prepared in boiled and cooled tap water. At each of the given concentrations, at least two replicates comprising 25 larvae each were exposed. Results were scored after 24 h of continuous exposure to the test solution and expressed as per cent mortality. The data obtained were subjected to log-Probit regression analysis, to calculate the median lethal concentration (LC_{50}) and LC_{90} values[20].

Hundred per cent larval mortality was observed within 24 h of exposure period in *An. culicifacies* species A, *Cx. quinquefasciatus* and *Ae. aegypti* at a concentration of 0.2%. The LC_{50} values for I and II instar larvae of *An. culicifacies* species A and *Cx. quinquefasciatus* were 0.0167% and 0.019%, respectively and for III and IV instar larvae it was 0.027% in both the species. For III and IV instar of *Ae. aegypti*, it was 0.032% (Table 1). The LC_{90} values for I and II instar larvae of *An. culicifacies* species A, and *Cx. quinquefasciatus* were 0.169% and 0.185%, respectively. The LC_{90} values for III and IV instar larvae of the three species were 0.176%, 0.205% and 0.212%, respectively (Table 1). From the LC_{50} and LC_{90} values, the extract was found to be relatively more toxic to the larvae of *An. culicifacies* species A, followed by *Cx. quinquefasciatus* and *Ae. aegypti.*

Results of this preliminary study with the crude extract of the leaves of this plant have exhibited its toxicity to the three important disease vector species and warrants further investigations. Sukumar *et al.*[15] have stated the existence of variations in the toxicities of phytochemical compounds on target species *vis-à-vis* plant parts from which they are extracted, responses in species and developmental stages of species to the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction, etc. Keeping in view the above variations, it will be of importance to study the variations in toxic effects of extracts and also to characterize the active ingredients responsible for the toxicity.

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ACKNOWLEDGEMENTS. We acknowledge the technical help rendered by Mr R. S. Sharma, Mr K. B. Masiwal, Mr H. D. Joshi, Mr Narender Kumar and Mr Kamal Dev. We also thank Mr T. G. Thomas, NICD for his critical comments on the design of the experiments.

Received 29 June 2001; revised accepted 28 September 2001

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Acclimatization of Asiatic hybrid lilies under stress conditions after propagation through tissue culture

Efforts are going on worldwide to boost floriculture industry using biotechnology[1] and the attention is focused on development of new flower colour and novel plant morphology, as these are the main features which determine consumer interest[2]. One of the major constraints of floriculture industry is non-availability of constant supply of quality bloom in all the regions of the country. All floricultural crops are climate-specific and flowers are transported from one climatic zone to another for sale[3]. Acclimatization of any crop from one climatic zone to another, is normally done through conventional breeding. Work has been initiated at the Floriculture Lab, NBRI, Lucknow to

acclimatize different temperate ornamentals using the biotechnological approach which may provide an attractive alternative to conventional breeding. Asiatic hybrid lily has been selected as the target species for acclimatization through *in vitro* culture and the present note reports the experimental results. Micropropagation of *Lilium longiflorum* has already been reported through somatic embryogenesis[4], stem node and pseudobulblet culture[5] and *in vitro* bulbing[6,7], whereas there are no reports on Asiatic hybrid lilies, except few hybrids (e.g. *Lilium* × *formolongi*), where plants were regenerated from protoplasts using meristematic nodular cell clumps[8]. Asiatic hybrid lilies are among one of the most popular lilies which have very good demand in the world cut-flower trade. These lilies generally grow and flower under temperate climate and require a low temperature of 10–15°C at night and 20–25°C during the day. Bulbs of Asiatic hybrid lilies are imported from Holland to meet the demand of the Indian market. These bulbs grow and flower mainly in the temperate climate of the hills, where the climate is similar to that required by Asiatic hybrid lilies. The temperature in subtropical North Indian plains (here, Lucknow) ranges from 2°C in extreme winters (December–January) to 45–46°C in extreme summers (May–June) and these lilies do not flower at such a high temperature. Sometimes, it becomes very difficult to even save the plants at this temperature, under normal field conditions. One more drawback of these imported bulbs is that there is no further new underground bulb formation after blooming of the original bulb. Propagation of Asiatic hybrid lilies through tissue culture and their acclimatization from the very beginning in the open field has been found beneficial in developing some adaptability towards higher temperature.

Propagation of Asiatic hybrid lily, cv. Orange Pixie has been done through tissue culture, using the segments of bulb scales as the explants. Bulb scales were separated from the bulbs, washed properly in running water and with 5% liquid detergent. Explants were dipped in 70% alcohol for one minute and then surface-sterilized with 0.1% $HgCl_2$ for 15 min. Washing was done at least three times at 5 min intervals, with sterilized double distilled water. The explants were inoculated in the Murashige and Skoog's[9] medium supplemented with 1 mg/l indole-3-acetic acid (IAA) + 0.5 mg/l 6-benzyladenine (BA) + 10 mg/l adenine sulphate (AdS). The pH of all the media used was adjusted to 5.8 before autoclaving at 1.08 kg/cm^2 for 15 min. The cultures were incubated at 26 ± 2°C under 3 klux light through fluorescent tubes, for 16 h photoperiod (50–60 mol m^{-2} s^{-1}).

After 3 weeks of incubation in the medium, the explants showed regeneration of shoots, which was generally from their abaxial surface and more from the lower end of the segment. Differentiation of shoots was a nonsynchronous process and many developmental stages of the shoots could be seen at one time. All the explants along with the differentiated shoots and shoot buds were subcultured in the fresh medium, after every 40 days. Within 3–4 months, a fairly good number of shoots (10–25) proliferated from one segment (Figure 1 *a*). Results of the effect of different concentrations and combinations of BA and IAA on shoot regeneration are presented in Table 1. It has also been found that 1 mg/l concentration of IAA added in the proliferation medium was effective in inducing rooting of the grown-up shoots among the proliferating mass of shoots. These shoots form a pseudobulb at their base. On an average, during every subculture of the proliferated shoots, approximately 10–15 shoots were rooted and had a pseudobulb at their base, and could be used directly for hardening. The groups of smaller shoots or shoot buds were further subcultured in the proliferation medium to grow and proliferate. On MS medium + NAA (0.5 mg/l), sufficient rooting in the isolated shoots within 10 days was noticed.

In another experiment, a separate liquid medium having 9% of sucrose + 0.25 mg/l NAA (IAA was inducing little callus) + 100 mg/l m-inositol and 0.1 mg/l kinetin was also found suitable for the growth of the bulblets in the isolated unrooted shoots. Due to the high concentration of sucrose, the size of the bulblets increased from less than 0.5 cm in diameter to approximately 1–1.5 cm in size, within 2 months of incubation (Figure 1 *b*). This was against the results of Marinangeli and Curvetto[6], where double-MS medium was required for bulblet growth, thus reducing the cost of the medium. During this period the plantlets grew up to 10–15 cm in height inside the culture tube with good

Table 1. Effects of BA and IAA on regeneration of shoots in Asiatic hybrid lily, cv. Orange Pixie in MS basal medium

Treatment (conc. mg/l)	No of shoots regenerated/ responded explant*	Associated callusing	Rooting in grown-up shoots
Control MS basal medium	–	–	–
BA 0.25 IAA 0.5	12 ± 1.538	–	–
BA 0.5 IAA 0.5	18 ± 1.614	–	–
BA 1.0 IAA 0.5	20 ± 2.01	+	–
BA 0.25 IAA 1.0	22 ± 2.124	–	+
BA 0.5 IAA 1.0	25 ± 2.326	–	+
BA 1.0 IAA 1.0	20 ± 1.837	+	+
BA 0.25 IAA 2.0	10 ± 0.938	+	+
BA 0.5 IAA 2.0	14 ± 1.538	++	+
BA 1.0 IAA 2.0	16 ± 1.624	++	+

*Average of 25 replicate cultures; culture period: 45 days; '+' sign denotes positive, while '–' sign denotes negative response.

Table 2. Comparison of growth pattern in *in vitro*-raised and *in vivo*-raised bulbs of Asiatic hybrid lilies cv. Orange Pixie under Lucknow climate

Type of planting material	Germination percentage in the field	Growth pattern during			Flowering	Regeneration of new bulb
		0–6 months	6–12 months	12–18 months	20–24 months	in the soil
In vitro-raised bulbs	100	Rosette of leaves; no stem formation	Leaves enlarged; no stem formation	Stem formation with nodes and internodes	Present	6–7 new bulblets are being formed
In vivo-raised bulbs	20	Rosette of leaves; no stem formation	Leaves enlarged; stem formation having nodes and internodes	Plants started necrosis from the shoot tip	Absent	No bulb formation

Figure 1. Cultures and flowering in Asiatic hybrid lily, cv. Orange Pixie. ***a***, *In vitro* proliferation of shoots; ***b***, *In vitro* rooting and bulblet formation; ***c***, 18-month-old bulb of lily (size 3.5 cm diameter); ***d***, Tissue culture-raised potted plant of lily in flowering; ***e***, Lily flower.

root, a bulblet at the base and thick green leaves having 0.5–0.7 cm of width.

These plantlets were transferred to pots containing a potting mixture of leaf mould : soil (3 : 1). The shoots were hardened within 15 days, by gradually increasing the temperature from 25 to 30°C and decreasing the humidity from 80 to 50% in the hardening chamber. After hardening, the tissue culture-raised plants having bulblets of 0.5–1.0 cm diameter were transplanted both in pots and in the field, where they grew and formed a rosette of leaves without any visible stem, within 6 months of transplantation. The stem was not visible above the ground for nearly 16–18 months. Leaves were shed-off during extreme winters, summers and monsoons, but the bulbs continued to grow and enlarge in the soil and after each season their size was increased. When the bulbs attained a size of 3.0–3.5 cm diameter within 16–18 months of transplantation (Figure 1 *c*), a stem with clear-cut nodes and internodes was formed in the next 3–4 months. Formation of the stem (height approximately 40 cm) was prerequisite for flowering, while 18 months incubation was required for stem formation. These tissue culture-raised Asiatic hybrid lilies flowered in subtropical climate during the last week of April to the second week of May at 43°C (Figure 1 *d*) and the quality of the bloom was also appreciable, having 14 cm diameter (Figure 1 *e*). A parallel experiment was also set up for *in vivo*-grown (imported) bulbs, to see their performance in the field. A comparison of response in growth pattern of *in vitro*-raised and *in vivo*-raised bulbs is presented in Table 2. The mature bulbs available in the market were 5.0–6.0 cm in diameter. When transplanted in the field, only 20% bulbs germinated, while the rest died. However, the germinated shoots grew up to 35–40 cm in height, but before the onset of flowering the tips started turning brown, and slowly the whole plant died. The underground bulb neither survived nor formed any new bulb. It could be concluded from these experiments that acclimatization of *in vitro*-raised plants from the very beginning in the open field developed some kind of adaptability in them, which enables them to flower at such a high temperature. This is a report of blooming of *in vitro*-raised Asiatic hybrid lilies under Lucknow conditions during extreme hot climate, when no other flowers are available for commercial purposes. Similar efforts are in progress for large-scale develop-

ment of quality plant materials and/or flowers of gerbera, carnation, orchid, etc. through *in vitro* acclimatization under adverse conditions, i.e. subtropical instead of temperate climate. The method reported here could provide an economic boost for the floriculture industry.

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ACKNOWLEDGEMENTS. We thank the Director, National Botanical Research Institute, Lucknow, for the facilities provided.

Received 18 July 2001; revised accepted 20 September 2001

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