Micropropagation of *Eustoma*

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INTRODUCTION

Eustoma (syn. *Lisianthius*) has been propagated and longterm stored in vitro at our laboratory as a service for our traditional breeding. The aim was to store important breeding lines in the laboratory and to use the in vitro lines for micropropagation of parent plants in the production of F_1 hybrids. In vitro storage is more convenient than keeping stock plants in the greenhouse and better than storage of seed because the lines are not totally inbreed (homozygotic) and the offspring will not be identical to the parents.

MICROPROPAGATION

It is not difficult to start tissue cultures of *Eustoma* as long as the stock plants are healthy and are growing well. Leaves and flowers are removed from the shoots before they are surface disinfected in a 3% korsolin solution. Due to the very smooth stems it is very easy to disinfect *Eustoma*. Nodes are used as explants. The same medium is used during culture establishment and for further propagation. The MS medium (Murashige and Skoog, 1962) is supplied with 6-benzylaminopurine (0.1 mg $liter^{-1}$). Subculture is performed every 5 weeks. Shoot clusters are divided into smaller pieces at each subculture. Temperature during propagation is 23C, and daylength is 16 h. Rooting is done on MS medium supplied with 3-indolebutyric acid (1.0 mg $liter^{-1}$).

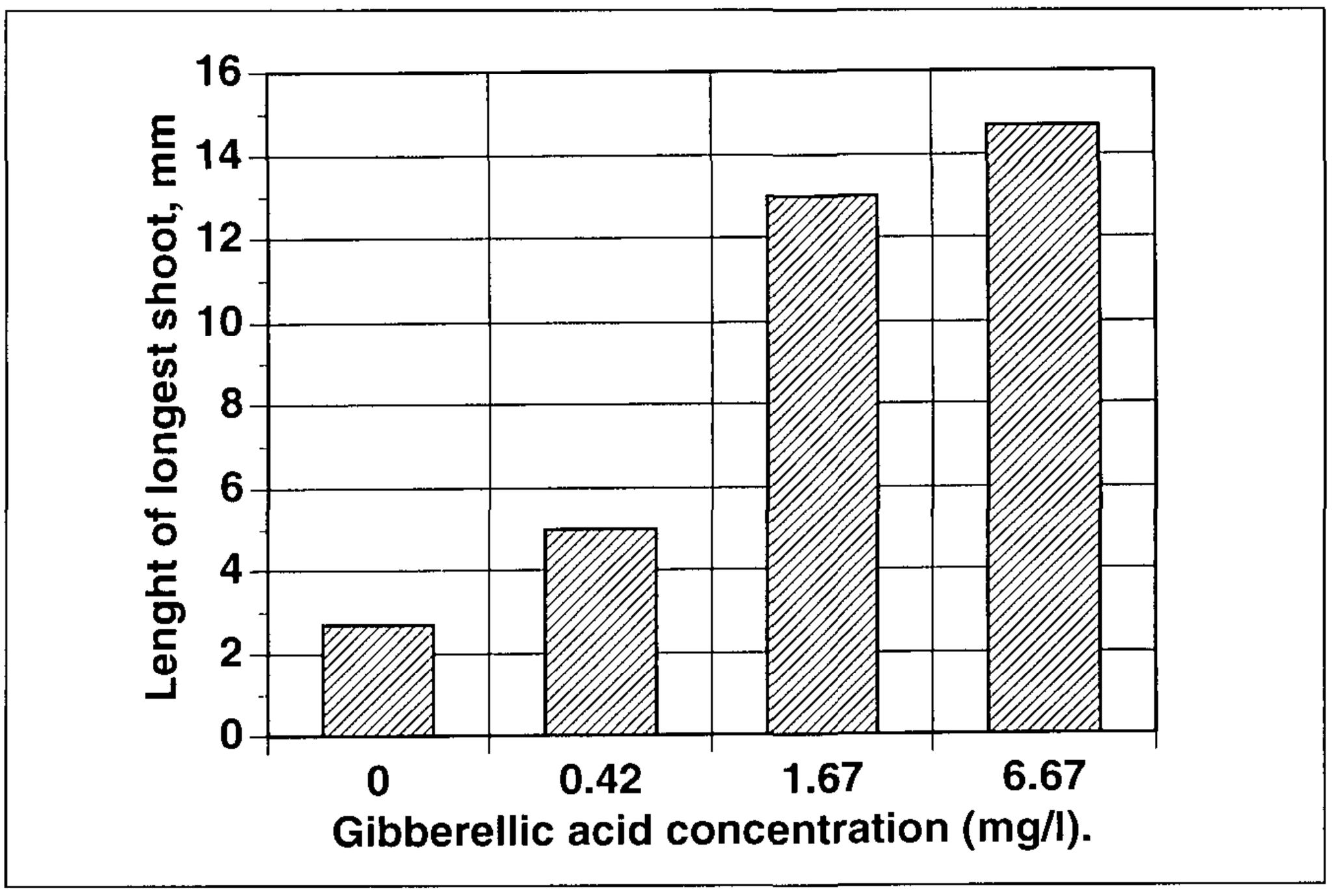

Figure 1. Effect of gibberellic acid concentration on in vitro shoot elongation of *Eustoma*.

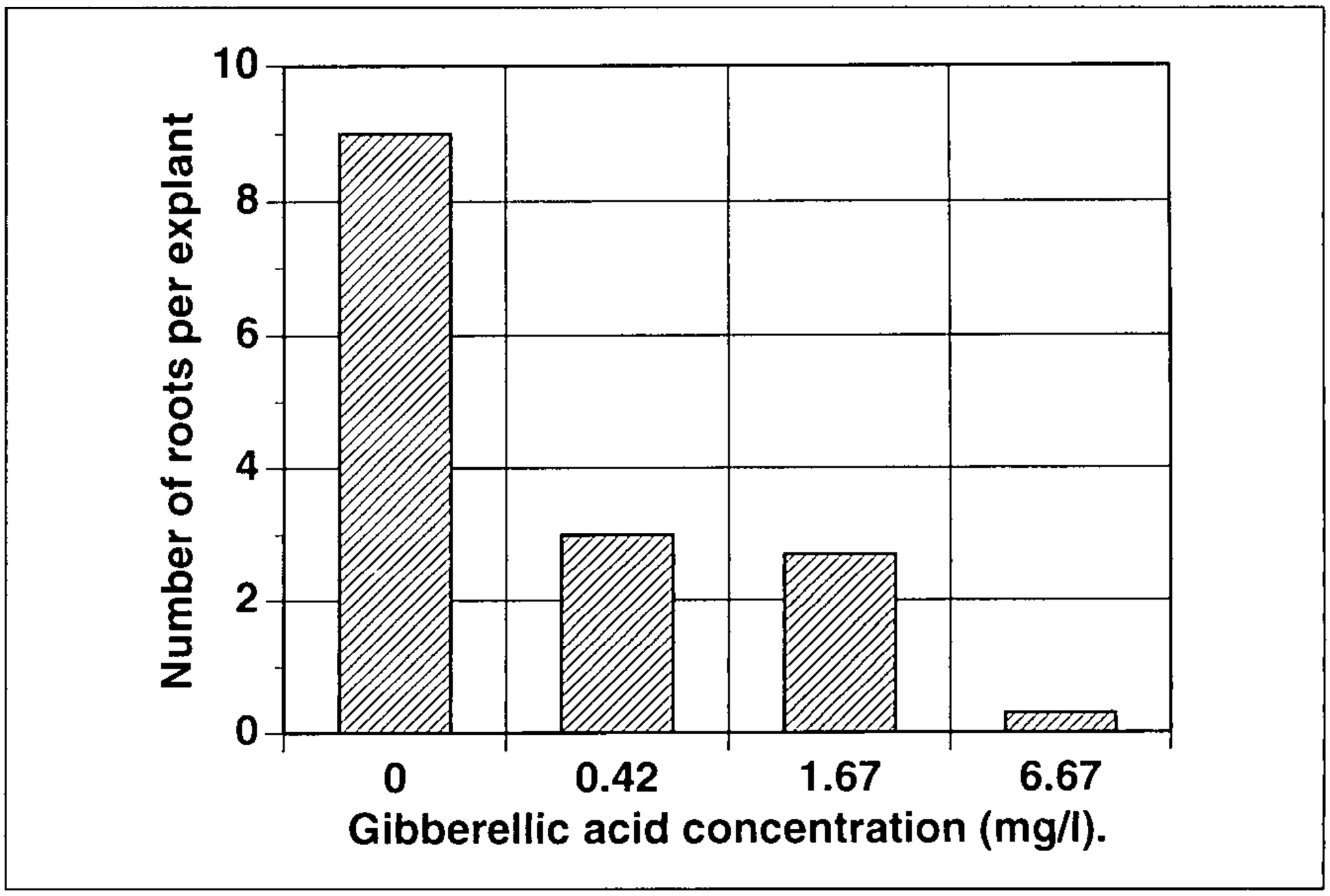

Figure 2. Effect of gibberellic acid concentration on in vitro root formation of *Eustoma*.

AXILLARY AND ADVENTITIOUS SHOOT FORMATION

Shoot formation is abundant but both axillary and adventitious shoots are produced. Adventitious shoots emerge on leaves and petioles. One has to be careful not to use adventitious shoots for further multiplication and thereby risk the production of abnormal plants. It can, however, be very difficult to identify whether a shoot is of adventitious or axillary origin. Both shoot types are easily multiplied and possible diverging plants can only be identified after their development in the greenhouse.

There is a great variation in the ability of different genotypes to produce quality plants in tissue culture. Generally, the optimal concentration of cytokinin and temperature differ, callus formation varies, different compactness of shoot clusters is observed, and the rooting ability can vary as well.

In order to distinguish whether shoots in very compact clusters were of axillary or adventitious origin we tried to increase shoot elongation by adding gibberellic acid (GA_3). Furthermore we hoped that the plants would show a subsequent increase in stem elongation in the greenhouse. Shoot elongation is stimulated by GA_3 in vitro (Fig. 1) and the increase in shoot length increased with increasing GA_3 concentration. This made it easier to distinguish between axillary and adventitious shoots. However, GA_3, inhibited root formation as shown in Fig. 2. This made initial establishment of the plants more difficult in the greenhouse and resulted in poorer growth. The stimulating effect on stem elongation was not persistent during subsequent growth.

ELONGATION AND FLOWER INDUCTION

In order to obtain stem elongation and flower induction in vitro plants were grown at either 18 or 23C during in vitro root formation. The experiment demonstrated that the temperature during root formation had a distinct effect on subsequent

growth in the greenhouse. When rooting occurred at 23C plants continued to grow as rosettes after transfer to the greenhouse at 22C. When rooting occurred at 18C some of the genotypes had a normal stem elongation during subsequent growth at 22C in the greenhouse (Fig. 3). There were large differences between the two genotypes tested.

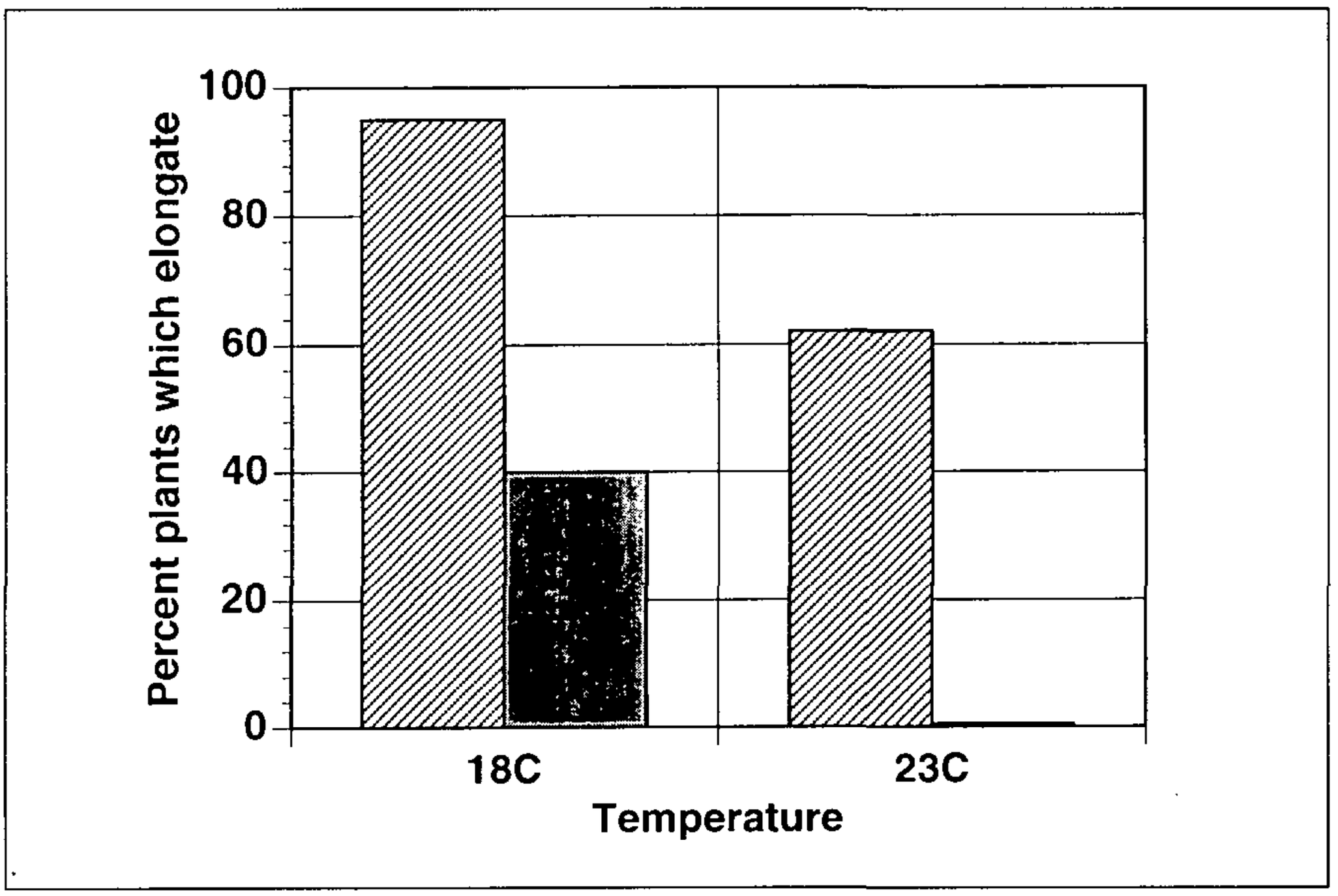

Figure 3. Effect of in vitro rooting temperature on ex vitro shoot elongation of the *Eustoma* genotypes 53425 ▨ and 53518 ■.

DISCUSSION

The addition of GA_3 to in vitro cultures of *Eustoma* makes it possible to distinguish between axillary and adventitious shoots which is important for the application of in vitro propagation in *Eustoma*. Research also demonstrated that it is possible to control stem elongation of *Eustoma* in the greenhouse through the temperature during in vitro rooting. This can be used to control transplanting dates of the various parent lines for seed production to make sure that the parent lines in an F_1 hybrid flower simultaneously.

LITERATURE CITED

Murashige, T. and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.