Cytokinin Effects on Shoot and Root Formation in *Miscanthus ×ogiformis* 'Giganteus'

Jeanette Møller Nielsen
Department of Ornamentals, Research Centre Årslev, Danish Institute of Plant and Soil Science, Kirstinebjergvej 10, DK-5792 Årslev

INTRODUCTION

Miscanthus ×ogiformis Honda 'Giganteus' is a sterile cultivar which traditionally has been propagated through rhizome division (Nielsen, 1987). In vitro propagation is an alternative method which could become important for large scale propagation. For in vitro propagation the type and concentration of the cytokinin used are of great importance. In the present study the short- and long-term effects of 6-benzyladenine (BA), thidiazuron (TDZ), kinetin (KIN), and isopentenyladenine (2iP) on in vitro cultures of *M. ×ogiformis* are reported.

MATERIALS AND METHODS

Actively growing shoots of *M. ×ogiformis* Honda 'Giganteus' were selected from greenhouse-grown plants. Nodal segments of about 15 mm in length were cut and surface sterilized for 20 min in 1.5% (w/v) sodium hypochlorite followed by three rinses in sterile deionized water. The nodal segments were trimmed to give 5- to 10-mm-long explants.

Murashige and Skoog medium (MS) (Murashige and Skoog, 1962) with 20 μM BA, 1.3 μM naphthaleneacetic acid (NAA), 58.4 mM sucrose, 3 g liter^{-1} Gelrite (Phytagel, Sigma), and 3.7 mM $MgCl_2$ was used. The pH of the medium was adjusted to 5.5 before Gelrite was added. After melting, 50 ml of the medium was dispensed into each 12-cm-high and 8-cm-diameter jar and autoclaved for 15 min at 121C.

Four explants were grown in each jar at a temperature of 27±1C, a photon fluence of 50 μmol m^{-2} s^{-1} provided by the Philips fluorescent TLD 58/33 tubes, and a photoperiod of 16 h. After 28 days of growth, the developed shoot clusters were divided. Single shoots were trimmed to a length of 15 mm and subcultured on the BA-containing MS medium at least four times.

Trimmed axillary shoots from the 20 μM BA-containing medium were transferred to a new type of cytokinin-containing medium. Other growth conditions were as described above. After 28 days, a representative shoot from each shoot cluster was trimmed and transferred to a new jar with the same or new type of medium. After each subculture the number of shoots per shoot cluster were recorded.

Experiment 1 (Nielsen et al., 1993). The concentration of 20 μM BA in the medium was replaced with different concentrations (0.01, 0.1, 1, 10, 30, or 100 μM) of BA, TDZ, KIN, or 2iP. Shoots were grown for four subcultures on the same cytokinin-containing medium. After the 4th subculture, shoots from 30 μM BA- or 30 μM TDZ-containing media were transferred to rooting medium consisting of MS medium with the following modifications: 1/2 strength macronutrients, 5.4 μM NAA, 58.4 mM sucrose, 3 g liter^{-1} Gelrite, 3.7 mM $MgCl_2$, and 5 g liter^{-1} activated charcoal. After each subculture the length of each shoot from the base to the upper

end of the longest leaf sheath, percentage of shoots with chlorosis on a least one leaf, and number of roots per shoot cluster were recorded.

Experiment 2 (Nielsen et al., 1995). The concentration of 20 μM BA in the medium was replaced with 30 μM BA, 30 μM TDZ, 10 μM KIN, or 100 μM 2iP (optimum concentrations for axillary shoot formation) and shoots were grown for four subcultures on the same cytokinin-containing medium. After the 4th subculture shoots were transferred to a 30 μM BA-containing medium.

Experiment 3 (Nielsen et al., 1995). The concentration of 20 μM BA in the medium was replaced with 30 μM BA, 22.5 μM BA + 7.5 μM TDZ, 15 μM BA + 15 μM TDZ, 7.5 μM BA + 22.5 μM TDZ, or 30 μM TDZ. This series of media contained a total of 30 μM cytokinin due to optimum axillary shoot formation on media containing 30 μM BA or TDZ. To test if the response was dependent on one specific cytokinin, the total cytokinin concentration or the ratio between the two cytokinins, another series of media was set to contain a total of 60 μM cytokinin. This was obtained by using 60 μM BA, 30 μM BA + 30 μM TDZ, or 60 μM TDZ.

RESULTS

Experiment 1 (Nielsen et al., 1993). The mean number of shoots formed per shoot cluster for all concentrations of TDZ, KIN, and 2iP in subculture 1 was significantly higher compared to the number of shoots formed in subculture 2, 3, or 4. At each BA concentration, a constant number of shoots per shoot cluster was produced in all four subcultures. The improved shoot formation on TDZ-, KIN-, or 2iP-containing media which was observed only in subculture 1 indicates that the shoots were influenced by the previous subculture with 20 μM BA in the medium.

The optimum concentration for shoot formation in subculture 2 to 4 was 30 μM BA, 30 μM TDZ, 10 μM KIN, and 100 μM 2iP. In descending order regarding shoot formation, the four cytokinins at the optimum concentration could be ranked as follows: BA, TDZ, KIN, and 2iP. Root formation was generally absent at these optimum concentrations. Kinetin and 2iP did not inhibit root formation at 1 μM. Generally, mean shoot size as well as the percentage of chlorotic shoots decreased with increasing concentration of the cytokinins. This indicates that cytokinin concentrations that produce many shoots also produce smaller and less chlorotic shoots.

Although shoot formation in subculture 4 differed significantly between BA and TDZ, no difference in shoot formation was observed after transfer from cytokinin-containing medium to rooting medium. Shoots grown previously on TDZ-containing medium formed a significantly lower number of roots and taller shoots on rooting medium compared to shoots previously grown on BA-containing medium, although the number of roots and shoot size were not significantly different when grown on the two types of cytokinin-containing media. The percentage of chlorotic shoots on BA- or TDZ-containing media and after transfer to rooting medium were not significantly different.

Experiment 2 (Nielsen et al., 1995). The carry-over effect observed in subculture 1, Experiment 1, was also observed in Experiment 2 (Table 1). When transferring shoots from BA-containing media to KIN- or 2iP-containing media the number of shoots formed corresponded to the number of shoots formed by continuous culture on BA-containing medium. The movement from BA- to TDZ-containing medium

resulted in a doubling in the number of shoots formed. When transferring shoots from KIN- or 2iP-containing medium to BA-containing medium the number of shoots formed corresponded to the number of shoots formed by continuous culture on KIN- or 2iP-containing medium, respectively. Shoots exposed to TDZ acted differently because transference to BA-containing medium resulted in a much reduced number of shoots. Continuous culture on TDZ-, KIN-, or 2iP-containing media induced fewer shoots compared to continuous culture on BA-containing medium (Table 1).

Table 1. Axillary shoot formation when transferring shoots between different cytokinin-containing media.

Cytokinin-containing media			
Previous subculture	Subsequent subculture	Number of shoots per shoot cluster	Treatment
BA	BA	5.3	1
BA	TDZ	10.9	1
BA	KIN	5.3	1
BA	2iP	6.2	1
BA	BA	6.2	2
TDZ	TDZ	3.0	2
KIN	KIN	2.9	2
2iP	2iP	3.1	2
BA	BA	6.2	3
TDZ	BA	2.3	3
KIN	BA	3.8	3
2iP	BA	3.2	3

Treatments:

1= Shoots grown on a medium containing 20 μM BA were transferred to a medium containing 30 μM BA, 30 μM TDZ, 10 μM KIN, or 100 μM 2iP.

2= Mean values of four subcultures of transfer from one of these cytokinin-containing media to the same medium.

3= Mean values after transfer from one of the four media to a medium containing 30 μM BA.

Experiment 3 (Nielsen et al., 1995). Different combinations and concentrations of BA and TDZ in one medium were used to test whether the synergistic effect of BA and TDZ in the first subculture could be obtained and maintained in subsequent subcultures (Table 2).

Generally, in the first subculture, shoot formation increased with increasing concentration of TDZ, whereas increasing concentrations of BA increased shoot formation in the second and third subculture. Neither the ratio of TDZ to BA in the medium, nor the combined concentration of cytokinin had any systematic effect on shoot formation. Again the change from BA- to TDZ-containing medium caused the carry-over effect with significantly higher shoot formation on media containing

more than 7.5 μM TDZ. However, shoot formation fell to a lower constant level in subsequent subcultures, also on media containing both cytokinins.

Table 2. Mean number of axillary shoots formed per shoot cluster. Shoots grown on a medium containing 20 μM BA were transferred to media containing different concentrations of BA, TDZ, or BA+TDZ and grown for three subcultures. All cytokinin concentrations are in μM.

	Axillary shoots/shoot cluster Subculture		
Cytokinin-containing media	1	2	3
30 BA	7.7	10.3	10.6
22.5 BA + 7.5 TDZ	10.9	9.4	9.9
15.0 BA + 15 TDZ	13.0	7.7	8.8
7.5 BA + 22.5 TDZ	15.4	7.4	6.9
30 TDZ	15.8	5.6	4.3
60 BA	9.6	10.2	9.5
30 BA + 30 TDZ	14.7	9.3	10.7
60 TDZ	13.9	5.5	6.0

DISCUSSION

The commonly observed cytokinin effects on shoot formation, inhibition of root formation, reduction of stem growth, and delay of senescence occurred in *M. ×ogiformis* 'Giganteus' with BA, TDZ, KIN, and 2iP. The activity of TDZ was comparable to the effects of the three ordinarily used cytokinins in vitro cultures.

When shoots were grown on medium containing one type of cytokinin and transferred to a medium containing a different cytokinin the carry-over effect was only seen in one subculture. By large-scale production of *M. ×ogiformis* it is advisable to grow shoots on 30 μM BA-containing medium and transfer them to a 30 μM TDZ-containing medium in one subculture before the shoots are transferred to a rooting medium.

From the results with BA and TDZ effects on axillary shoot formation in *M. ×ogiformis* a model for mode of cytokinin action in the plant cell is proposed in Nielsen et al. (1995).

LITERATURE CITED

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

Nielsen, J.M., K. Brandt, and **J. Hansen**.1993. Long-term effects of thidiazuron are intermediate between benzyladenine, kinetin or isopentenyladenine in *Miscanthus sinensis*. Plant Cell Tissue Organ Cult. 35:173- 179.

Nielsen, J.M., J. Hansen, and **K. Brandt.** 1995. Synergism of thidiazuron and benzyladenine depends on sequence of application in *Miscanthus ×ogiformis* 'Giganteus'. Plant Cell Tissue Organ Cult. 41:165-170.

Nielsen, P.N. 1987. Vegetative propagation of *Miscanthus sinensis* 'Giganteus'. Tidsskr. Planteavl 91:361-368.