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BRIEF COMMUNICATION

Multiple Shoot Formation from Almond Embryos

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Abstract. Stimulation of multiple shoot formation from almond embryos using 6-benzylaminopurine was studied. Maximum shoot numbers and shoot growth were obtained with 2.5 μM in the growth medium. Continuous multiple shoot formation from cut shoots was also achieved.

Recently CHENG *et al.* (1980), reported that soybean seeds formed multiple shoots in a medium containing 6-benzylaminopurine (BAP) and indolebutyric acid (IRA). This study was extended using selected plant seeds, including almond seeds and led to the finding that multiple shoot formation stimulated by BAP alone is a common phenomenon in plant seeds (HISAJIMA 1981). In the present study it is shown that multiple shoots are formed by almond embryos when stimulated by BAP. In addition, cut shoots derived from BAP-stimulated almond embryos were themselves stimulated by BAP to produce multiple shoots thus achieving a continuous propagation system.

Almond seeds (*Prunus amygdalus* BATSCH) were permitted to swell in tap water overnight. The seeds were sterilized for 15 min in 70% ethanol and for 30 min in 20% Chlorox, successively. After removing excess sterilant with sterile water, the embryos were removed and inoculated in a sterilized medium (modified Murashige and Skoog medium), containing BAP at various concentrations (0—50 μM), 3% sucrose and 0.6% agar (pH 5.5). Modified Murashige and Skoog medium contains per litre: 1650 mg NH_4NO_3, 1900 mg KNO_3, 440 mg $CaCl_2.2H_2O$, 370 mg $MgSO_4.7H_2O$, 170 mg KH_2PO_4, 12.4 mg H_3BO_4, 33.8 mg $MnSO_4.H_2O$, 21 mg $ZnSO_4.7H_2O$, 1.66 mg KI, 0.5 mg $Na_2MoO_4.2H_2O$, 0.05 mg $CuSO_4.5H_2O$, 0.05 mg $CoCl_2.6H_2O$, 15 mg $FeSO_4 . 7H_2O$. 18 mg Na_2EDTA, 0.5 mg inositol and 5 mg thiamine. HCl. The culture conditions consisted of a constant temperature of 25 °C, with

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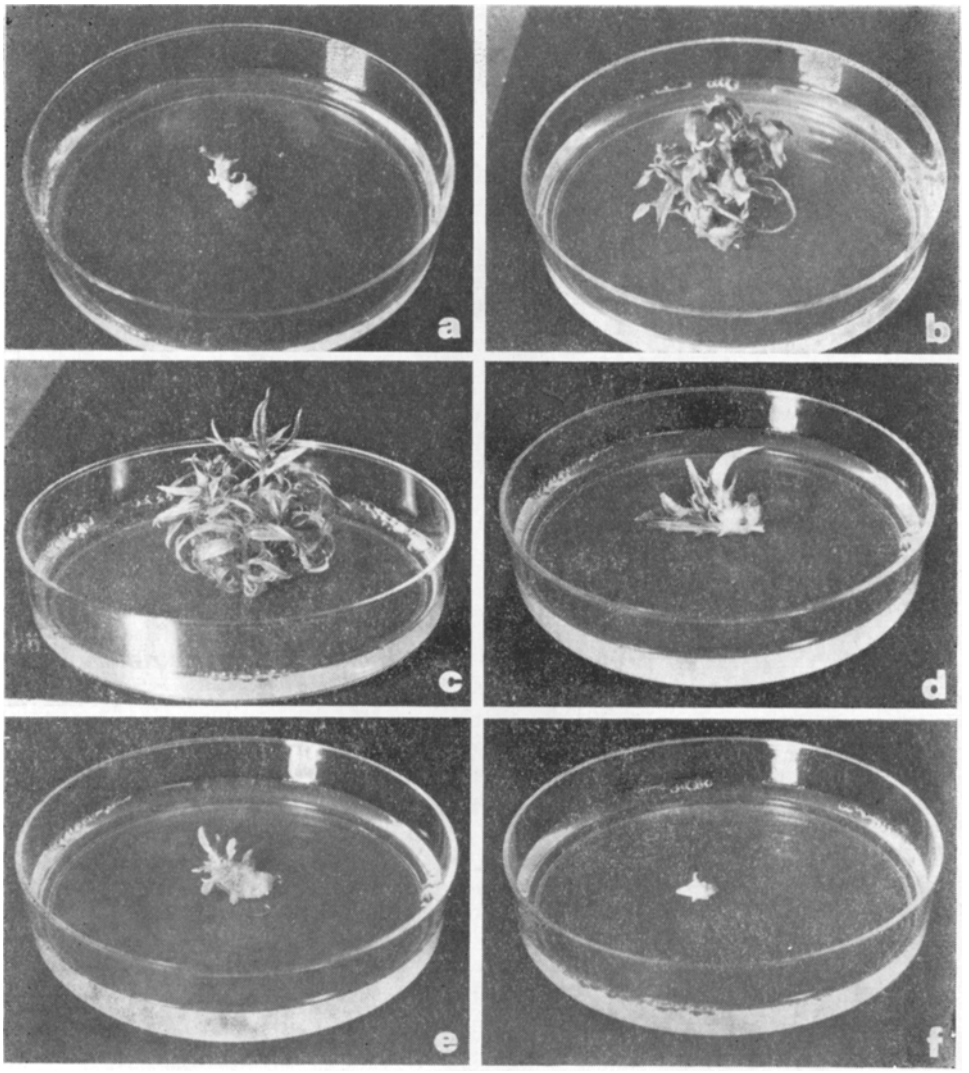

Fig. 1. Effect of BAP on multiple shoot formation. — a: BAP 0 μM; b: BAP 1 μM; c: BAP 2.5 μM; d: BAP 5 μM; e: BAP 25 μM; f: BAP 50 μM. Pictures were taken after shoots had been cultured for four weeks.

18-h photoperiod (21 klx) and a 6-h dark period. Each experimental group consisted of at least 12 cultures and each experiment was repeated at least twice.

Embryo development was sensitive to BAP concentration (Fig. 1, Table 1). The germination rate was about 30% in the absence of BAP and was maximal at 1—2.5 μM BAP; the germination rate decreased when the BAP concentration was greater than 2.5 μM.

TABLE 1
Effect of BAP concentration on multiple shoot formation

	BAP concentration [μM]					
	0	1	2.5	5	25	50
Shoot number						
> 5 mm	only main	3	10	2	main shoot	only main
< 5 mm	shoot	many*	many*	5	4	shoot
Shoot growth	+−	++	+++	+	+−	−
Rate of germination	30%	80	80	50	under 20	under 20

* Many small buds. Four-week culture.

Multiple shoots formed from almond embryos stimulated by a wide range of BAP concentrations (1—25 μM, Fig. 1 and Table 1). However, no shoot growth occurred in the higher BAP concentration range. Taking into account the numbers of shoots and buds formed and the shoot growth, effect of BAP attained the optimal was obtained around the 2.5 μM level. A suitable BAP concentration for multiple shoot formation from almond seeds was around 25 μM (HISAJIMA unpublished data). Thus, embryos form multiple shoots at a ten fold lower range concentration of BAP than do seeds. TABACHNIK and KESTER (1977) reported that BAP at 0.1 mg l^{-1} (0.5 μM) produced shoot growth of dormant buds and that shoot proliferation occurred at 1 mg l^{-1} (about 5 μM) of BAP.

Using multiple shoots obtained by stimulating almond embryos with 2.5 μM BAP shoot subculture and shoot multiplication were examined using a medium augmented with combinations of BAP (0—50 μM) and IBA (0—1 μM). Low concentrations of BAP appeared to favor bud formation and shoot growth. Low IBA concentrations also appeared to be more effective than high IBA concentrations. Using a combination of BAP at 1 μM and IBA at 0.025 μM, cut shoots were subcultured and continuous shoot multiplication was achieved. When embryos were first cultured on medium containing 2.5 μM BAP for four weeks and then transferred to fresh medium containing BAP (1 μM) and IBA (0.025 μM), 90—140 shoots (shoot size >0.5 mm) were obtained from one embryo.

The application of these results may provide a new almond propagation method: microplant propagation through multiple shoot formation from embryo. Preliminary studies on almond tree propagation through tissue culture techniques have been published previously (METHRA and METHRA 1974, TABACHNIK and KESTER 1977).

Multiple shoots were not obtained by BAP stimulation of seeds and embryo of *Prunus armeniaca* ansu. Almond embryos may be a better experimental system for analytical studies of effect of BAP on multiple shoot formation from plant embryos.

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BOOK REVIEWS

WAGNER, J. C. (ed.): BIOLOGICAL EFFECTS OF MINERAL FIBRES; 1, 2. — International Agency for Research on Cancer, Lyon 1980, Vol. 30. 494 pp. + 496—1007 pp., clothbound Sw. fr. 60.— + 60.—; US $ 35.00 + 35.00.

In spite of the known mutagenic and carcinogenic hazards associated with the use of mineral fibres, especially asbestos, its commercial value has ensured its continuing use. The 30th volume of the Scientific Publications of the International Agency for Research on Cancer comprises the Proceedings of a meeting held under the sponsorship of the National Institute of Health and Medical Research, France, and of the IARC in Lyon 1979. The aim of the meeting was to review the current knowledge in the whole field of the biological effects of mineral fibres and to stress the need of establishing or improving regulations, which could reduce the possible risks to a minimum. The mineral fibres discussed can be divided into 4 groups: asbestos, other asbest-form fibres, other naturally occurring fibrous minerals, and the man-made mineral fibres. The introductory sections of the Proceedings present contributions on the physics and chemistry of asbestos dust. This is followed by sections on the pathology and cytogenetic effects of asbestos fibres, including papers on the relation of the dimension of the fibres to their biological activity, and on the syncarcinogenicity of various substances such as benz(a)pyrene, N-nitrosodiethyl-amine, cigarette smoke and radiation with asbestos. Results of cytogenetic studies suggest that chromosomal damage is not caused by a direct interaction between fibres and DNA, but that probably these fibres act as disturbing elements, perhaps during DNA replication. The concluding chapters deal with fibres other than asbestos, shown to have biological activity, *e.g.* amosite, chrysolite, tremolite, crocidolite, anthophyllite *etc.* Each section of the book is preceded by a review that sumarizes the contributed papers.

T. GICHNER (*Praha*)

JAUHAR, P. P.: CYTOGENETICS AND BREEDING OF PEARL MILLET AND RELATED SPECIES. — Alan R. Liss, Inc., New York 1981. 310 pp. £ 32.80, DM 144.00.

Pearl millet is a dual-purpose crop. It provides grain for human consumption and fodder for cattle. It is the sixth most important grain and forage crop which is mainly cultivated in Asia and Africa and has a remarkable potential of growing under some of the driest agricultural conditions. In the sub-Saharan Africa, harvests of pearl millet were obtained with as little as 250 mm of annual rainfall. The need for the genetic improvement of this important economic crop as well as its potential as a research tool has been fully realized recently. The purpose of this book was to summarize and integrate the available information on the genetics and cytogenetics not only of pearl millet but also of other *Pennisetum* species, particularly of those of greater importance to man. The introductory chapters deal with the taxonomy and karyology of the genus *Pennisetum*. These are followed by chapters on meiosis, abnormal meiosis and its genetics, haploidy, aneuploidy, structural changes in chromosomes, B chromosomes, hybridization and chromosome relationship and apomixis. Further sections of the book cover the physiology, genetics of qualitative and quantitative characters, induced mutations and floral biology. The concluding chapter reviews the early breeding work and the current breeding strategy. The most important task of breeding pearl millet today is the production of dwarf hybrids and the improving of the nutritive quality of grain with particular reference to protein content and amino acid balance. According to the author," with further research, pearl millet will, hopefully, emerge as one of the leading economically viable crop".

Readership: plant breeders, plant geneticists and cytogeneticists.

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