Question and Answer Period: Saturday AM General Session I

Jim Kresler: Have you done any studies where gypsum has been added to the potting mix?

Jim Downer: No. Others have worked on that When coarse gypsum (crushed dry wall) was added to make up to 5% of the medium, the physical and chemical properties of the mix change One is that the porosity of the mix is altered. That immediately has an effect on reducing the water content of the medium which is critical for eliminating *Phytophthora* diseases The other effect seems to be a chemical/fungicidal, calcium ion-based effect directly on the fungus that reduces the size of the sporangia and the number of spores produced.

Rooting Media and Plant Acclimatization ex Vitro

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INTRODUCTION

The transfer of plants from a sterile environment to a greenhouse is known as acclimatization, which corresponds to the Stage IV in the process of micropropagation Due to its technical requirements this phase is considered one of the most expensive of the micropropagation process and, consequently, a possible limiting factor on a commercial scale (Lewandowski, 1991)

In vitro culture is done under artificial conditions like a constant long-day photoperiod (generally about 16 h day^{-1}) with a low luminance level (1000 to 2000 lux) and a narrowed light spectrum (fluorescent lamps without red and infrared waves). The usual temperature ranges from 20 to 25C Inside the flask the air humidity is very high and the CO_2 level is low. The culture medium fixes the plants in the right position and releases to them nutrients, vitamins, amino acids, and sugar. Under these conditions, the plant is considered heterotrophic (Fugiwara, Kozai, and Watanabe, 1988) or mixotrophic (Deng and Donnely, 1993) Leaf anatomy and morphology show typical culture-induced phenotype (CIP) reduced palisade tissue, no epicuticular waxes on the leaf surface, abnormal linkage between the conduction vessels from the root-shoot sequence, roots with less absorbent hairs and stomata with low photosynthetic efficiency (Waldenmaier, 1988, Preece and Sutter, 1991, Hartmann et al., 1997)

For the horticulturist, this stage of acclimatization involves transplanting, which needs special care to avoid plant stress and contamination from pathogens Preece and Sutter (1991) suggest the following parameters be considered for successful acclimatization: control of light and air humidity, use of antitranspirants, prevention of contaminants, reduction of fertilization, and selection of substrates Among these factors the selection of substrates is one of the least studied (Avanzato and Cherubini, 1993).

ADVENTITIOUS ROOT FORMATION

Acclimatization depends on the development of adventitious roots and this is affected by the substrate Basically, the process is divided into two phases: root initiation (in vitro) and root elongation. The last phase depends especially on the properties of the rooting medium. Hartmann et al. (1997) listed four functions for the rooting medium: to hold the cuttings in place during the rooting period, to provide moisture, to permit air exchange, and to reduce the light penetration to the cutting base.

According to Drew (1990), one single cell produced by the meristem can complete the root initial development in a few hours. The root elongates through the continuous pores of the substrate and, if necessary, its tip can dig channels by displacing solid particles. The resistance of the particles against this movement can stress the root. As a consequence, apical dominance can be broken and the speed of cell division decreased. Hence, the roots will be shorter with more lateral branches. Considering these observations, we may add some items to Hartmann's list (Table 1) Each function is related to one or more physical or chemical properties of the medium, which must be considered in the evaluation of substrate quality.

Table 1. Functions of the root medium and related properties

Functions	Related properties
To fix the plant, cutting, or microshoot	Bulk density
To retain and deliver water	Meso and micropores
To permit gas exchange	Macropores (air space)
To promote root growth	Fertility (salinity), pH value
To permit root penetration	Penetrability (mechanical impedance)
To maintain the physical structure	Resistance to deformation

ROOTING MEDIUM

In Brazil, the substrate industry is just beginning and at this stage is unable to attend to all demands. So, growers prepare their own mixtures using local, available components. The most commonly used components we have are black and fibrous peat, mineral soil, vermiculite, organic composts and residues of the agroindustry like rice hulls (carbonized or burned), and *Pinus* and *Acacia mearnsii* bark The guidelines (Table 2) to evaluate these materials, pure or mixed, follow the concepts of De Boodt and Verdonck (1972), Roeber and Schaller (1985), Hartmann et al (1997), and Ballester-Olmos (1992).

Acclimatization can be done under mist or in indoor tents. The required properties of the medium for each situation are different. Under mist quick drainage is required. We use pure carbonized rice hulls (about 75% of water volume runoff) with good results for *Limonium platyphyllum* (syn. *latifolium*). Under the tents, besides drainage, the water retention can be a limiting factor, as demonstrated by the two following examples, *Eustoma grandiflorum* (prairie gentian) and *Fragaria* ×*ananassa* 'Campinas' (strawberry).

Table 2. Desired properties of root media

Properties	Values	Unity
Dry density	200 to 500	g liter^{-1} substrate
Total porosity	0 80 to 0 90	cm cm^{-3}
Air space	0 10 to 0 30	cm cm^{-3}
Easily available water	0 20 to 0 30	cm cm^{-3}
Water retention after drainage at 100 hPa	0 20 to 0 25	cm cm^{-3}
pH value	>5 0 to <6 5	
Total soluble salts substrate	<1,0	g KCl liter^{-1}
Mechanical impedance	<400	kPa

PLANTS

Eustoma grandiflorum.

In vitro-rooted microshoots of prairie gentian were transferred to three substrates based on mixtures of a mineral soil (classified as Cumulic Haplumbrept) + carbonized rice hulls or *Pinus* sawdust or a sugarcane compost. The medium varied significantly in the following properties. pH value (5 0 to 6.0), total soluble salts (0.7 to 4.2 g liter^{-1}), air space (0 18 to 0.41) and remaining water after drainage by suction at 50 hPa (0.17 to 0.44) The correlation of these properties and plant growth revealed a significant effect of air space and the remaining water volume on the biomass production (Fig 1). The results showed the importance of aeration in the root medium for acclimatization of *Eustoma*, suggesting a value of 0 40 cm^3 cm^{-3} for air space and 0.17 cm^3 cm^{-3} for remaining water at 50 hPa.

Fragaria ×ananassa 'Campinas'.

In this trial we compared the development of in vitro rooted microshoots of strawberry 'Campiñas' after transplant into eight media (Table 3), seven of them based on the following components: peat — a high mineralized black peat, corresponding to H9 in the von Post' Scale (Roeber and Schaller, 1985) and a fibrous red peat, corresponding to H3 in the same scale, carbonized or burned (ash) rice hulls; vermiculite with 0.5 mm diameter; and partially decomposed *Acacia mearnsii* bark, sieved to 5 mm particle size A commercial mixture of *Pinus* bark, black peat, and vermiculite was used as the control.

All the mixtures showed similar density and total porosity values, but differed significantly in air space (0.05 to 0.35), available water (0.15 to 0 38), and total soluble salts (1.14 to 2 15 g liter^{-1}). The acclimatization, evaluated by percentage of the surviving plants, dry and fresh mass of roots and shoots, plant height, and number of leaves was clearly affected by the quality of the substrates. The rice hulls ash mixes produced better plants with more roots and leaves. The results submitted to correlation analysis (Table 4) indicated that the number of surviving plants, the plant height and the number of leaves are significantly related to the volume of available water and air space. Increasing the medium air space decreased significantly the percentage of surviving plants and plant height. The opposite occurred with the amount of available water The curves in Fig 2 suggest that the medium for acclimatization of strawberry 'Campiñas' must have 15 ± 5% air space and 33 ± 5% available water.

Table 3. Components of the media used as substrates for acclimitization of *Fragaria ×ananassa* 'Campinas'.

Treatments	Components (% by volume)
1	Black peat (50) + rice hull ashes (40) + vermiculite (10)
2	Black peat (50) + carbonized rice hulls (40) + vermiculite (10)
3	Black peat (45) + red peat (22 5) + rice hull ashes (22 5) + vermiculite (10)
4	Black peat (50) + red peat (25) + carbonized rice hulls (25)
5	Black peat (45) + rice hull ashes (22 5) + vermiculite (10) + *Acacia* (22 5)
6	Black peat (45) + carb rice hulls (22 5) + vermiculite (10) + *Acacia* (22 5)
7	Black peat (50) + rice hull ashes (25) + *Acacia* (25)
8	Black peat + Pinus bark + vermiculite + perlite (control)

Table 4. *Fragaria ×ananassa* 'Campinas' - correlation between substrate properties and plant growth

Properties		Plant growth parameters	Coefficient (r)	P (≥0.05)
Air Space	×	Plant high	-0 88	*
		Surviving plants (%)	-0 88	*
Available water	×	Number of leaves	0 74	*
		Shoot dry weight	0 76	*
		Plant high	0 78	*
		Surviving plants (%)	0 89	*

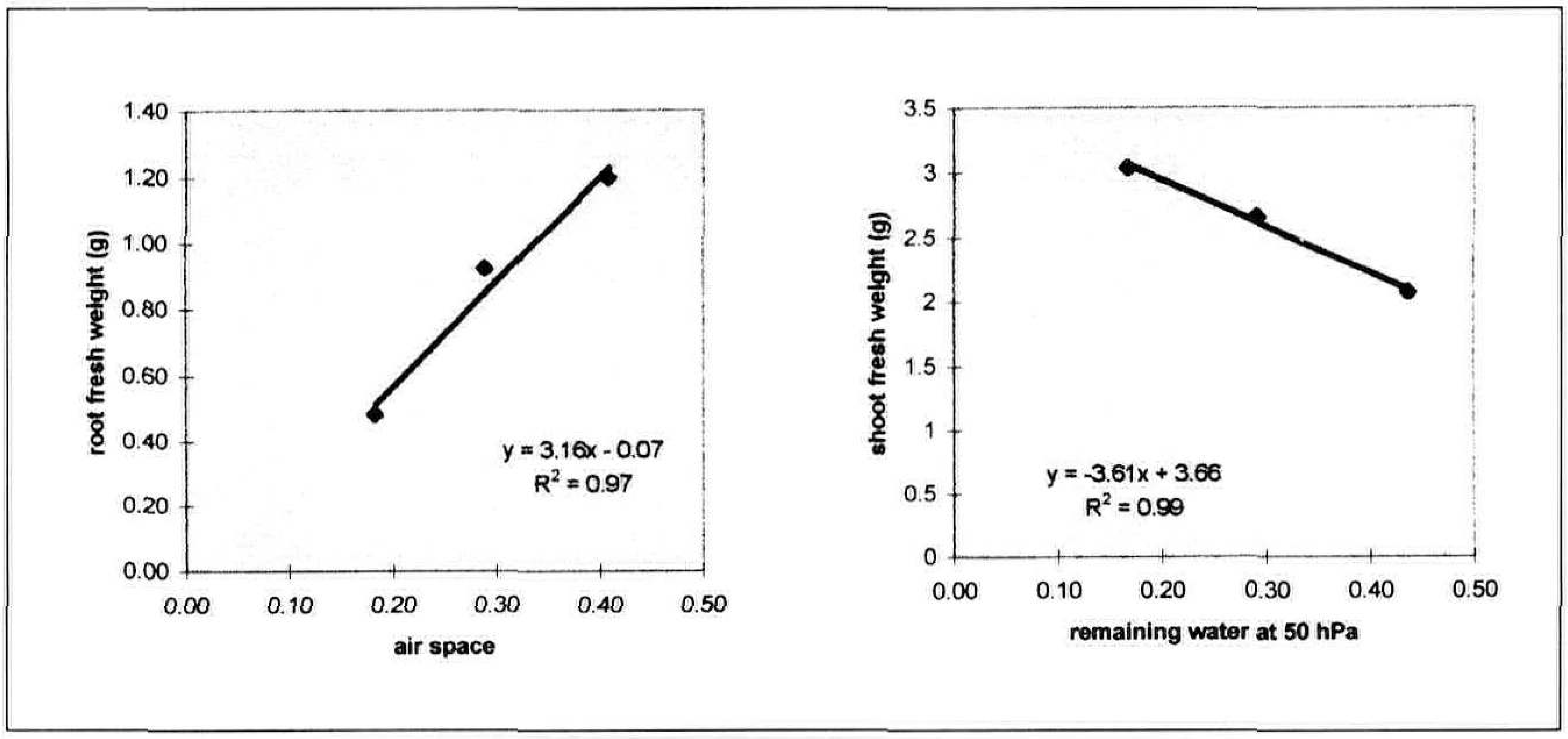

Figure 1. Acclimatization of *Eustoma grandiflorum* ex vitro. Left: Effect of air space volume (cm^3 cm^{-3}) of the rooting medium on the root fresh weight (g). Right: Effect of remaining water volume (cm^3 cm^{-3}) of the rooting medium on the shoot fresh weight.

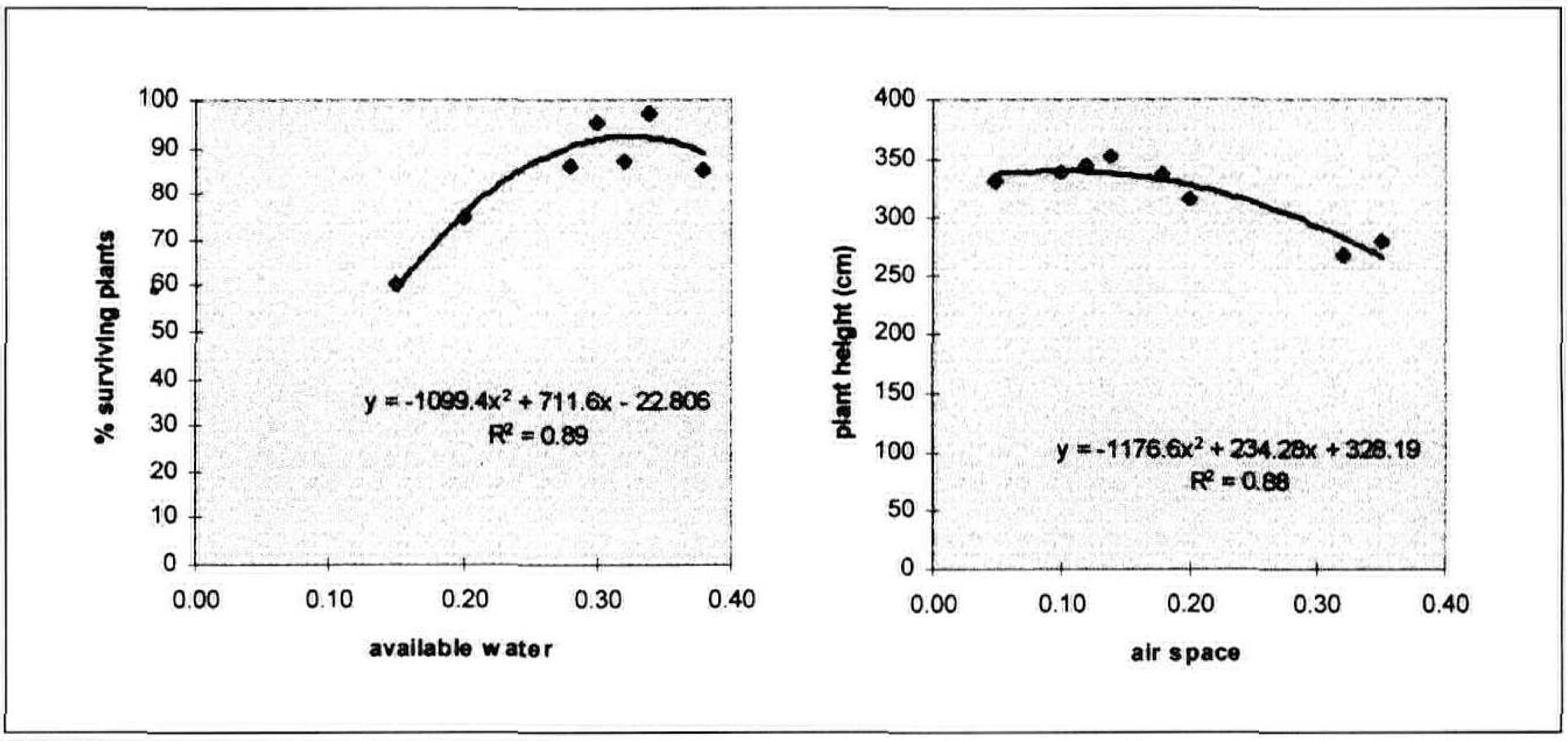

Figure 2. Acclimatization of *Fragaria* ×*ananassa* 'Campinas' ex vitro. Left: Effect of available water volume (cm^3 cm^{-3}) on the percentage of surviving plants. Right: Effect of air space volume (cm^3 cm^{-3}) on the plant height.

CONCLUSION

Knowledge of the requirements for adventitious root formation from each species and the selection of rooting media based on analysis of its properties can improve the ex vitro acclimatization of microshoots.

LITERATURE CITED

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