Peroxidase activity in relation to *in vitro* rhizogenesis and precocious flowering in *Bambusa arundinacea*

Bambusa arundinacea, a multipurpose, monocarpic, woody perennial bamboo, exhibits gregarious flowering once after a prolonged intermast period of 30–45 years[1]. Incidentally, we noticed *in vitro* flowering in one out of ten seedling lines of *B. arundinacea*. The physiological basis of such flowering in bamboos has not been investigated. However, Gaspar, while studying variation in peroxidase activity in relation to *in vitro* rhizogenesis in several plant species, proposed that rhizogenesis and flowering are two antagonistic phenomena, preceding respective minimum and maximum levels of peroxidase activity[2,3]. Therefore, we sequentially followed peroxidase activity *vis-à-vis* the *in vitro* rhizogenesis and precocious flowering in *B. arundinacea*.

Ten subculture-cycle-old clonal explants from the seedling line of *B. arundinacea*, which exhibited *in vitro* precocious flowering during the 6–7 subculture cycle, were chosen for the present study. The clonal explants were maintained on 0.7% agarified and 3% sucrose enriched MS medium[4] supplemented with 2 mg l^{-1} BA for shoot multiplication and 3 mg l^{-1} NAA for rooting of multiplied shoots. pH of the medium was adjusted to 5.8 before autoclaving, and the cultures were kept at 25 ± 2°C under 16 h photoperiod from white fluorescent tubes. Root and flower primordia were visible within 10 days and after 45 days of inoculation on rooting medium respectively. The sampling for peroxidase activity[5] and protein content[6] was done daily for an initial ten days and on every third day after eighteenth day onwards until the appearance of flower primordia, taking three samples each of 100 mg shoots from cultures maintained on rooting medium. The data presented here represent mean of three experiments, using independent sets of proliferated shoots, and have been subjected to statistical analysis, employing Student's t test at $p = 0.05$ level of significance for comparing mean values of peroxidase activity recorded at various days after inoculation.

The peroxidase activity of *in vitro* proliferated shoots transferred on rooting medium exhibited a definite pattern, attaining significant minimum values at 7 days and 42 days; but the *in vitro* root and flower primordia were visible at 10 days and 45 days respectively after inoculation (Figure 1). In fact, *de novo* organogenesis is a complex phenomenon, involving a cascade of cytological events, e.g. cell division, elongation and maturation (differentiation). The observed depression in peroxidase activity prior to appearance of root or flower primordia presumably indicates initial events such as cell division and elongation, whereas its subsequent elevation until formation of roots and flowers (Figures 1 and 2) seems to be associated with lignification of differentiating cells, i.e. cell maturation. The role of peroxidase in lignification of cells is well established as it catalyses the oxidation of cinnamyl alcohols into lignin precursors[7]. There is no report in the

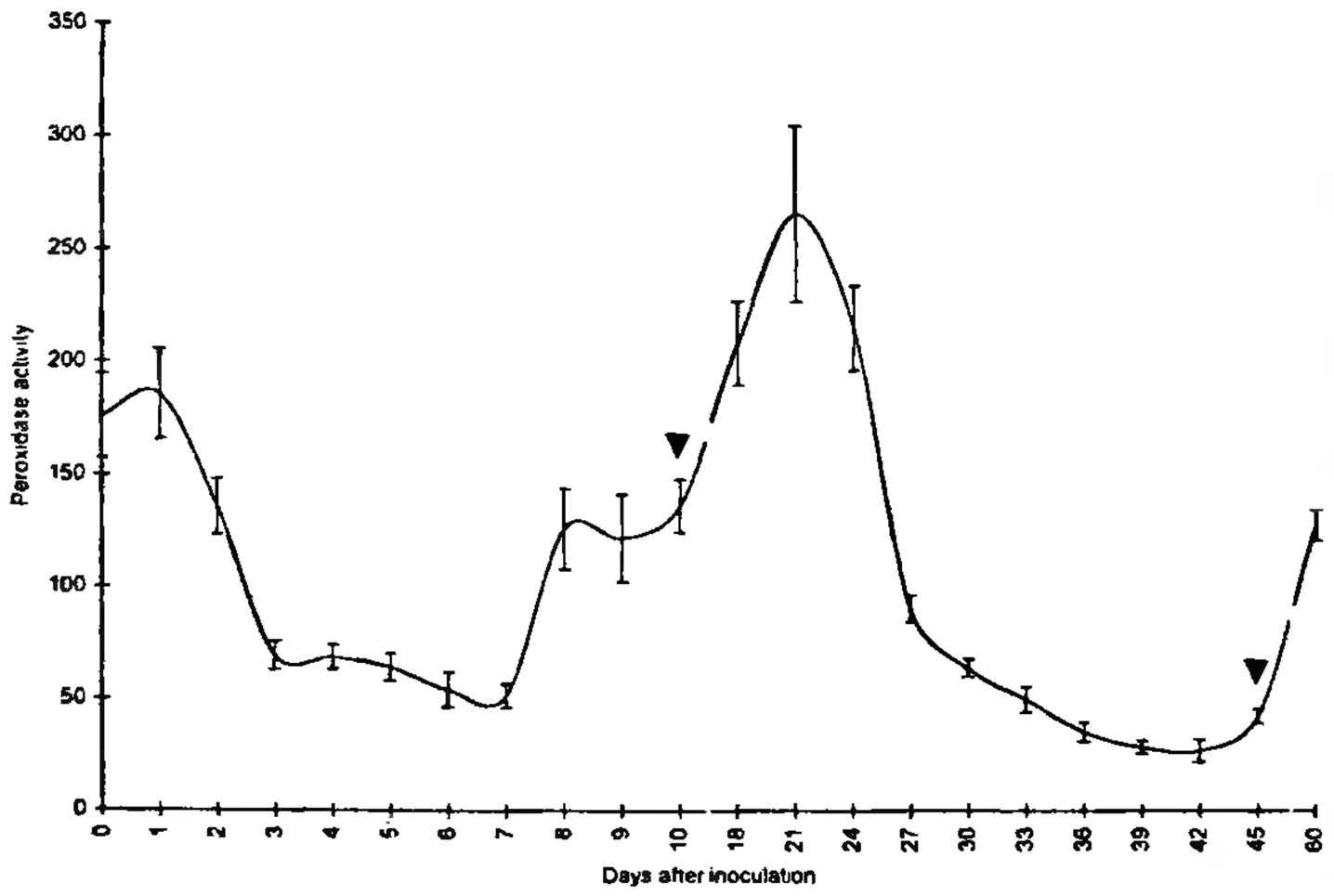

Figure 1. Peroxidase activity [A470 min^{-1} (mg protein)$^{-1}$] in relation to *in vitro* rhizogenesis and precocious flowering in *Bambusa arundinacea* explants inoculated on rooting medium (MS + NAA). Arrow heads at 10 days and 45 days indicate the appearance of root and flower primordia respectively. Data represent mean ± SE of three experiments.

Figure 2. Plantlets of *Bambusa arundinacea* showing (*a*) adventitious roots (10-day-old), (*b*) precocious flowers with stamens (45-day-old).

literature, establishing a relationship between peroxidase activity and various stages of organogenesis. However, Rama Rao *et al.*[5] have shown that the peroxidase activity remains minimum at fiber initiation and elongation and becomes maximum at fiber maturation in cotton.

Further, our observation that the formation of *in vitro* roots and flowers precedes the minimum level of peroxidase activity is in agreement with that on *in vitro* rhizogenesis in several plant species[7] and cyclic bud formation in date palm[8]. However, the present data do not support Gaspar's assumption[2,3]. It seems likely that the *de novo* organogenesis precedes a circumstantial minimum level of peroxidase activity.

In the present study, the multiple shoots of *B. arundinacea* exhibited the *in vitro* precocious flowering when they were maintained on rooting medium (MS medium + *NAA*) for a long period of 45 days. These results indirectly confirm those of Nadgauda *et al.*[9], who reported the *in vitro* precocious flowering in this species on cytokinin-enriched multiplication medium. It may be possible that multiple shoots of the seedling line used in the present study possessed the genetical potential for precocious flowering which got expressed on accumulation of sufficient cytokinin (BA) level due to undergoing several cycles on the multiplication medium. Therefore, the *in vitro* precocious flowering in bamboos as reported in the present study and elsewhere[9–11] seems to be independent of culture conditions (light, medium, temperature, etc)., and cytokinin (BA) probably helps only in expression of this potential.

Hence, we conclude that the low peroxidase activity reflects early events (e.g. cell division and elongation) in the sequence of *de novo* organogenesis rather than organ-specific differentiation of root or flower. Further, the *in vitro* precocious flowering in bamboos is probably genetically controlled, with cytokinin (BA) playing a complementary role in the process.

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S. A. ANSARI
S. KUMAR
K. PALANISAMY

Tropical Forest Research Institute,
P.O. RFRC,
Jabalpur 482 021, India