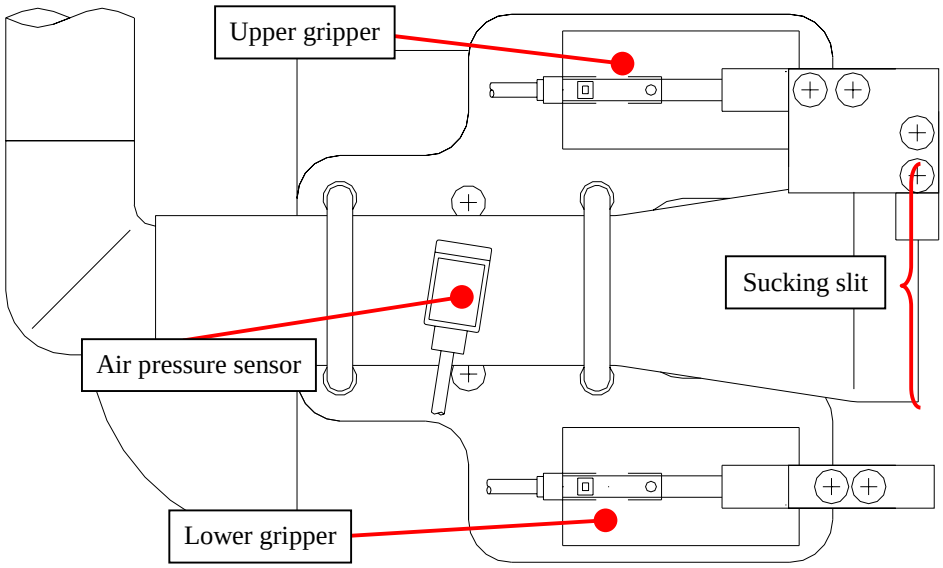

Figure 11. Assembly drawing of the SSS (single shoot separator)

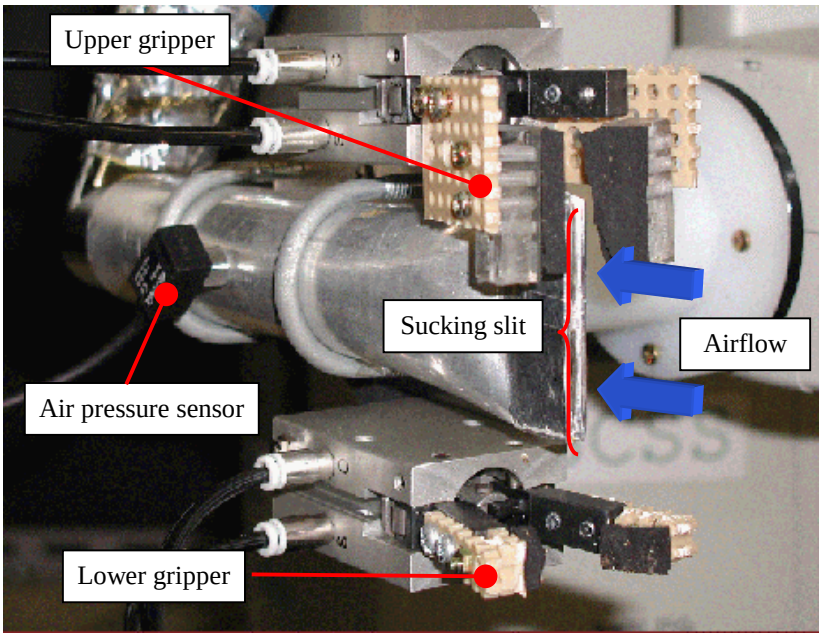

Figure 12. An image of the SSS (single shoot separator)

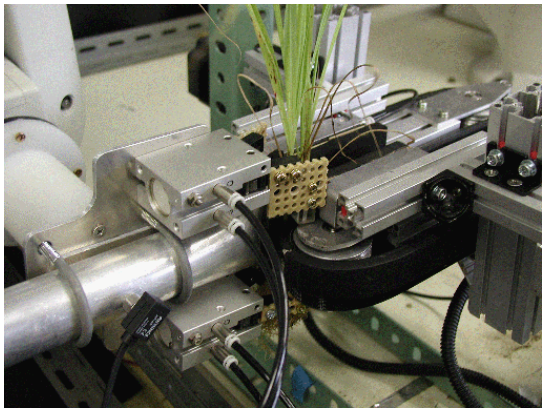

Figure 13. The SSS sucks the front shoot

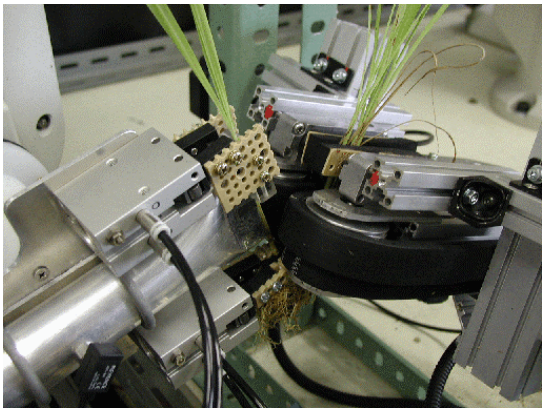

Figure 14. The pneumatic grippers close and then hold the shoots

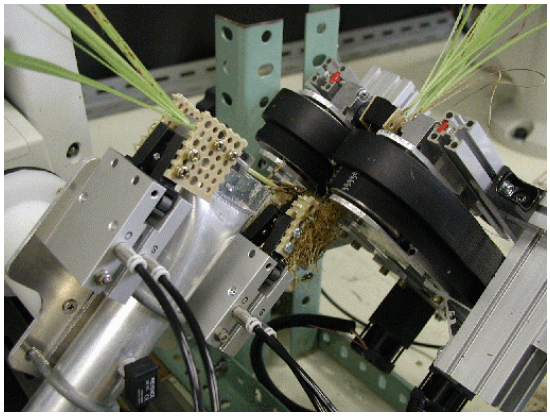

Figure 15. The SSS and the intermediate conveyer rotate synchronously

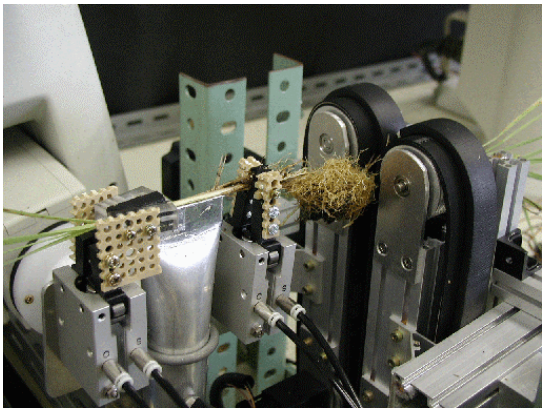

Figure 16. The shoot is completely separated

Experiments

Picking up the plug of shoots by the CSPM

The number of plugs of the shoots that were properly picked up by the CSPM from the cell plug tray was recorded.

Separating the shoots by the SSS

A test was done as explained below to evaluate the capability of the SSS. For the test, the shoots, which had been raised for 2 months since the last transplanting, were used.

1. Put one plug into the cell plug tray.
2. The CSPM cut the root ball and blew off the residue as mentioned above.
3. The SSS separated the shoots. We recorded the number of shoots, which were categorized into four groups: one shoot, two shoots, three or more shoots and a broken shoot.
4. The stem length and stem diameter was measured. Those of broken shoots were not recorded.

Rooting rate of the shoots

The rooting rate of the shoots that were separated by this system was measured to examine the shoot damage caused by the division. Ten plugs of the shoots that were separated by the system and 10 that were separated by hand were transplanted into a new cell plug tray and raised for one month in the open air.

Results and discussions

Materials

Table 1 shows the number of shoots in a cell. Table 2 shows the principal dimensions of the separated shoots.

Table 1 Number of shoots in a cell

	Mean	Maximum	Minimum	SD
Number of shoots (Number/cell)	4.7	9	2	1.9

Table 2 Principal shoot dimensions

	Mean	Maximum	Minimum	SD
Stem length (mm)	82.9	138	25	27.6
Stem diameter (mm)	2.7	5.1	1.2	0.9

Picking up the plug of shoots by the CSPM

The results of the picking up rate are shown in Table 3. The CSPM sometimes involved blades of an adjacent plug of the shoots, and so two plugs were picked up at the same time. Cutting the blades before picking up might solve this problem. A failed plug means that the shoots had not grown well. In this experimental configuration, the CSPM could hold the plug having a total height including the root ball of more than 115 mm. From a practical point of view, the total height of the plugs must be controlled to some extent.

Table 3 Number of picked plugs by CSPM

	Number of plugs	Percentage
1 plug (plug)	33	86.8%
2 plugs (plug)	4	10.5%
Failed (plug)	1	2.6%
Total (plug)	38	100%

Cutting the root ball and removing the residue

The band saw cut the root ball without mistake. In most cases, the compressed water and air washed off the residual potting compost and the exsected roots well. No bruises or cuts were observed on the stems, and very few shoots were broken during the washing. The preliminary experiment showed that the degree of washing greatly influences the success rate of the separation, since the potting compost bonds the roots of the shoots. Two thirds of the processing time, 77 seconds, was consumed for washing and blowing off the residue completely. The flow rate of the water and the air should be enlarged for speeding up these processes.

Separating the shoots by the SSS

Table 4 shows the result of separation by the SSS.

Table 4 Number of separated shoots by SSS

	Number of shoots	Percentage
1 shoot	91	77.1%
2 shoots	8	6.8%
3 or more shoots	9	7.6%
Broken	10	8.5%
Total	118 (25 plugs)	100.0%

Twenty-five plugs, 118 shoots in total, were separated. As a result, 77.1% of the shoots were divided into individual shoots. The reasons why two or more shoots were separated at a time were as follows. First, when the front shoot was too small, the air pressure did not drop under the threshold pressure, and so the belts were not stopped and the next shoot was also sucked at the same time. Second, conversely, when the next shoot was too small, the intermediate conveyer could not keep it well and it followed the front shoot. Third, when the blade of the rear shoot was sucked by the SSS before the front shoot was sucked, both shoots were picked off by the SSS. Fourth, when two shoots were crossed between the belts of the intermediate conveyer, they were also picked off at the same time. The shoots tend to cross each other as the number of shoots in a cell increases. Table 5 shows the number of separated shoots that were 4 or less in a cell. Table 6 shows the number of separated shoots that were 5 or more in a cell.

Table 5 Number of separated shoots by SSS (4 or less shoots in one cell)

	Number of shoots	Percentage
1 shoot	34	89.5%
2 shoots	0	0.0%
3 or more shoots	0	0.0%
Broken	4	10.5%
Total	38 (12 cells)	100.0%

Table 6 Number of separated shoots by SSS (5 or more shoots in one cell)

	Number of shoots	Percentage
1 shoot	57	71.3%
2 shoots	8	10.0%
3 or more shoots	9	11.3%
Broken	6	7.5%
Total	80 (13 cells)	100.0%

When the number of shoots in one cell was 4 or less, separation of two or more shoots did not occur. On the other hand, when the number of shoots was 5 or more, 21.3% of the shoots were picked off with two or more shoots connected. Therefore it is desirable to transplant 4 or less micropropagated shoots into the cell to achieve a high separation rate.

The reasons why the shoots were broken during the separation were as follows. First, when the water stream struck the root ball, the shoot was broken, but this was very rare. Second, when the SSS and the intermediate conveyer divided the multiple shoots, bending of the stems broke some shoots.

We are going to use more samples to know the performance of the system well in the future.

Operating time

It took 2 minutes for picking up, cutting and washing a plug of shoots, the most of which time was consumed for washing as already explained. It took 30 seconds to divide one shoot. But for the following reasons, it sometimes took more than 3 minutes for the division. The first was that some shoots have browned blades. When the SSS sucked the browned blade, the SSS misunderstood it to be a normal shoot and detached it in the usual way, because the SSS detects the contact of the shoot solely by the air pressure. Some optical sensors should be adopted to distinguish browned blades in the future. The second was that when the shoots were crossed or were attached firmly at their bottom, if the SSS once sucked one shoot, it would be detached from the SSS by the backward movement of the intermediate conveyer belts. In this case, the SSS and intermediate conveyer had to repeat the procedures. To solve this problem, the flow rate of the vacuum cleaner needs to be enlarged.

Rooting rate of the shoots

The rooting rate of the shoots separated by hand and by SCSS is shown in Table 7. Removing the browned leaves and bending the stem during the separation were thought to be reasons that the rooting rate of the shoots separated by the SSS was lower than that of shoots separated by hand. Some browned leaves have vital sheaths. If the SSS picked these leaves, their sheaths were also picked off. Peeling the vital sheaths spoils the shoot growth. We must improve the separation method not to damage the shoots for the practical use.

Table 7. Rooting rate of shoots separated by hand and by SCSS

	By hand	By SCSS
Rooted (shoots)	72.1% (44)	66.7% (36)
Unrooted	27.9% (17)	33.3% (18)
Total	100.0% (61)	100.0% (54)

Conclusions

A new robot system that automatically separates a clump of *ex vitro* micropropagated sugarcane into individual shoots without using a machine vision system was developed and tested. The following conclusions were drawn from the results.

1. The CSPM (continuous shoot picking mechanism) that was attached to the SCARA type robot picked up the plug of thickly grown shoot clump from the cell plug tray without overlapping or damaging them. The success rate of picking up was 86.8%.
2. The band saw was capable of cutting the root ball at the setup height. The compressed water and the compressed air were able to efficiently remove the potting compost and the unwanted thin roots.
3. The SSS (single shoot separator) that was attached to robot 2 and the intermediate conveyer were synchronously controlled, separated multiple shoots into individual shoots in collaboration without using a machine vision system.
4. 77.1% of the shoots were properly separated into individual shoots. The number of shoots in a cell influenced the success rate.
5. Rooting rate of shoots that were separated by the SCSS was 66.7%. Though the rooting rate of the shoots that were separated by the hand was 72.1%. The separation process damaged the shoots. We should improve the separation method for the practical use.
6. The new techniques that were developed in this research are useful for the separation of *ex vitro* rooted micropropagated sugarcane shoots. Future development and evaluation are required to enhance end-effecters, improve separating method, shorten operation time and improve the reliability of the whole system.

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