Development of a Prototype Automated Cutting and Placing System for Tissue Culture Multiplication

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A prototype cutting and placing device has been developed at the New Zealand Institute for Crop & Food Research Ltd and the Agricultural Engineering Institute. *It is not intended to be a complete tissue culture system but* it may be able to be integrated into existing laboratory procedures. The workstation comprises a vision system, a robotic arm incorporating the cutting device, and computer control hardware. It is mobile and can be situated adjacent to a laminar flow cabinet enabling the robotic arm to move into the cabinet to operate. The robotic hand has been designed for the compact multi-meristematic form of plantlet growth in conjunction with a range of conventional plastic tissue culture containers. Preliminary tests have shown good growth of explants subsequent to cutting and placing with the robot, and 0.9% contamination compared with 1.7% when cut and placed manually.

INTRODUCTION

Micropropagation is a labour-intensive method of producing plants and a large proportion of the total cost can be labour. A number of automated tissue culture systems have been developed but few are currently in use commercially (Aitken-Christie, 1991). There have been some sophisticated systems developed, but economic feasibility has not often been a consideration (Kurata, 1992). We perceived a need for a machine that was inexpensive, mobile, and would fit into a conventional laminar flow hood. The machine developed here was not intended to be a complete tissue culture system, but one that would be able to be easily integrated into an existing laboratory. The programme was funded under the Foundation for Research Science Technology Priority Research Contracts Scheme and work was jointly performed by Crop & Food Research and the Agricultural Engineering Institute. It focused on the cutting and handling aspects of micropropagated plantlets. The associated tasks of handling containers was not part of this programme, as there are a number of conveyor belt systems available that have been designed to move containers and these could be adapted if necessary. This programme targeted the compact multimeristematic form of plant growth in tissue culture as it is the simplest to automate.

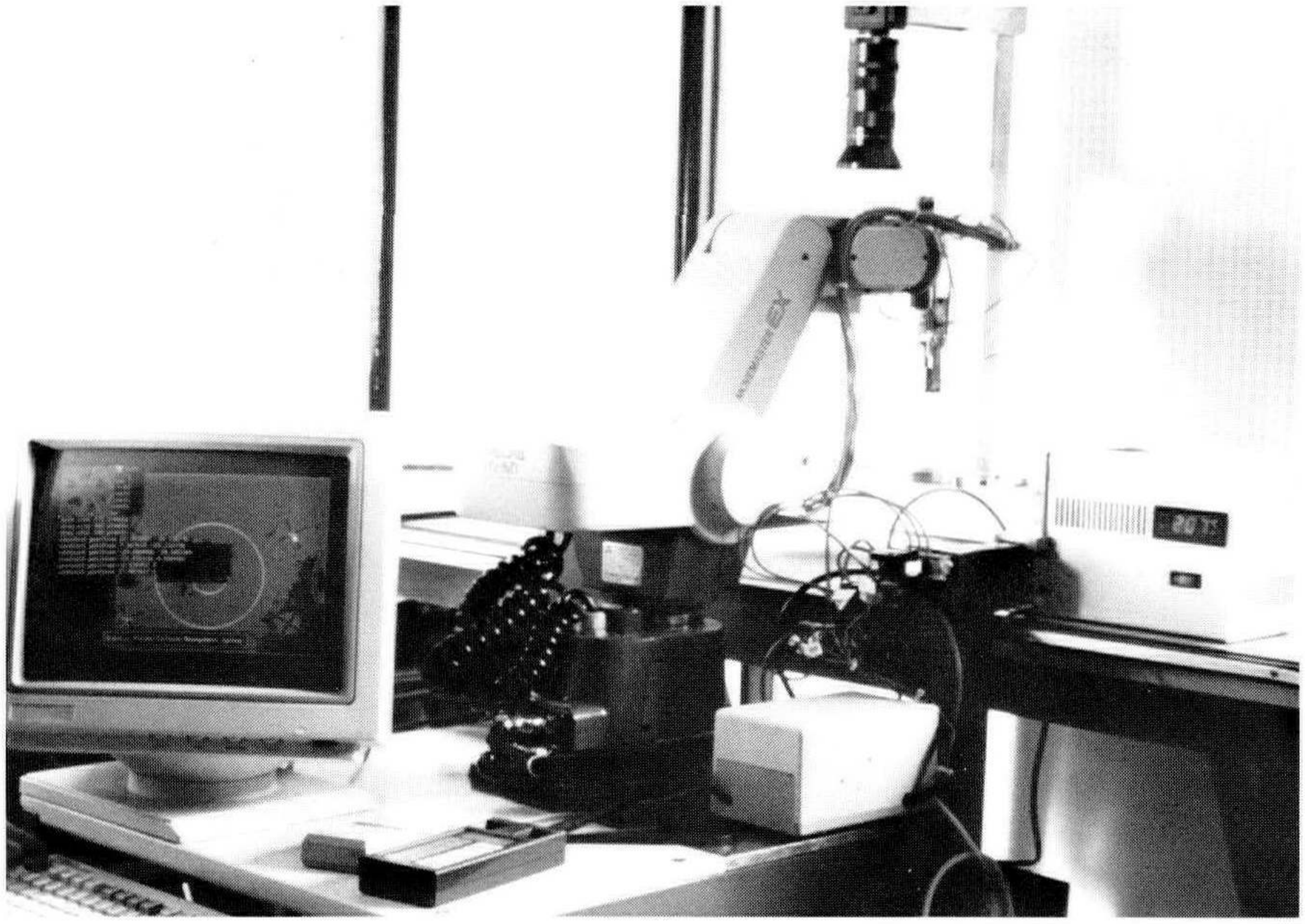

Figure 1. Prototype robot situated in laminar flow hood.

THE ROBOTIC SYSTEM

The workstation consists of the robot and a robotic hand, a vision system, a computer, and software written for the operation of our machine (Fig. 1)(Kerr et.al,1992). It is mounted on a trolley, adjacent to the laminar flow hood, so that it is mobile and can be easily moved. For pragmatic reasons, it was decided to buy an off-the-shelf Mitsubushi RVM1 vertical articulated robot with 5 degrees of freedom, operated by the robot controller or hand held teaching box. The load capacity is 1.2 kg, accuracy ± 0.3 mm, maximum speed 1 m/sec. The software directs the robot, issuing speed and position information. Coordinates are defined externally for fixed locations such as the source container location, planting positions, and sterilizer. Plantlet locations, identified by the vision system, are communicated to the robot by the software.

The computer is an IBM compatible 386 SX PC, with i/o cards to operate the pneumatic system. The software coordinates and controls all the other components of the system. The testbed software is implemented with Microsoft C, and Cscape screen libraries. The system has pull down menus, editing screens for system parameters, and scripts for sequencing actions. The scripts provide the flexibility to change sequences and timing without making coding changes. Two methods for invoking scripts are implemented — single pass and repeated. Single pass scripts allow the setting up of sequences for initialising the apparatus and testing components. The repeated scripts start from the beginning and, after reaching the end, return to the beginning allowing for production sequences.

The imaging system employs a Matrox IP-8/AT video graphics system board connected to a CCD camera, providing 256 grey scale images through a Fujinon 12.5 to 75 mm zoom lens. Diffuse back lighting was employed to obtain good

Figure 2. Robotic hand shown here cutting *Eucalyptus camaldulensis*.

contrast of the plant material compared with the background. An image is obtained from the vision routine and thresholded to produce a binary image. The threshold used is predetermined, and adjustment made to the image through the aperture and focus setting. Radial scan lines are traced from the edge to the centre of the petri dish at specified intervals, to contain each plantlet within a sector. Each resultant sector is scanned, and the pixels, representing plant material, are averaged to determine the centroid.

The hand is an integrated device, which cuts, transports, and places the explants into fresh media (Fig. 2). The three-bladed cutter is directed to the centroid and presses down onto the plantlet (still in its original container) to divide it into three explants. These are each held by a needle and a finger. The hand moves across to the new container and the explants are deposited in sequence evenly spaced on a 50 mm pitch diameter. Explant release is effected by a pusher which presses the explant into the media. This sequence repeats filling the preset positions in the container. Fresh containers of plantlets and new media are requested when required. The hand is pneumatically operated and constructed from stainless steel, gauge plate and has plastic (PEEK) pushers.

A wash and sterilization sequence, to sterilize the cutting hand, can be scheduled as required. Currently this occurs before a source container of plantlets is processed. The cutter is first immersed in ethanol that is agitated by an air sparge to dislodge pieces of plant material and media. Then the robot traverses to the hot bead sterilizer where the cutter is sterilized at 250°C for 20 sec. A pulsing sequence after each operation shakes off excess ethanol and glass beads.

PROGRESS TO DATE

We have used three species, *Asparagus officinalis, Eucalyptus camaldulensis*, and a *Zantedeschia* hybrid (calla lily), in our trials. Their growth form was manipulated by changes in media components to achieve the compact multimerisematic form for the multiplication phase (Grant et al., 1992). The containers we routinely use are clear plastic petri dishes (90 mm diameter × 14 mm deep) and tubs (95 × 60 mm), but the robot will operate in other containers when parameters such as explant position, planting depth, and container dimensions are changed. The plantlet layout within the container is set at six plantlets equidistant on the circumference of a 50-mm diameter circle. However, this layout was designed to be flexible and can be changed by the user.

The system is operational and initial tests have been conducted. So far, after the production of 1,400 explants by the robot, there has been 0.9% rate of contamination using the robot which is less than the manual rate at 1.7% for the same number produced. The operational rate of the robot is approximately 18 sec per explant, which is approximately 180 new explants produced per hour. With parallel processing of the imaging while the robot operates, time taken to yield one explant should reduce to approximately 14 sec. The robot at the moment only operates at about 30% to 40% of full speed. Speed will be increased at a later date when current trials have finished. A pilot study to compare growth, health, and contamination rates of robot-cut plantlets versus manual-cut plantlets over four subculture cycles is underway. Growth is being assessed twice weekly using image analysis, and quantitative data will be collected.

A three-bladed cutter was chosen because a 3-fold multiplication rate was an average rate in a 2 to 3 week subculture cycle. When a species has a fast growth rate, for example *Zantedeschia*, subculture is at 2-week intervals. *Asparagus officinalis* and *E. camaldulensis* are subcultured at 3-week intervals. Some investigation is needed to optimise length of subculture period. We planned to have interchangeable or removable cutter heads to allow for differing plant types, varying growth rates, and to enable sharpening. However, our prototype has operated for 4 months with the original stainless 3-bladed cutter. When the cutter was tested on a rhododendron cultivar 'Surrey Heath' and a *Rubus* hybrid 'Kotata', it initially cut the material, but in later trials it was unsuccessful. Improvements to the cutting blades would be needed to deal successfully with woodier species. Laser cutting has been successfully used in several systems (Holdgate, 1992) and could be an improvement, solving the problem of cutting woody species.

With regard to machine vision, the present radial lines system cannot distinguish between overlapping plantlets and, as a result, a group of such plantlets are treated as one. The number of explants per container was set at six due to the limitations of the vision system; however, we would anticipate putting many more explants per container in a commercial operation with an improved vision system. A new image processing programme is now available and should be substituted to distinguish effectively the original individual clumps when overlapping occurs. Other improvements such as automatic thresholding of the image to maintain image quality could also be incorporated.

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