Ancymidol Hastens in Vitro Bud Development in Melon

Victor Gaba¹, Chassia Elman¹, and Abed A. Watad²

Agricultural Research Organization, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

Dennis J. Gray

Institute of Food and Agricultural Sciences, Central Florida Research and Education Center, University of Florida, 5523 University Avenue, Leesburg, FL 34748-8232

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Abstract. Cotyledonary explants of melon (*Cucumis melo* L. cv. Galia) regenerate primordia and buds in vitro induced by benzyladenine. The anti-gibberellin ancymidol can stimulate the rate of regeneration on melon explants in the presence of benzyladenine. Concentrations of benzyladenine plus ancymidol that are individually ineffective can act synergistically to stimulate regeneration. Gibberellic acid reduces the rate of regeneration induced by benzyladenine or benzyladenine plus ancymidol. Chemical names used: *N*-(phenylmethyl)-1*H*-purine-6-amine (benzyladenine); alpha-cyclopropyl-alpha-(4-methoxy-phenyl)-5-pyrimidine methanol (ancymidol).

There are several reports on the effect of compounds with anti-gibberellin activity on in vitro regeneration (see George, 1993). Generally, the use of anti-gibberellins leads to a stimulation of morphogenetic processes, such as production of somatic embryos in *Citrus sinensis* Osb. callus (Spiegel-Roy and Saad, 1986), promotion of *Gladiolus* spp. cormel formation (Steinitz et al., 1991; Ziv, 1989), *Albizia julibrissin* Durazz. shoot proliferation (Sankhla et al., 1993), and *Philodendron hastatum* × *imble* × *wendelandii* bud multiplication (Ziv and Ariel, 1991).

The initiation of regeneration is important in plant transformation. While investigating the control of the initiation of in vitro bud production by growth regulators in melon (*Cucumis melo* L.), we found that gibberellin status is important in regeneration. Here we report that an inhibitor of gibberellin biosynthesis also affects the kinetics of regeneration in melon.

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¹Department of Virology.

²Department of Ornamental Horticulture.

Materials and Methods

Seeds of the commercially important Israeli melon 'Galia' had the seedcoats removed and were sterilized for 20 min in a solution of 1% sodium hypochlorite, with two drops of Tween 20 per 100 mL solution. After washing with sterile distilled water, seeds were germinated on Murashige and Skoog (1962) medium with 3% sucrose, vitamins and Bacto agar (Difco) at 8 g·L⁻¹ in a growth room at 26 °C, 16-h photoperiod, 100 μmol·m⁻²·s⁻¹ cool-white fluorescent light. After 4 days, the hypocotyl, apical bud, and adjoining 2 to 3 mm of the cotyledons were excised from seedlings and discarded, and the remaining cotyledons

halved transversely. Only experiments using explants derived from the part proximal to the apex of the seedling are reported, as only these explants gave a consistently high regeneration response. Explants were placed in 9-cm-diameter petri dishes with the abaxial side on agar-solidified medium. The basal medium was amended with *N*-(phenylmethyl)-1*H*-purine-6-amine (benzyladenine) (BA), with or without 1.08 μM alpha-cyclopropyl-alpha-(4-methoxy-phenyl)-5-pyrimidine methanol (ancymidol) (Elenco), or 14.5 μM GA₃ (Sigma Chemicals Co., St. Louis). Explants were observed daily by a dissecting microscope and scored for the first appearance of primordia. In each treatment, 16 or 20 explants were used, four explants per petri dish. Experiments were repeated once. Results were consistent, but the timing of the response between each set of experiments varied. Means of individual pairs of results were separated by chi-squared test.

Results and Discussion

After 12 days in culture (Fig. 1a), regeneration of samples treated with ancymidol + BA was significantly better ($P \leq 0.001$) than of samples with the intermediate concentrations (0.44 and 1.1 μM) of BA alone. At 4.4 and 0.11 μM BA, ancymidol did not affect the response. After 28 days in culture, there was still a significant effect ($P \leq 0.03$) of ancymidol at 0.44 μM BA (Fig. 1b). Melon explants placed on growth regulator-free medium did not regenerate shoot buds, as also noted by Leshem et al. (1994). Medium containing ancymidol by itself did not promote bud regeneration (data not shown). Another compound with anti-gibberellin activity, (2*RS*, 3*RS*)-D(4-chlorophenyl)-4,4-dimethyl-2 (1*H*-1,2,4-triazyl-1-yl pentan-3-ol) (paclobutrazol), by itself did not stimulate bud regeneration in melon (Leshem et al., 1994).

The morphogenetic effect of ancymidol was observed at an early developmental stage (appearance of the first primordia) (Fig. 1a).

Fig. 1. Effect of benzyladenine (BA) on regeneration of melon explants in the presence of ancymidol. Percentage of explants regenerating with and without 1.08 μM ancymidol were scored after 12 days (a: primordia) and 28 days (b: buds). Individual pairs of results were separated by chi-squared test. Mean separation within pairs in a at $P \leq 0.01$, and in b at $P \leq 0.05$. Solid bars = without ancymidol; hollow bars = with ancymidol.

Therefore, inhibitors of gibberellin biosynthesis affect not only the final growth response (Sankhla et al., 1993; Spiegel-Roy and Saad, 1986; Steinitz et al., 1991; Ziv, 1989), but can also affect the rate of developmental processes. Also, the interaction between BA and the inhibitor of gibberellin biosynthesis is only observable at nonsaturating levels of BA (i.e., 1.1 μM or less). Additionally, after 12 days in culture there is a synergism at a BA concentration of 0.44 μM between the cytokinin and the anti-gibberellin (Fig. 1a): i.e., the compounds separately cannot elicit any response (0%), but together the response is large (88%). BA activity is only evident after 12 days at the highest concentration (4.4 μM), and effects of the lower concentrations are only noticeable after 28 days (Fig. 1). However, ancymidol action started more rapidly, and to about the same extent at 0.44 μM BA at 12 days to 28 days (Fig. 1).

Ancymidol functions by specifically blocking the pathway of gibberellin biosynthesis (Graebe, 1987), thereby reducing the level of endogenous gibberellins. Theoretically, the reduction of endogenous gibberellin permits BA to stimulate primordia and bud development. This theory is bolstered by the fact that addition of gibberellins to regeneration media often reduces morphogenesis (see George, 1993). We also find this with in vitro development of melon. In another series of experiments, in the presence of 4.4 μM BA, 95% of explants began regeneration after 13 days in

Table 1. Regeneration of primordia on melon cotyledons under the influence of benzyladenine (BA), ancymidol (ANC), and gibberellic acid (GA_3).

Days of treatment	Explants with primordia (%)		
	BA ^z	BA + ANC	BA + ANC + GA_3
13	0 a ^y	60 b	5 a
17	0 a	80 c	40 b

^zExplants were incubated in the presence of BA (0.44 μM), or with the addition of ancymidol (1.08 μM), or ancymidol and GA_3 (14.5 μM), and the appearance of primordia was scored.

^yMean separation at $P \leq 0.01$ by paired chi-squared test.

culture, compared to 40% of explants incubated with 4.4 μM BA + 14.5 μM GA_3 (statistically significant at $P \leq 0.01$). Additionally, at a concentration of BA at which the ancymidol has a strong effect we find that the response to ancymidol is reversed by the simultaneous application of GA_3 and that the effect of GA_3 is reduced with time (Table 1).

In conclusion, these results demonstrate that ancymidol promotes regeneration in melon at low levels of exogenous BA, demonstrating a synergistic effect between an inhibitor of gibberellin synthesis and a cytokinin.

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