Cytokinins and *in vitro* induction of flowering in bamboo: *Bambusa arundinacea* (Retz.) Willd

Mohini Joshi and Rajani S. Nadgauda

Tissue Culture Pilot Plant Division, National Chemical Laboratory, Pune 411 008, India

Four-week-old seedling explants of *Bambusa arundinacea* were cultured on Murashige and Skoog (MS) basal medium with different cytokinins to study the effect on *in vitro* induction of flowering. The cytokinins used were adenine hemisulphate (AdS), 6-[γ,γ-dimethylallylamino]-purine (2iP), kinetin (Kin), zeatin individually and in combination with 6-benzyl adenine (BA).

BA was found to be the only cytokinin inducing flowering *in vitro* when added individually in the culture medium. The induction of flowering in media containing other cytokinins, when added individually, was not observed. However, induction of flowering was observed in all the cytokinin combinations whenever BA was added. Presence of BA in the culture medium was observed to be absolutely essential for induction of flowering. 2iP showed synergistic effect in combination with BA although it was ineffective in the presence of flower induction alone, while zeatin showed antagonistic effect on induction of flowering in combination with BA.

BAMBOOS have a peculiar flowering behaviour. Most of them flower at the end of a very long vegetative phase followed by death of the clumps. Several workers have tried to explain this peculiar behaviour[1]. Nevertheless it has remained one of the least-understood phenomena. It is usually believed that the flowering in bamboos is controlled by an internal physiological calendar rather than an external weather cue[1,2].

Improvement of bamboos by breeding is still a big challenge. Induction of flowering under *in vitro* conditions has been reported in species of bamboo, viz. *Dendrocalamus strictus*[3], *Bambusa arundinacea*, *Dendrocalamus brandisii*[4], *Dendrocalamus hamiltonii*[5], *Bambusa vulgaris*, *Dendrocalamus giganteus*, and *Dendrocalamus strictus*[6]. A report from our laboratory on comparison of *in vitro* flowering and natural flowering phenomenon in *B. arundinacea* describes in detail the similarities and differences between the two[7]. Potential of *in vitro* flowering in bamboos has already been dicussed[7–10].

There is an extensive literature on different physiological and biochemical signals involved in induction of flowering. It has been shown that cytokinins have promotory as well as inhibitory effects on induction of flowering in a number of plant species, although promotory effects are much more than inhibitory ones[11].

The reports on *in vitro* flowering in *B. arundinacea*, *D. brandisii*[4] and *D. hamiltonii*[5] have one striking similarity, i.e. use of BA in culture media whereas for *B. vulgaris*, *D. giganteus* and *D. strictus* Rout and Das[6] have used adenine hemisulphate (AdS), IBA and GA_3 in combination for induction of flowering. However in all the reports, other cytokinins were not tested. We report here the results of the work carried out to study the effect of different cytokinins, viz. AdS, 6-benzyl adenine (BA), 6-[γ,γ-dimethylallylamino]-purine (2iP), kinetin (Kin) and zeatin individually and AdS, 2iP, Kin and zeatin in combination with BA on *in vitro* induction of flowering in *B. arundinacea*.

Material and methods

Plant material

Seeds of *B. arundinacea* were obtained from Empress Garden, Pune, India. Dehusked seeds were washed in 2% solution of Labolene (Qualigens, India) and treated with 4% Cetrimide (ICI, India) for 10 min. The seeds were then surface-sterilized with 0.1% mercuric chloride solution for 10 min. This was followed by 3–5 rinses with sterile distilled water. The surface-sterilized seeds were inoculated on half-strength MS basal medium[12] without any plant growth regulators. The culture tubes were incubated in the dark at 25 ± 2°. The seeds germinated and reached three leaf-stage within four weeks. Seedlings at this stage were used for further experiments.

Experiment 1. Effect of cytokinins on in vitro induction of flowering

To test the effect of individual cytokinin on induction of flowering, five different cytokinins, viz. BA, 2iP, Kin zeatin were added to control media at concentrations ranging from 2.2 to 9.3 μM excepting zeatin, which was added at the concentration 0.26 μM (Table 1). AdS was tested at 2.71, 27.1, 271 μM.

To study the effect of cytokinins in combination with BA, four of the cytokinins AdS, 2iP, Kin, zeatin were

Table 1. Addition of cytokinins in M1 and M2 media and flower induction response

Cytokinin	Concentration (μM)	Flower induction response
BA	2.22–8.88	+
AdS	2.71–271.0	–
Kin	2.32–9.30	–
2iP	4.90	–
Zeatin	0.26	–
BA + AdS	4.93	+
BA + Kin	4.54	+
BA + 2iP	7.21	+
BA + zeatin	2.5	+

+ Flower induction; – No flower induction.

added to control media containing BA at 2.22 μM as the maximum response of flower induction was observed in media containing BA when added individually. The concentrations of AdS, 2iP, Kin and zeatin chosen were same as the experiment above.

Control media used were MS basal liquid medium with 2% sucrose and with or without CW termed as M1 and M2 respectively. There were in all 20 combinations including two controls, M1 and M2.

Experiment 2. Effect of BA on induction of flowering in cultures when added at the end of third subculture

Similarly, cultures grown in M1 and M2 media containing different cytokinins other than BA were subcultured in the same media but by adding BA at the end of third subculture, i.e. after 16 weeks.

Maintenance of cultures and parameters for observations

Ten seedlings were inoculated in each of the media combinations and the cultures incubated on a rotary shaker at 25 ± 2°C and 60 rpm under continuous illumination of 6.25 μE m^{-2} s^{-1}. Multiple shoots produced in all the combinations of experimental media, including controls M1 and M2, were maintained by subculturing at 4 weeks interval in the respective media. The cultures were regularly observed for any visual signs of emergence of floral buds. Mature florets, at the time of anthesis, were dissected under microscope and observed for the presence of floral parts. All the experiments were replicated thrice and the observations were noted at the end of the third subculture. The data were analysed using a χ^2 test for significance. Correlation coefficient between the root elongation and flower induction was calculated using Microsoft Excel (version 5.0).

Results and discussion

Flowering is a unitary and integrated process. It is generally divided into two steps: induction or initiation and expression. These two steps react differently to and are controlled by numerous environmental and physiological stimuli. *In vitro* induction of flowering has been achieved in a number of plant species[11]. In this process there is a sudden shift from juvenile to mature phase even when other characters of maturity such as an inability of shoots to form adventitious roots and an adult leaf morphology are not observed[13]. Different factors which can influence *in vitro* induction of flowering include absence of roots[14,15], correct combination of plant growth regulators[16], physical condition of the medium[17], etc.

Effect of cytokinins

It has been shown that cytokinins, BA and its conjugates in particular, play a significant role in induction of flowering in tobacco thin cell layers[18], cotyledon derived shoots of 'burpless hybrid' cucumber[19], *Arachis hypogaea*[20] and *Boronia megastigma Nees*[21].

In the present study the three leaf-stage seedlings produced multiple shoots when they were inoculated in different media combinations tried. The multiple shoots when subcultured in the respective media showed induction of flowering only after third subculture. Table 1 describes the flower induction response under *in vitro* conditions at different concentrations of cytokinins used. BA at 2.22 μM gave maximum response of flower induction, i.e. 23.07% of cultures compared to other concentrations of BA used, i.e. no induction response in absence of BA to 10.0% at 8.88 μM BA. Table 2

Table 2. Percentage of cultures showing root elongation and induction of flowering in M1 and M2 media with different cytokinins when added individually and in combination with BA

Cytokinin(s)	Concentration (μM)	% Cultures showing RE	% Cultures showing flower induction	No. of spikelets/ No. of vegetative shoots
Zeatin	0.26	100	0	–
AdS	2.71	100	0	–
Kin	2.32	100	0	–
2iP	4.90	100	0	–
BA	2.22	7.69	23.07	0.23
Zeatin + BA	2.5	25.0	6.25 s	0.21 ns
AdS + BA	4.93	20.0	13.33 ns	0.18 ns
Kin + BA	4.54	14.28	21.42 ns	0.085 ns
2iP + BA	7.21	0	44.44 s	0.48 s

M1, MS basal medium + 2% sucrose; M2, MS basal medium + 2% sucrose + 5% CW; RE, root-elongation; s, significant; ns, non-significant at 0.05 probability level according to a χ^2 test.

Figure 1. *a*, Profuse rooting in culture which did not show induction of flowering; *b*, Culture showing induction of flowering; *c*, Culture with spikelet showing florets at anthesis stage; *d*, Close-up of a floret at anthesis stage (× 3.5) (Bar represents 1 mm).

summarizes the data of effect of different cytokinins on flower induction and root elongation when the cytokinins were added individually and in combination with BA in M1 and M2 control media. The concentrations of other cytokinins used in this experiment were equimolar to BA, which gave maximum flower induction response, i.e. 2.22 µM except zeatin. It is evident from Table 2 that except BA none of the other cytokinins tested were able to induce flowering when added individually or with CW. The control media M1 and M2 also failed to induce flowering.

An inverse relationship was observed between the cultures showing induction of flowering and those showing root elongation. The shoots produced in all the cultures, except those in BA-containing medium, showed root elongation (Figure 1 *a*) when cytokinins were tested individually for their ability to induce flowering. The induction of flowering was not observed in these cultures. Media containing only BA showed induction of flowering (Figure 1 *b*) where the percentage of cultures showing root elongation was 7.69 (Table 2).

Similarly when the cytokinins were added in combination with BA, root elongation was observed in the cultures excepting those grown in media containing BA in combination with 2iP. The flower induction in this combination, i.e. BA + 2iP, was significantly higher compared to BA alone. As the percentage of cultures showing root elongation increased from 0.0 in media with BA + 2iP to 25.0 in media with BA + zeatin, the percentage of cultures showing flower induction decreased from 44.44 to 6.25 respectively (Table 2). The correlation coefficient of –0.96 was observed between the cultures with root elongation and cultures showing flower induction for all the combinations which showed positive flower induction response. The root elongation whenever observed was from the first subculture of the explants in the respective media.

Besides the correlation between the root elongation and induction of flowering, it was observed that the addition of Kin and AdS to medium in combination with BA did not significantly affect the induction response while addition of zeatin and 2iP in combination with BA did, where zeatin showed negative while 2iP showed positive induction response. Also, the ratio of number of spikelets to that of vegetative shoots produced in medium with BA and 2iP in combination was significantly higher than BA alone. Other combinations, however, did not show any significant increase in the number of spikelets produced (Table 2).

The florets, at the time of anthesis from induced cultures, when dissected showed the presence of all the floral parts, i.e. lemma, palea, lodicules, gynoecium consisting of ovary, style and stigma, and androecium with 6 anthers at the tip of long filaments (Figure 1 *c*, *d*).

When the cultures were treated with cytokinins other than BA for three subcultures, i.e. 16 weeks from initiation and then exposed to BA at the end of the third subculture, there was no induction of flowering. This has confirmed that the presence of BA in the culture medium was essential from the initiation stage for optimum flower induction response in this species.

Conclusions

The present study revealed the following important points:

- BA was the only cytokinin among the different cytokinins tested effective in induction of flowering. Cultures grown on individual cytokinin-containing media other than BA failed to induce flowering.
- All cultures grown in individual cytokinin-containing media, except BA, showed root elongation and no induction of flowering. The percentage of cultures showing root elongation was less in BA-containing media where flower induction was also observed.

- Root elongation and flower induction had an inverse relationship. The cultures grown in media containing 2iP in combination with BA never developed roots and maximum percentage of cultures showed flower induction (correlation coefficient = – 0.96).
- BA and 2iP showed synergistic effect on induction of flowering. 2iP alone was found to be ineffective in the process of *in vitro* induction of flowering. This synergistic effect of 2iP with BA was not apparent when BA was applied to the cultures grown on 2iP alone for 16 weeks.

From the present study and other reports on *in vitro* flowering in bamboos[3–5], it has become clear that BA plays an important role in induction of flowering when applied exogeneously to seedling cultures. Therefore, endogenous status of different hormones at the time of shift from vegetative to flowering stage remains to be studied.

1. Janzen, D. H., *Annu. Rev. Ecol. Syst.*, 1976, **7**, 347–391.
2. John, C. K., Nadgauda, R. S. and Mascarenhas, A. F., *Curr. Sci.*, 1995, **68**, 885–896.
3. Nadgauda, R. S., Parasharami, V. A. and Mascarenhas, A. F., *Nature*, 1990, **344**, 335–336.
4. Rao, I. V. R. and Rao, I. U., in *Bamboos: Current Research* (eds Rao, I. V. R., Gnanaharan, R. and Sastry, C. B.), Proceedings of the International Bamboo Workshop, International Development Research Center, Canada, 1990, Nov. 14–18, pp. 151–158.
5. Chambers, S. M., Heuch, J. H. R. and Pirrie, A., *Plant Cell Tissue Organ Cult.*, 1991, **27**, 45–48.
6. Rout, G. R. and Das, P., *Plant Cell Rep.*, 1994, **13**, 683–686.
7. Nadgauda, R. S., John, C. K., Parasharami, V. A., Joshi, M. S. and Mascarenhas, A. F., *Plant Cell Tissue Organ Cult.*, 1997, in press.
8. Nadgauda, R. S., John, C. K. and Mascarenhas, A. F., *BIC – India Bull.*, 1993, **3**, 14–20.
9. Nadgauda, R. S., John, C. K. and Mascarenhas, A. F., *Tree Physiol.*, 1993, **13**, 401–408.
10. Nadgauda, R. S., John, C. K., Joshi, M. S., Parasharami, V. A. and Mascarenhas, A. F., in 'The Bamboos', An international symposium held at the Linnean Soc. of London, London UK, March 25–29, 1997, p. 1.
11. Bernier, G., *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1988, **39**, 175–219.
12. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
13. Gerorge, E. F., in *Plant Production by Tissue Culture* (ed. George, E. F.), Exegetics Limited, UK, 1993, part 1, pp. 243–247.
14. Henrickson, C. E., *Plant Physiol.*, 1954, 536–538.
15. Dennin, K. A. and McDaniel, C. A., *Dev. Biol.*, 1985, **112**, 377–382.
16. Gertsson, U. E., *J. Hortic. Sci.*, 1988, **63**, 497–502.
17. Coussan, A. and Tran Thanh Van, K., *Physiol Plant.*, 1981, **51**, 77–84.
18. Van der Krieken, W. M., Croes, A. F., Smulders, M. J. M. and Wullems, G. J., *Plant Physiol.*, 1990, **92**, 565–569.
19. Al-Juboory, K., Skirvin, R. M. and Williams, D. J., *Hortsci.*, 1991 **26**, 1085.
20. Kachonpadungkitti, Y., Hisajima, S. and Arai, Y., *Biosci. Biotech. Biochem.*, 1992, **56**, 543–546.
21. Roberts, N. J., Luckman, G. A. and Menary, R. C., *J. Plant Growth Regul.*, 1993, **12**, 117–122.

ACKNOWLEDGEMENT. We acknowledge UGC for grant of fellowship to Mohini Joshi. We wish to thank Dr A. Kharshikar, Department of Statistics, University of Pune, India for his valuable help during statistical analysis of data. Thanks are due to Mrs S. V. Kendurkar and Dr C. K. John for suggestions during preparation of the manuscript. Thanks are also due to Ms Shilpa Gogte, Mr Gaurav Mathur for their help during this work and to Mr Parag Akkadkar for photography.

Received 24 March 1997; revised accepted 15 July 1997