Effects of 2,4-D and zeatin riboside on pollen callus induction in barley anther culture

Ye, J. M.; Harvey, B. L.; Kao, K. N.

Publisher's version / Version de l'éditeur:
https://doi.org/10.4141/cjps85-004
Canadian Journal of Plant Science, 65, 1, pp. 29-32, 1985-01-01

EFFECTS OF 2,4-D AND ZEATIN RIBOSIDE ON POLLEN CALLUS INDUCTION IN BARLEY ANTHER CULTURE

J. M. YE[1], B. L. HARVEY[1], and K. N. KAO[2]

[1]*Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Sask. S7N 0W0; and* [2]*Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Sask. S7N 0W9.* *Received 17 Nov. 1983, accepted 9 July 1984.*

YE, J. M., HARVEY, B. L. AND KAO, K. N. 1985. Effect of 2,4-D and zeatin riboside on pollen callus induction in barley anther culture. Can. J. Plant Sci. **65**: 29–32.

Anthers of two barley (*Hordeum vulgare* L.) cultivars, Bonanza and Elrose were cultured in media A or K1 with different levels of 2,4-D and zeatin riboside (Z-r). The results indicated that a combination of 2,4-D and Z-r in their optimal ranges (0.25–1.0 mg/L and 0.125–0.25 mg/L, respectively) significantly increased pollen callus induction in Bonanza anthers. Addition of 2,4-D alone (optimal range 0.25–2.0 mg/L) increased the frequency of callus formation in Elrose, while Z-r (0.125–2.0 mg/L) had no obvious effect on callus induction. When Z-r concentration was higher than 2.0 mg/L it decreased callus formation. At high concentrations (2.0–4.0 mg/L) 2,4-D alone induced loose calli which were not able to develop into plantlets.

Key words: Barley, anther culture, 2,4-D, zeatin riboside

[Effets du 2,4-D et du riboside de zéatine sur l'induction du callus pollinique dans les cultures d'anthères d'orge.]

Titre abrégé: Culture d'anthères d'orge.

On a cultivé des anthères des cultivars d'orge (*Hordeum vulgare* L.) Bonanza et Elrose sur les milieux A et K1 à une concentration variable de 2,4-D et de riboside de zéatine (Z-r). Les résultats montrent que le mélange de 2,4-D et de Z-r à leur concentration optimale (respectivement 0,25–1,0 mg/L et 0,125–0,25 mg/L) augmente sensiblement l'induction du callus pollinique dans les anthères de Bonanza. L'addition de 2,4-D seul (concentration optimale 0,25–2,0 mg/L) aumente la formation du callus chez Elrose, mais celle de Z-r (0,125–2,0 mg/L) ne semble pas avoir d'effet à ce niveau. Lorsque la concentration de Z-r dépasse 2,0 mg/L, on note une réduction de la formation du callus. A concentration élevée (2,0–4,0 mg/L), le 2,4-D employé seul entraîne la formation de callus lâches qui ne produisent pas de plantules.

Mots clés: Orge, culture d'anthères, 2,4-D, riboside de zéatine

Since Clapham (1973 succeeded in producing barley (*Hordeum vulgare* L.) plants from another culture, attempts to increase the efficiency of pollen callus and plantlet formation have been made by a number of scientists (Pearson and Nilan 1975; Wilson 1977; Gonzalez-Medina and Bouharmont 1978; Xu and Sunderland 1981; Xu et al. 1981). Huang and Sunderland (1982) found that cold pretreatment of barley anthers increased the frequency of callus formation. Kao (1981) floated anthers on liquid media containing Ficoll and this also resulted in a high frequency of pollen callus formation.

In this publication the effects of 2,4-dichlorophenoxyacetic acid (2,4-D) and zeatin riboside (Z-r) on pollen callus formation in Ficoll media are reported.

MATERIALS AND METHODS

Two barley cultivars, Bonanza (six-row) and Elrose (two-row) were used. Ye (1982) indicated that these two genotypes differed in their media requirements. Based on this work and that of Kao (1981) liquid medium A (Kao and Horn 1982) with 10% Ficoll was selected for Bonanza and liquid medium K1 (Kao 1981) with 10% Ficoll for Elrose. Anthers were obtained from field-grown plants in 1981 and 1982.

For each basic medium, six levels of 2,4-D (0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/L) and six levels of Z-r (0, 0.125, 0.5, 1.0 and 2.0 mg/L) were tested. A total of 36 combinations (6 × 6) from the different concentrations of the two hormones were prepared for each basic medium. All liquid media were filter sterilized with a 0.22 μm Millipore filter.

Since the pollen of three anthers within a floret are in the same developmental stage, spikelets from one side of a spike are almost developmentally equal to the equivalent spikelets from the opposite side of the spike (Bennett and Finch 1971). This makes it possible to compare six treatments at the same time. Because six treatments were tested at one time the comparison of 36 combinations of the two hormones was divided into six parts. That is, at each level of Z-r, six levels of 2,4-D were tested. Similarly, at each level of 2,4-D, six levels of Z-r were tested. A sample consisted of eight anthers. The number of cultured spikes (i.e. replications) for each test varied from 8 to 28. Anthers containing tetrads and pollen at uninuclear stage were placed on the media in the wells of Micro Test II tissue culture plates. The developmental stage of pollen in the anthers was determined by observing the live anthers under an inverted microscope. The plates were incubated in darknss at 25°C.

The number of anthers with pollen calli and total number of calli per 100 anthers were counted after 30 days of culturing. These data were analyzed using the two-way analysis of variance. Least significant differences (LSD) was used for the multiple comparisons of treatment means in each experiment (Steel and Torrie 1980).

Calli over 1 mm in diameter were transferred to plant growth regulator-free medium A or K1 containing 0.8% agar and reduced sucrose and glucose concentrations (5% and 0%, respectively). They were then incubated under constant light (4000 lx cool white fluorescent) at 25°C. The extent of plantlet formation from the calli for both cultivars was assessed.

RESULTS AND DISCUSSION

The results showed that both Bonanza and Elrose anthers could produce pollen calli without exogenous growth regulators and the frequencies of callus formation were 16% and 13%, respectively (Table 1). Addition of 2,4-D at appropriate levels to media A and K1 greatly promoted callus formation. For Bonanza in medium A, the optimal concentration range of 2,4-D was from 0.25 to 1.0 mg/L. It was not possible to test for Z-r effects in the experiment designed to test 2,4-D effects because the Z-r effects were confounded by spike effects (Chien and Kao 1983). Another experiment was carried out to assess the effects of Z-r

Table 1. Effect of 2,4-D on pollen callus formation in cultured anthers with zeatin riboside varying from 0 to 2.0 mg/L

Range of Z-r (mg/L)	Level of 2,4-D (mg/L)	Mean number of anthers producing pollen calli per 100 cultured anthers			
		Bonanza		Elrose	
0-2.0	0	16*c*	(55)†	17*b*	(34)
	0.25	28*ab*	(98)	27*a*	(56)
	0.50	29*ab*	(99)	27*a*	(62)
	1.0	32*a*	(103)	26*a*	(57)
	2.0	26*b*	(85)	26*a*	(54)
	4.0	15*c*	(34)	21*ab*	(55)

†The data in parentheses are the total number of calli per 100 cultured anthers.
a-c Means within the same column followed by the same letter are not significantly different ($P<0.05$).

Table 2. Effect of zeatin riboside on pollen callus formation in cultured anthers with 2,4-D varying from 0 to 4 mg/L

Range of 2,4-D (mg/L)	Level of Z-r (mg/L)	Mean number of anthers producing pollen calli per 100 cultured anthers			
		Bonanza		Elrose	
0-4.0	0	27*bc*	(98)†	24*a*	(54)
	0.125	35*a*	(156)	23*a*	(53)
	0.25	30*ab*	(116)	24*a*	(51)
	0.5	27*bc*	(100)	22*a*	(48)
	1.0	28*b*	(102)	24*a*	(46)
	2.0	22*c*	(75)	23*a*	(54)

†The data in parentheses are the total number of calli per 100 cultured anthers.
a-c Means within the same column followed by the same letter are not significantly different ($P<0.05$).

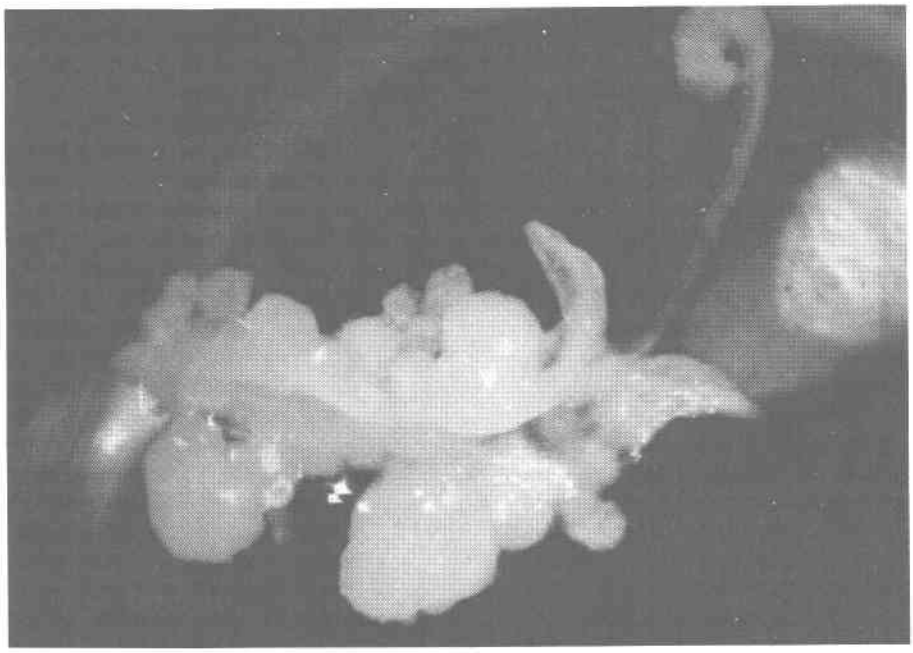

Fig. 1. Embryo-like calli. The compact, milk-white, embryo-like calli, from an anther of Bonanza barley, are growing in medium A containing 0.25 mg/L 2,4-D and 0.25 mg/L zeatin riboside. Magnification 510×.

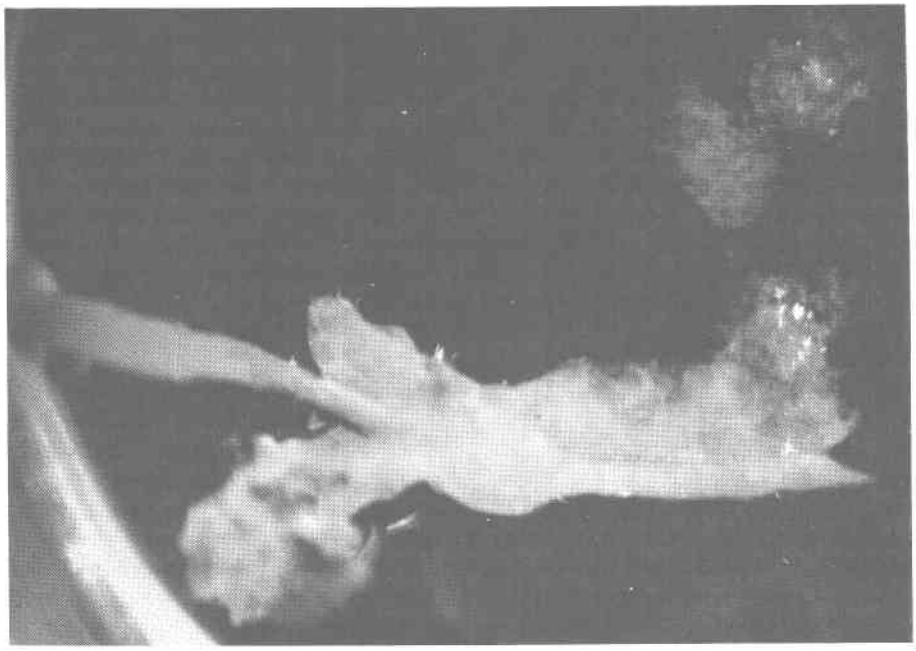

Fig. 2. Loose calli, formed from an anther of Bonanza barley, growing in medium A containing 4.0 mg/L 2,4-D alone. Magnification 540×.

at different levels of 2,4-D. The results indicated that Z-r at 0.125–0.25 mg/L Z-r had a promoting effect on pollen callus formation in anthers from Bonanza but no obvious effect on anthers from Elrose (Table 2).

In both cultivars the effects of the combined use of these two growth regulators within their optimal rage resulted in the formation of embryo-like calli (Fig. 1). Higher concentrations of Z-r (2 mg/L or over) decreased callus formation in Bonanza, while higher levels of 2,4-D (over 2 mg/L) caused the formation of loose callus in both cultivars (Fig. 2). These loose calli were not able to develop into plantlets.

The frequency of plant formation was very low in both cultivars. Many calli ceased to develop further after they were transferred onto plant-growth-regulator-free medium. It may be that the calli still require exogenous growth regulators for survival or develpment. Kao and Horn (1982) obtained a high frequency of pollen plant formation when the pollen calli were cultured on media containing low levels of exogenous 2,4-D and Z-r.

A total of 891 calli of Bonanza were transferred onto agar medium and 52 plants were obtained but all of them were albino. Among 828 calli of Elros, 34 green and 19 albino plants were obtained.

Bennett, M. D. and Finch, R. A. 1971. Duration of meiosis in barley. Genet. Res. Camb. **17**: 209–214.

Chien, Y. C. and Kao, K. N. 1983. Effects of osmolality, cytokinin and organic acids on callus formation in Triticale anthers. Can. J. Bot. **61**: 639–641.

Clapham, D. 1973. Haploid *Hordeum* plants from anthers in vitro. Z. Pflanzenzücht. **69**: 142–155.

Gonzalez-Medina, M. and Bouharmont, J. 1978. Experiments on anther culture in barley. Influence of culture methods on cell proliferation and organ differentiation. Euphytica **27**: 553–559.

Huang, B. and Sunderland, N. 1982. Temperature-stress pretreatment barley anther culture. Ann. Bot. **49**: 77–88.

Kao, K. N. 1981. Plant formation from barley anther culture with Ficoll media. Z. Pflanzenphysiol. **103**: 437–443.

Kao, K. N. and Horn, D. C. 1982. A method for induction of pollen plants in barley. Pages 529–530 *in* Akio Fujiwara, ed. Proc. 5th Int. Con. Plant Tissue Cell Culture. Abe Photo Printing Co., Ltd., Tokyo, Japan.

Pearson, O. W. and Nilan, R. A. 1975. Barley pollen culture. Barley Genet. Newsletter **5**: 35–39.

Steel, R. D. and Torrie, J. H. 1980. Principles and procedures of statistics. A biometrical approach. 2nd ed. McGraw-Hill Book Company, New York. pp. 173–177, 195–201.

Wilson, H. M. 1977. Culture of whole barley spikes stimulates high frequency in anthers of *Hordeum vulgare* L. J. Exp. Bot. **29**: 227–238.

Xu, Z. H., Huang, B. and Sunderland, N. 1981. Culture of barley anthers in conditioned medium. J. Exp. Bot. **32**: 767–778.

Xu, Z. H. and Sunderland, N. 1981. Glutamine, inositol and conditioning factor in the production of barley pollen callus in vitro. Plant Sci. Lett. **23**: 161–168.

Ye, J. M. 1982. The improvement of barley anther culture in liquid media. M.Sc. thesis. Department of Crop Science, University of Saskatchewan, Saskatoon, Sask.