Table 1. Effect of amooranin against the growth of mammary adenocarcinoma in Sprague–Dawley rats

Drug	Dose (mg/kg)	Maximal T/C% (day)*	Number of rats survived after tumour transplantation		Mean survival time (MST) ± S.D.	% ILS†
			90 days	125 days		
PBS	—	—	6/6	0/6	96.00 ± 5.83	—
	20	56.0 (16)	6/6	1/6	120.33 ± 7.53	25.34
	10	60.1 (14)	6/6	1/6	107.00 ± 8.89	11.46
Tamoxifen	0.5	17.3 (19)	6/6	6/6	164.00 ± 9.80	70.83

The treatment was started on day 50 after tumour transplantation and when the tumour volume was 50–150 mm^3.

*The day at which difference between T and C was maximal. All values were significantly different ($P < 0.01$) from controls.

†Percent increased life span: [(Mean survival time of treated group/mean survival time of control group) × 100] – 100.

were measured by Caliper measurement every 3–4 days and volumes were calculated by multiplying length × width × height × 0.5. Inclusion of height in the calculation improved the accuracy of measurements. Survival was recorded daily for 172 days. Antitumour activity was evaluated from mean survival times and by calculation of the T/C (tumour size of treated rats divided by tumour size of control rats)[2].

The results of this study are shown in Table 1. Control rats treated with PBS had a mean survival time of 96 days, while in rats treated with 10 or 20 mg of amooranin per kg, the mean survival time was 107 or 120.33 days with % ILS of 11.46 or 25.34, respectively. The rats treated with tamoxifen at 0.5 mg/kg had a mean survival of 164 days with % ILS of 70.83. Amooranin at a dose of 10 or 20 mg/kg achieved 60.10% or 56% T/C for tumour size ($P < 0.01$). Tamoxifen at the dose of 0.5 mg/kg achieved 17.3% T/C for tumour size. Although the first treatment with amooranin at 10 mg/kg usually caused a tumour growth delay from day 7, this effect was not longlasting and the tumours soon reached sizes comparable to non-treated groups after the day 24, leading to death. The effects of the amooranin and tamoxifen at 20 mg/kg and 0.5 mg/kg were studied. Observed tumour growth delay at these doses could be attributed to the better antitumour activity. The tumour growth delay was longlasting; the tumour did not reach sizes comparable with the non-treated tumours.

Although the *in vivo* activity seen with amooranin was inferior to that of tamoxifen, amooranin has some antineoplastic activity against N-nitrosomethyl urea induced Sprague–Dawley rats mammary adenocarcinoma. The mechanism through which amooranin induces its biological activity is not known.

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Flowering process in generative buds of woody perennials: An experimental approach

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The timing and extent of reproductive determination of generative buds of woody perennials not conceivable by conventional anatomical methods can be obtained by using the bud culture technique. Potentially generative buds are taken for *in vitro* culture at sequential stages of development through the annual cycle and an imagery of the change in their morphogenetic status visualized by examining the sprouts produced. The traditional technique of microscopic examination is only a superficial indicator of their reproductive status as the ripe-to-flower state may precede or succeed the histologically discernible stages.

FOWERING in woody perennials, particularly the temperate ones, is an extended process with a long time lapse between the initiation of potentially generative buds on the branches and subsequent anthesis and bud burst months later. The basic questions regarding the time of reproductive determination and the extent of irre-

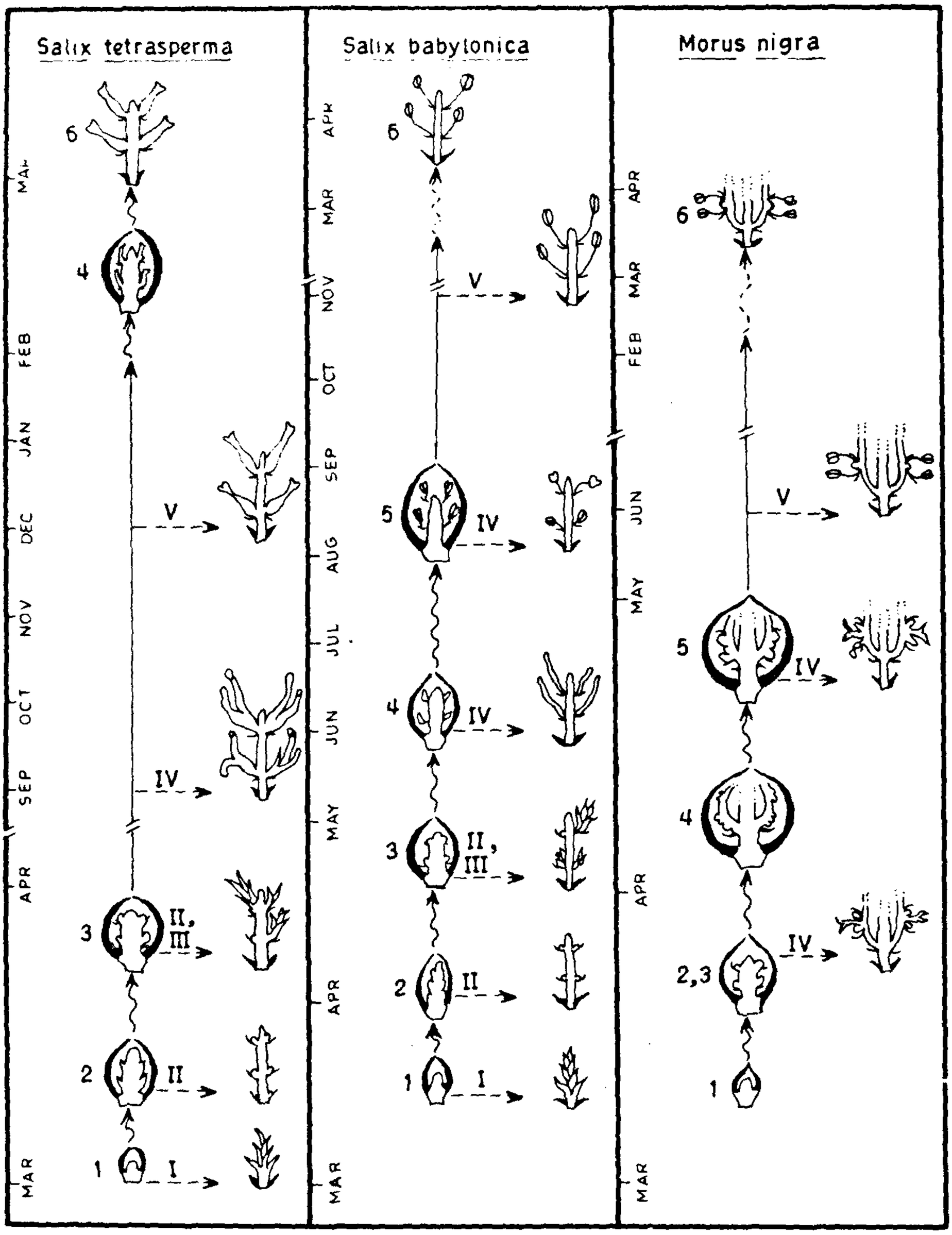

Figure 1. Diagrammatic representation of the sequence of histological events (vertical arrows) in the buds of *Salix tetrasperma*, *S. babylonica* and *Morus nigra* through the annual cycle and their *in vitro* morphogenetic response (broken line horizontal arrows). Vertical arrows depict active histogenesis (wavy lines), lack of apparent histological activity (straight lines) and expansion of preformed flower axis (broken wavy lines). Bud histogenesis stages are denoted by: 1, bud meristems; 2, inflorescence primordia; 3, floral meristems; 4, flower primordia; 5, preformed flowers; 6, anthesis. Structures produced from buds cultured *in vitro* are denoted by: I, buds producing vegetative shoots; II, floral meristems remaining quiescent on inflorescence axis; III, floral meristems producing vegetative structures; IV, partly reproductive structures; V, flowers.

versibility, along with the nature of stimulus, need to be answered. This would have implications also in horticulture[1–5]. Anatomical method due to limitation is not useful in identification of reproductive primordia during early stages of bud development[4,6]. It is also uncertain whether histological events indicate a stepwise progression or that the whole processes remain suppressed due to dormant state of buds, which achieve anthesis when conditions for their growth are favourable. Therefore the results of *in vitro* experiments on specific structures have helped a better understanding of reproductive development in woody perennials[7–9]. Therefore, we examine relevant information from literature in the light of studies conducted in Department of Botany, Panjab University.

Histologically identifiable changes in the buds are taken as criteria for reproductive determination[1]. During vegetative growth the current year's branches develop potentially generative buds. However, the extent of further development of meristems of these buds during dormancy varies between two extremes. Meristems in the first category remain undifferentiated in a quiescent state. Active histogenesis leading to differentiation of flower parts, occurs prior to bud burst as observed in species like *Actinidia*[10], *Callistemon*[11], *Citrus*[12,13], *Dalbergia*[14], *Juglans*[15], *Quercus* (female)[6], *Salix* (female)[16] and *Vitis*[17]. The second category is represented by *Cupressus*[18], *Salix* (male)[19], *Machilus*[14], *Malus*[2], *Morus* (male)[20] and *Quercus* (male)[6], where the development progresses in apparently quiescent buds, leading to preformation of flowers with essential parts well before the bud burst. This is followed by rapid elongation of axis during anthesis.

The novel approach of sequential *in vitro* culture of excised bud explants was adopted by Nanda *et al.*[21] to elucidate the details pertaining to the transformation of vegetative bud into generative one and subsequent complete flower formation.

Three axillary flowering species, i.e. female *Salix tetrasperma*[16], male *S. babylonica*[19] and male *Morus nigra*[20] have contrasting schedules of reproductive differentiation (Figure 1) with *S. tetrasperma* representing the first category and *S. babylonica* and *Morus nigra* the second category as described earlier.

In vitro culture of excised buds of *S. tetrasperma* just initiated in March produced only vegetative shoots (Figure 1), indicating the lack of any irreversible stimulus. However, excised older buds which had differentiated inflorescence primordia and even floral meristems gave sterile inflorescences with the floral meristems remaining quiescent or developing into leafy sprouts. A progressive change in the morphogenetic response was observed from September to November, when the floral meristems produce partly and predominantly reproductive structures. During December and later normal flowers, characteristic of the species, developed. Absence of clear stages in histological profile during the annual cycle is significant in *S. tetrasperma.*

On the other hand, aseptic culture of just initiated buds of *S. babylonica* at the meristem stage gave vegetative shoots (Figure 1), indicating lack of any irreversible stimulus. Older buds during March to May showed the development of sterile inflorescences, with floral meristems remaining quiescent or developing into leafy structures. Buds having preformed flower primordia showed the development of partly sterile anther stumps or anthers without pollen when cultured *in vitro* during June to September. The buds produced normal flowers with viable looking pollen in anthers when cultured in November and later. It is thus interesting to note that though the buds appear to be ripe-to-flower in the morphological sense in August, it is only towards November that they actually show anthesis. In *M. nigra* the youngest buds cultured from late March to May produced aberrant reproductive structures (Figure 1). However, perfect flowers were obtained from excised buds during June and later. Like *S. babylonica*, in this case also full reproductive potential of the buds is expressed after the differentiation of apparently mature preformed flowers.

It is clear from the above account that the histological differentiation of buds is not a measure of their intrinsic morphogenetic potential and the ripe-to-flower state of the buds may temporally precede (as in *S. tetrasperma*) or succeed (as in *S. babylonica* and *M. nigra*) the histologically discernible stages. However, buds can be forced to sprout *in vitro* at sequential stages through the annual cycle and an imagery of their developmental events visualized.

The buds seem to be continuously affected by endogenous and environmental factors *in situ* that result in their progressive transformation towards the ripe-to-flower condition and a self-perpetuating flowering stimulus does not seem to be present in the generative buds as the young differentiated buds do not produce perfect flowers[16,19,20]. Experimental studies with *Citrus* also show that the determined buds revert to vegetative sprouts when cultured *in vitro*[22] unless provided with a specifically permissive inductive stimulus of ringing[23] or low temperature[24]. Further evidence that this pattern could be of general occurrence comes from the consideration of sporadic reports where generative buds of trees when forced to sprout prematurely, by treatments such as excessive pruning, produce aberrant structures instead of flowers[25–29]. Naylor[30] generalized that in flowering plants a 'teeter-point' exists between vegetative and reproductive development and if the signal received for floral induction is insufficient, a mixture of leaves and flowers develop.

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Induction of hairy roots in tea (*Camellia sinensis* L.) using *Agrobacterium rhizogenes*

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***Agrobacterium rhizogenes* strain A4-mediated 'hairy roots' have been induced for the first time in leaves of *in vitro*-grown seedlings of *Camellia sinensis*. The hairy roots originated at the proximal end of the young leaves after 17 ± 2 days of bacterial inoculation. These hairy roots were cultured in hormone-free MS medium, and MS supplemented with 1 mg/l NAA. Root biomass production was better in auxin-containing medium.**

AGROBACTERIUM RHIZOGENES is the causal agent of the hairy root disease and is characterized by its ability to cause root proliferation at the site of infection of the susceptible hosts. Virulence of this species is dependent on the root-inducing Ri-plasmid a portion of which, called 'T-DNA', is transferred and integrated into the host plant DNA (refs. 1, 2). Susceptibility towards *A. rhizogenes* infection varies among different plant families and also with the bacterial strains[3]. Such hairy roots are characterized by their ability to grow profusely on a hormone-free culture medium under *in vitro* condition in a large number of plant species[4]. The major consideration behind induction and *in vitro* culture of *A. rhizogenes*-mediated hairy roots has so far been to device an alternate strategy for the production of commercially useful secondary metabolites of plant origin which are known to be synthesized and accumulated in roots[5,6]. In a few recent studies, however, the root-inducing capacity of Ri-plasmid has also been gainfully employed in vegetative cloning of some otherwise difficult to root, recalcitrant woody plant species such as almond, olive, *Actinidia deliciosa* and apple[7–11]. The approach was found to work satisfactorily both in species where self-rooted cuttings or efficient root-stocks were required.

Camellia sinensis (family Theaceae) is an important cash crop of India. Like other woody perennials, the rooting efficiency of the stem cutting of tea is low[12]. This, coupled with slow vegetative growth rates of cutting, poses a serious limitation in the early introduction of high-yielding clones of tea for their commercial plantation and cultivation. Cell culture-based micropropagation approaches such as axillary or apical bud proliferation, induction of somatic embryogenesis and multiple shoot cultures are being tried in many laboratories to overcome this problem[13–17]. While establishment and *in vitro* proliferation of microshoots is now a routine in tea tissue cultures[6,17], rooting in these shoots and regeneration frequency of complete plantlets is still very low[18]. Based on this information, it was thought logical to explore the possibility of improving