Facility for Growing Plants in Test Tubes at ICRISAT

Information Bulletin no. 18

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

Abstract

Rupela, O. P., Dart, P. J., Toomsan, B., Singh, D.V., Subramaniam, D. , and Sharma, B. K. 1984. *Facility for growing plants in test tubes at ICRISAT.* Information Bulletin no. 18. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Authentication of *Rhizobium* strains and the serial dilution-plant infection technique for determining *Rhizobium* population require the growing of plants under sterile conditions. ICRISAT staff have designed and made units for growing plants in test tubes using materials locally available in India. Two plant growth units are housed in a room cooled with two window air-conditioners. Each unit can accomodate 1824 test tubes of 25 x 200 mm size or 2448 test tubes of 18 x 150 mm size and are laterally illuminated by flourescent tubes. The same units can also be used for growing plants in small pots with illumination from above. At ICRISAT these units have been successfully used for 7 years. Details of construction, operation, and costs of the units are presented.

Résumé

Rupela, O. P., Dart, P. J., Toomsan, B., Singh, D.V., Subramaniam, D., et Sharma, B.K. 1984. (*Moyen efficace de culture des plantes dans des éprouvettes mis au point par l'ICRISAT*). Facility for growing plants in test tubes at ICRISAT. Information Bulletin no.18. Patancheru, A.P. 502 324, Inde : International Crops Research Institute for the Semi-Arid Tropics.

La vérification des souches de *Rhizobium* et la technique d'infection des plantes par la dilution en série afin de déterminer la population de *Rhizobium* exigent des conditions stériles pour la culture des plantes. Le personnel scientifique et technique de l'ICRISAT ont mis au point des unités pour cultiver des plantes dans des éprouvettes en utilisant du matériel localement disponible en Inde. Deux de ces unités sont placées dans une chambre qui est refroidie par deux climatiseurs. Chaque unité peut contenir 1824 éprouvettes de 25 x 200 mm ou 2488 éprouvettes de 18 x 150 mm qui sont illuminées latéralement par tubes fluorescents. Ces unités permettent également de cultiver des plantes dans des petits pots illuminés d'en haut. A l'ICRISAT ces unités sont utilisées avec succès depuis sept ans. Les données sur la construction, l'opération, et les coûts des unités sont présentées en détail.

Correct citation: Rupela, O.P., Dart, P.J., Toomsan, B., Singh, D.V., Subramaniam, D., and Sharma, B.K. 1984. Facility for growing plants in test tubes at ICRISAT. Information Bulletin no. 18. Patancheru, A.P. 502324, India: International Crops Research Institute for the Semi-Arid Tropics.

Facility for Growing Plants in Test Tubes at ICRISAT

O.P. Rupela, P.J. Dart, B. Toomsan,

D.V. Singh, D. Subramaniam, and B.K. Sharma

ICRISAT
Information Bulletin no. 18

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS
Patancheru P.O., Andhra Pradesh 502324, India

1984

Contents

Introduction

Soil microbiologists make considerable use of plants grown in test tubes. Plants can be grown axenically (i.e., in the absence of other organisms) by the process that involves sterilizing the root medium, surface-sterilizing the seeds, and raising the plants from these seeds in test tubes to prevent contamination from unwanted microorganisms. Tubed plants are widely used in the authentication of *Rhizobium* cultures where nodule formation indicates the presence of *Rhizobium* in the inoculum. The numbers of *Rhizobium* in soils or inoculants are also estimated by a serial dilution-plant infection technique using plants grown in test tubes.

However, the enclosing of plants within tubes, which is necessary for microbiological control, poses the problem of how to regulate the temperature inside the tubes when placed in natural light conditions and, hence, of how to create a controlled environment. Chambers for this purpose are commercially available but they are expensive and not locally purchasable in India. This bulletin describes the "Plant growth unit" designed and made at ICRISAT Center to grow tubed plants routinely for experimental purposes (Fig.1), and provides information about its construction and operation that may guide others who wish to install such a facility elsewhere. At ICRISAT two plant growth units have been used since 1978 mainly for growing chickpea, pigeonpea, and siratro plants.

Description of the plant growth unit

The unit consists of six metal pipe frames placed one above the other (a to f in Fig.2) separated by four pieces of metal pipe at each of the four corners. For operational convenience the four lower frames (or shelves) on which the wooden racks carrying plant tubes are placed, are fitted with wire mesh. The distance between any two shelves is determined by the height of the metal pipes at the four corners.

Above each shelf is suspended a movable metal frame of slotted angle iron containing 14 fluorescent tubes in 7 vertical pairs, spaced to allow wooden racks holding the plant tubes to stand between the pairs. The fifth frame from the bottom (e) supports the topmost slotted angle frame and the top frame (f) supports two 30-cm diameter exhaust fans (1420 rev/min).

We have based our plant growth unit on 150-cm (5-ft)65/80 W cool daylight tubes; but 120-cm (4-ft) 40 W cool daylight tubes can also be used. The slotted angle frames are suspended on nylon ropes through pulleys such that they can be raised or lowered individually during the placement and removal of wooden racks.

After fabrication in the workshop the metal pipe frames were installed initially in a room measuring 4 x 3 x 3 m. We now have two plant growth units in two rooms at different ambient temperatures. The walls of the rooms are insulated with expanded polystyrene (thermocole) sheets.

The wiring diagram of the electrical supply is shown in Figure 3, and of a single fluorescent tube in Figure 4. The ballasts (chokes) generate considerable heat and are mounted externally (Fig. 5). They are connected to the plant growth units by cables enclosed in flexible conduits.

Light and temperature

The lights are controlled by automatic time clocks (one per plant growth unit), though each movable frame with fluorescent lights has a manual on-off switch. The lights are kept on for 16 h per day. To reduce the heat load, the times of operation of each unit are staggered, so that at least one unit is always on. If both plant growth units in the room are on for more than 4-6 h continuously, the ambient temperature rises above 25°C, particularly in summer (the dry season). For this reason it is not practical to maintain strict plant photoperiodic diurnal cycles. The exhaust fans on the top of the plant growth unit blow cool air downwards over the fluorescent tubes.

The temperature in the room is controlled by

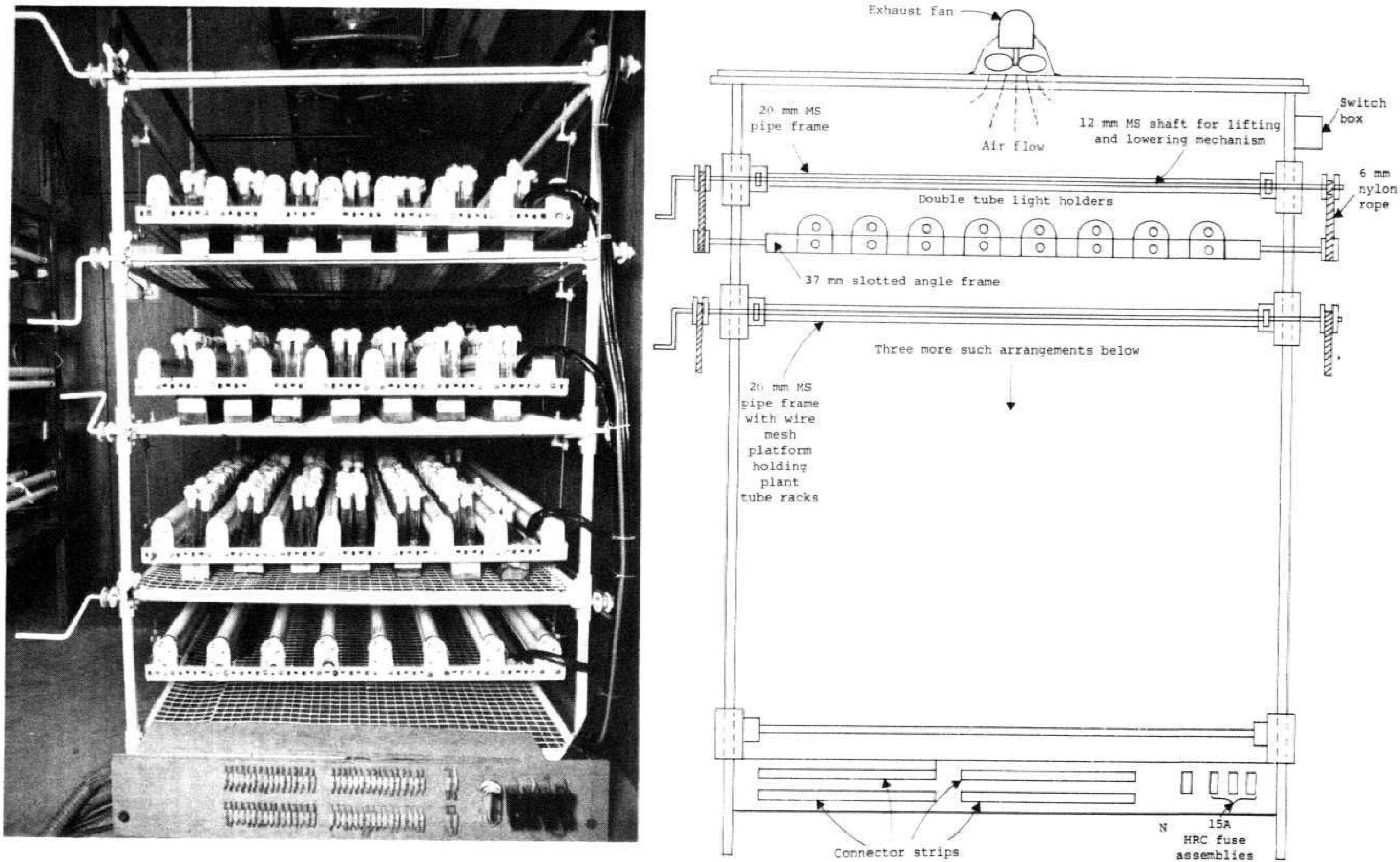

Fig. 1. Photograph and explanatory diagram of the ICRISAT plant growth unit.

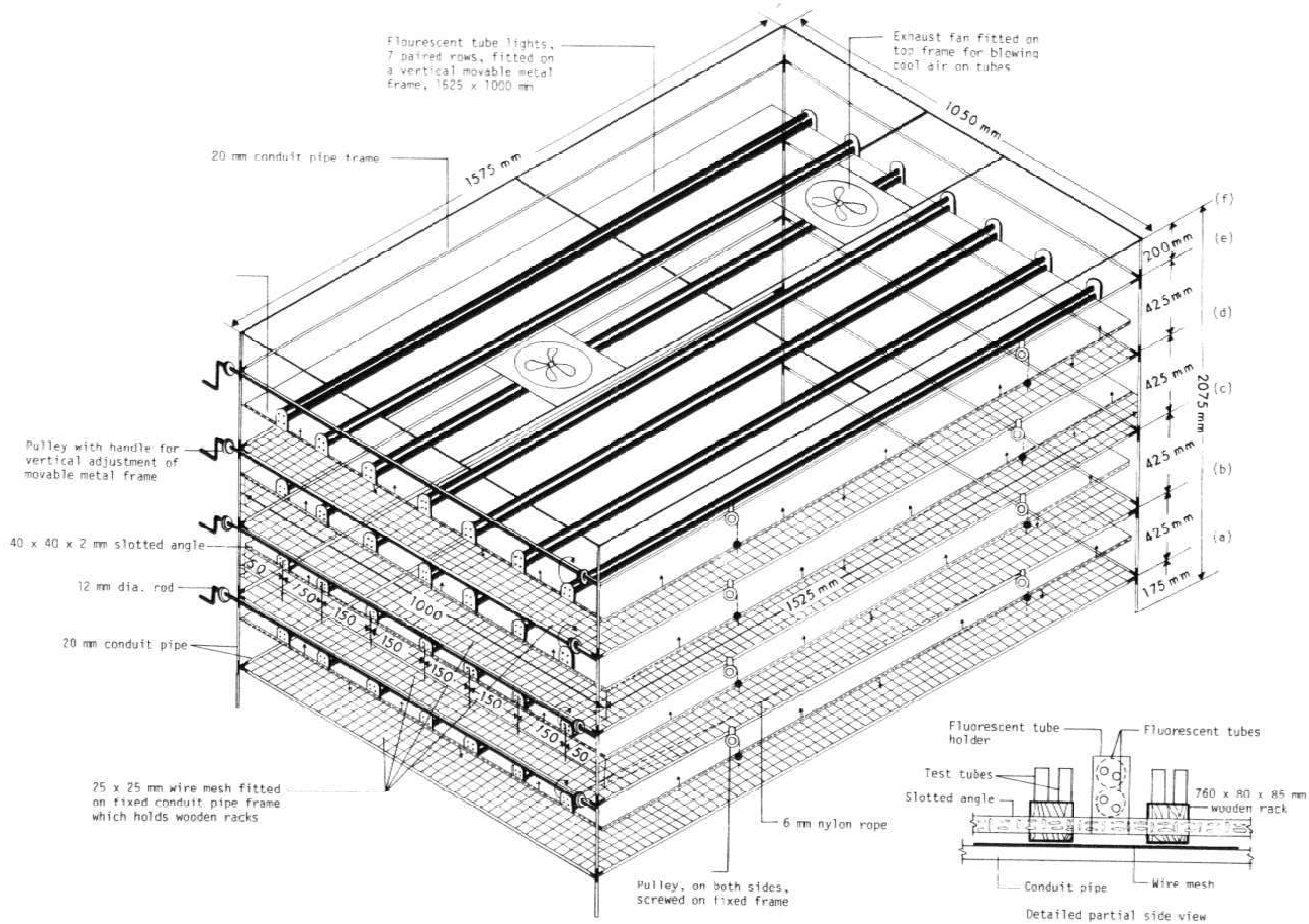

Fig. 2. Isometric view of the ICRISAT plant growth unit.

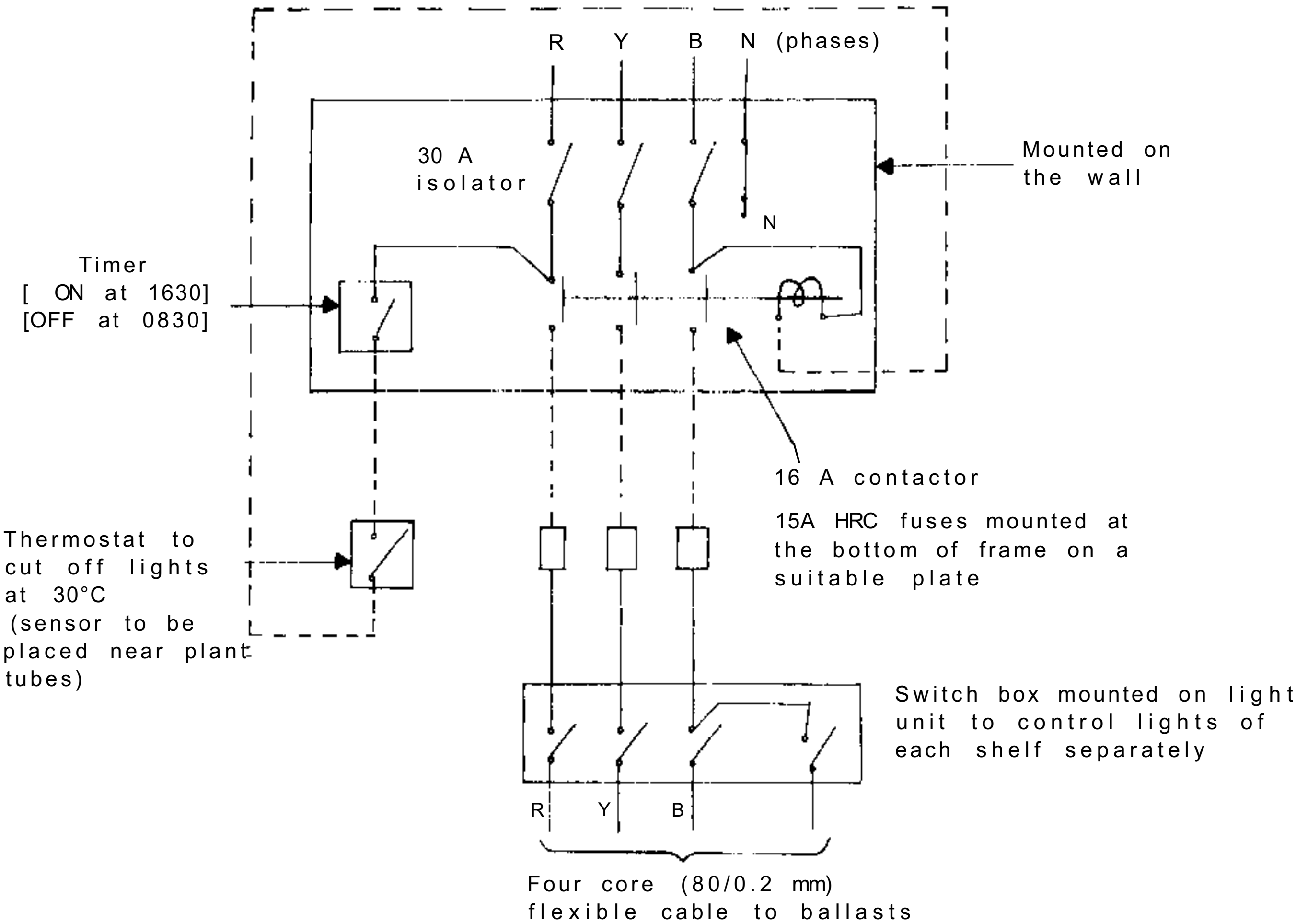

Fig. 3. Electrical supply switching arrangement.

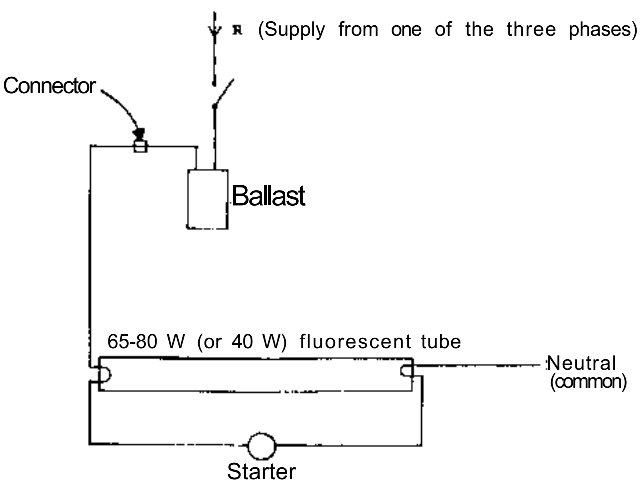

Fig. 4. Schemetic wiring diagram for a fluorescent tube.

two window air-conditioners, both of 2-tonne capacity, installed 215 cm above the floor. The temperature sensor of the thermostat, which is usually inside the air-conditioner, has been removed and placed near the plant growth unit. This has provided better control of room temperature. Normally the temperature inside the room is maintained at 20±2°C for growing temperate plants, such as chickpea (*Cicer arietinum*) and *Vicia sativa.* We use a similar separate room at 25±2°C for such tropical species as siratro (*Macropiilium atropurpureum*), pigeonpea (*Cajanus cajan*), mung bean (*Vigna radiata*), and soybean (*Glycine max*). Both the air-conditioners are operated to provide the required temperature throughout the year.

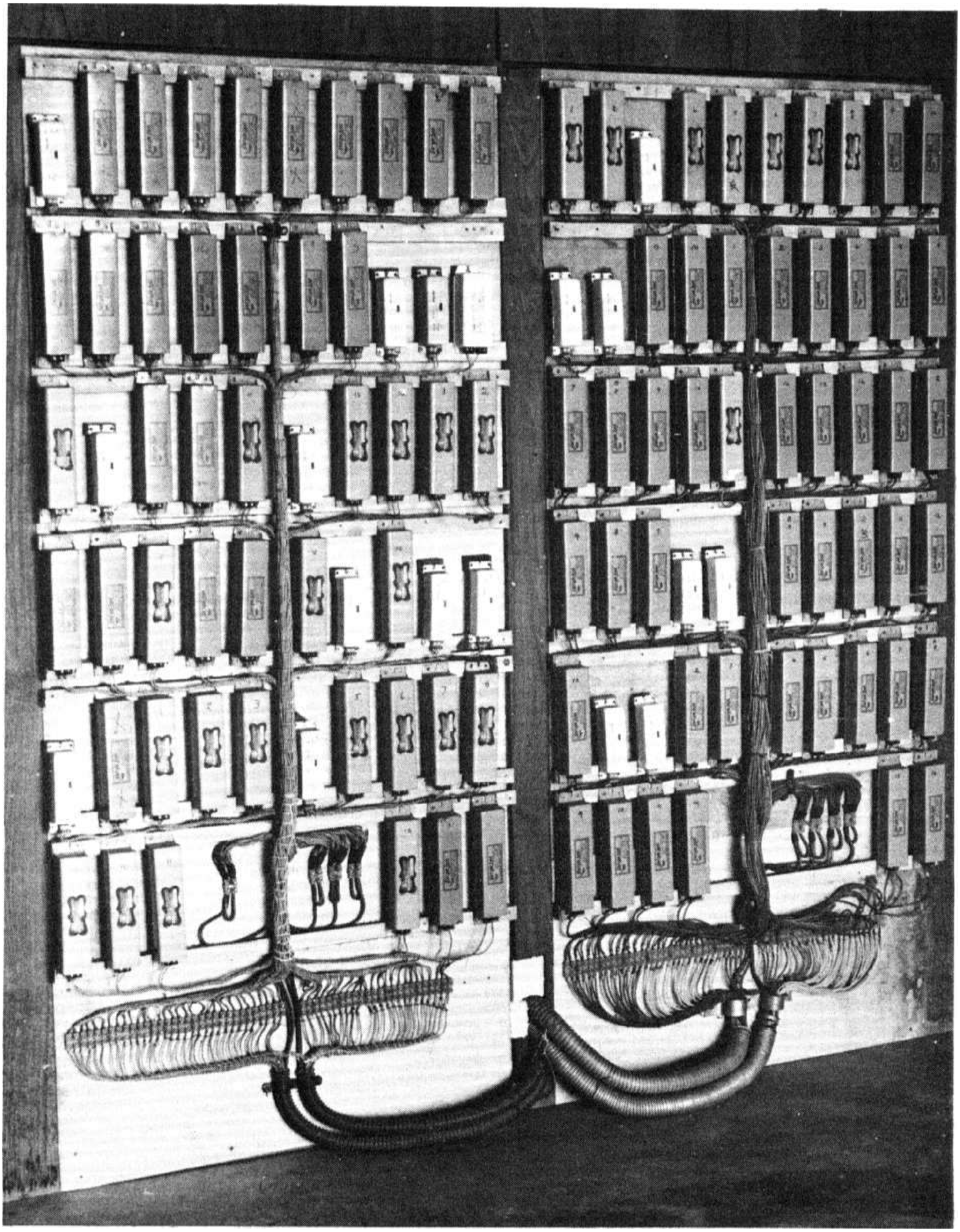

Fig. 5. External ballast boards.

The mean max/min temperatures at ICRISAT Center are about 27/14°C in winter (the postrainy season, November-January) and 37/24°C in summer (April-June). In summer, with day ambient temperature ranging between 35 and 43°C, we find it difficult to maintain a room temperature of 20±2°C for chickpea. In this case we reduce the heat load by disconnecting one of each pair of fluorescent tubes. This does not affect the nodulation of chickpea, pigeonpea, siratro, *Trifolium* spp, *Vicia* spp, or *Medicago* spp. We disconnect the power supply to one of the two plant growth units, and hence the heat load, when one of the two air-conditioners is not working. Each plant growth unit can accommodate 1824 plant tubes of 25 x 200 mm size or 2448 plant tubes of 18 x 150 mm size.

In addition to the thermostats of the air-conditioners there is another safety thermostat (differential type) which is set to switch the lights off when the temperature near the test tubes rises above the desired temperature of 25°C for temperate plants, and 32 °C for tropical plants. Lights are switched on again when the temperature drops. The differential of the thermostat gives a range of 0-10°C between on-off switchings.

Adequate ventilation for the quick dissipation of heat is an important feature of the facility. The shelves supporting the plant tube racks should therefore be of open wire mesh, as shown in Figures 1 and 2. One can use wire racks instead of wooden racks, but exposure of roots to light can affect nodulation. The temperature 1-2 cm below the surface of the plant growth medium inside the plant tubes is always about 5°C higher than the air temperature in the room. The root temperature inside the plant tubes also varies with its position on the shelf in the plant growth unit by up to 2°C.

Light intensity

The average illumination and photosynthetic photon flux density at the surface of a plant tube was 9600 lux and 350 μE $m^{-2}s^{-1}$ respectively when measured with a Li-COR meter (Model LI-185 A) equipped with photometric and quantum sensors. The reduction in light achieved by removing one of each pair of fluorescent tubes, as mentioned earlier, causes about a 50% reduction in these measurements. Dust on the fluorescent tubes can reduce the measured radiation levels by up to 24%.

A major advantage of these units is the flexibility they offer for the lateral or top illumination of tubes. We can use the same facility, for example, for growing chickpea plants in pots, plastic pouches or in test tubes. For capped or plugged plant tubes, lateral illumination is desirable. Also, the height of fluorescent tubes can be adjusted to the height of plants growing in the test tubes.

Components and cost of construction

Table 1 lists the items and their quantities required for making one plant growth unit. All the items used are available locally in India. At mid-1983 prices one unit costs about Rs.1,82,000 (approximately US$ 18,200) to make and install.

For successful operation of any laboratory equipment a dependable power supply is required. Table 2 therefore indicates that about 78% of the component costs are incurred for the air-conditioning plus the generator that is essential for providing a dependable power supply. The facility would obviously be cheaper if an emergency power supply is already installed in the building. Table 2 also gives the costs involved in setting up a suggested smaller facility. We have successfully used a smaller facility in the past for growing pigeonpea and siratro. This had three instead of four floors and used 120-cm (4-ft) fluorescent tubes. The size of such a facility can obviously be scaled down even further, if required, although the relative cost per unit of usable area would increase. The ICRISAT facility described in Table 2 is most cost effective, but any choice concerning size has, of course, to be

Table 1. List of materials required for one plant growth unit.

Qty	Unit	Material	Suggested source in India
10	100 m coils	Single core 1.5 mm^2 copper wire	Finolex, Radiant Cables
30	meter	80/0.2 mm 4-core flexible cable	Finolex
30	meter	37 mm (1.5 in) flexible conduit	Any best quality available
20	meter	25 mm (1 in) PVC sleeves	Any best quality available
56	no.	Single tube light holders	Philips, Crompton, GEC, Bajaj
56	no.	80 W ballasts	Philips, Crompton, GEC, Bajaj
56	no.	80 W starters	Philips, Crompton, GEC, Bajaj
11	no.	12-way 15 A nylon connectors	Any best quality available
4	no.	20 A HRC fuse assemblies	English Electric
1	no.	30 A neutral link	English Electric
1	no.	Timer	Escol
1	no.	16 A 3-pole contactor	Siemens, L and T
1	no.	30 A 3-phase isolator	GEC
4	no.	15 A on-off toggle switches	Anchor
56	no.	Tube light starter holders	Philips
2	no.	300 mm (12 in) exhaust fans	EPC, GEC, Bajaj
56	no.	80 W fluorescent tubes	Philips, Sylvania and Laxman
1	no.	Differential type thermostat with setting range 5-30°C and differential range 0-10°C	Ranco, Danfos
25	meter	40 x 40 x 2 mm slotted angle iron	Any best quality available
55	meter	20 mm conduit pipe	Any best quality available
6	meter	12 mm mild steel rod	Any best quality available
5	meter	25 mm mild steel pipe	Any best quality available
7	meter	120 cm wide, 25 x 25 mm welded mesh	Any best quality available
1	meter	75 mm mild steel rod	Any best quality available
1	kg	Nuts and bolts	Any best quality available
1	kg	6 mm nylon rope	Any best quality available
2	liter	Red oxide	Any best quality available
1.5	liter	White paint	Any best quality available

based on the requirements of the user.

An incubator fitted with lights (Percival, Boone, Iowa 50036, USA, model I 35 LL, with standard fittings) has also been successfully used to grow chickpea plants at ICRISAT Center and costs approximately US$ 6500 at 1983 prices, including freight. With modifications for extra lights one can accommodate 272 plant tubes of 25 x 200 mm or 352 plant tubes of 18x150 mm. In terms of the number of plant tubes accommodated, the facility developed at ICRISAT is at least 8 times cheaper than the Percival units if the cost of the generator is excluded from both the facilities.

Any electrical instrument can be hazardous. It is therefore stressed that the best quality of materials should be used for assembling the plant growth unit described.

Suggested references

Vincent, J.M. 1970. A manual for the practical study of the root nodule bacteria. IBP Handbook no.15. Oxford: Blackwell.

Downs, R.J., and Hellmers, H. 1976. Controlled climate and plant research. WMO Technical

Table 2. **Comparative component and installation costs of plant growth unit in use at ICRISAT Center, and suggested facilities for prospective users.**

Item	Rate	ICRISAT facility for 2 units		Suggested facility for 1 unit		Suggested facility for 2 units	
		No.	Cost (Rs.)*	No.	Cost (Rs.)*	No.	Cost (Rs.)*
Air-conditioner (2-t capacity)	22,000	3**	66,000	2**	44,000	2**	44,000
Ballasts (chokes)							
80 W	95	112	10,640	-	-	-	-
40 W	45	-	-	42	1,890	84	3,780
Fluorescent tubes							
1500 mm (5 ft)	35	112	3,920	-	-	-	-
1200 mm (4 ft)	25	-	-	42	1,050	84	2,100
Exhaust fans	640	4	2,560	2	1,280	4	2,560
Electrical wires	-	-	3,000	-	1,500	-	3,000
Misc. electrical items	-	-	5,600	-	2,500	-	5,000
Hardware: mild steel pipes, slotted angle iron, etc.	-	-	3,000	-	1,500	-	3,000
Labor	-	-	4,500	-	2,000	-	4,000
Generator: 18 kVA	-	1	83,000	-	-	-	-
12.5 kVA	-	-	-	1	65,500	1	65,500
Total cost (Rs.)			1,82,220		1,21,220		1,32,940
(Equivalent in US$)			(18,220)		(12,120)		(13,290)
Total capacity (25 x 200 mm test tubes)			3,648		1,224		2,448
Cost efficiency $\left\{\frac{\text{Capacity}}{\text{cost}} \times 100\right\}$			2.0		1.0		1.8

* Excluding sales tax. ** One of the air-conditioners is a standby for possible breakdown.

Note no.148. Geneva: World Meteorological Organization.

Gibson, A.H. 1977. The influence of the environment and managerial practices on the legume-*Rhizobium* symbiosis. Pages 393-450 *in* A treatise on dinitrogen fixation. (Hardy, R.W.F., and Gibson, A.H., eds.). New York and London: Wiley.

Gibson, A.H. 1980. Methods for legumes in glasshouse and controlled environment cabinets. Pages 139-184 *in* Methods for evaluating biological nitrogen fixation. (Bergersen, F.J., ed.). New York and London: Wiley.

Acknowledgments

We thank J.V.D.K. Kumar Rao, Shikha Mittal, and Usha Kiran for assistance in testing the performance of these units, and J.A. Thompson for his helpful suggestions in writing the text. Also the help of the electrical, mechanical, electronics, and refrigeration units of the Physical Plant Services Division of ICRISAT is gratefully acknowledged.

RA-0079

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics
ICRISAT Patancheru P.O.
Andhra Pradesh 502 324, India