蚕豆SCE检测环境诱变剂的方法学研究*

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摘要　通过大量探索性试验，对蚕豆根尖细胞SCE的Brdu-Feulgen法的试验条件，如Brdu浓度、Brdu标记时间及其长短，稀酸水解浓度、时间和温度等对分色的影响进行了详尽的研究，获得了最佳的实验条件及程序，克服了SCE研究沿用的FPG法程序复杂，难以普及的缺点。此外，还用其他遗传毒理学方法与本方法进行了比较研究，探索利用该技术检测环境中诱变因素的可能性。

关键词　蚕豆，SCE，Brdu-Feulgen法。

自从Kihlman等[1]用荧光-Giemsa法(Florescent plus Giemsa technique，FPG)研究蚕豆姐妹染色单体交换(SCE)以来，所有SCE研究均沿用了FPG法，并有不少报道[2—4]。但该法程序繁杂，且不够经济，难以推广。1982年张自立等简化了FPG法程序，发展成Brdu-Giemsa法[5]，但仍较复杂。其后佘其兴等[6,7]曾用Feulgen法研究了植物姐妹染色单体区分染色。为了克服植物SCE不能推广的缺点，本文对蚕豆SCE的Brdu-Feulgen法的实验条件及影响因素进行了详尽的研究，以探索利用蚕豆SCE技术检测环境中诱变因素的可能性。

1　材料与方法

1.1　试验材料

蚕豆：启豆2#，江苏省沿江地区农科所提供。

1.2　试验方法

(1) 按常规浸种催芽[8]待初生根长至3—5 cm时，剪去根尖生长点和胚芽顶端，移入盛有清水的10 ml小烧杯中，置25℃温箱内悬浮水培，促使侧根生长。

(2) 选择大多数侧根长至1—1.5 cm的蚕豆幼苗，浸入盛有实验所需的Brdu溶液的10 ml小烧杯中，继续在25℃避光条件下悬浮培养35 h左右。诱变剂处理时，侧根在Brdu溶液中培养一个细胞周期(20 h)后，取出浸入含诱变剂的溶液中避光培养1 h，流水冲洗后再转入Brdu溶液中继续培养生长一个细胞周期。

(3) 剪取侧根(约0.5—1 cm)，浸入盛有0.05%秋水仙素青霉素瓶中，25℃避光处理3.5—4 h，再转入新配制的卡诺氏固定液(冰醋酸-甲醇：1∶3)，在上述条件下固定8 h以上。

(4) 吸去固定液，用蒸馏水浸洗后，分别以不同HCl浓度、温度、时间进行处理，以摸索最佳水解条件。

(5) 以席夫氏试剂染色至根尖呈深紫红色(约1 h)，常规压片后镜检，观察分染效果并统计SCE频率。

2　结果与讨论

2.1　Brdu浓度对蚕豆姐妹染色单体分色(SCD)的影响

SCD等级标准为：分染清晰，能够准确计算互换率的为分色良好；能看出深浅不同，但计算互换率有困难的为分色差；看不出深浅差异的为未分色。从表1中可以看出，随着Brdu浓度的增加，分色良好的细胞数也增加，分色差的细胞数则减少，说明Brdu浓度与SCD有密

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表1 不同浓度的Brdu对蚕豆根尖细胞姐妹染色单体分色的影响

Brdu浓度(μg/ml)	观察的中期相染色体总数	分色良好(%)	分色差(%)	未分色(%)
10	5×25	4.00	18.40	77.60
30	5×25	28.80	22.40	48.80
40	5×25	59.20	18.40	22.40
50	5×25	78.40	11.20	10.40
100	5×25	82.40	9.60	8.00

切的关系。与50 μg/ml组相比，尽管100 μg/ml组浓度成倍增加，但分色良好的细胞组间差异并不大，说明在这2组中，有足够的未被降解的Brdu取代DNA中的胸腺嘧啶核苷，使姐妹染色单体分色，也就是说，50 μg/ml的Brdu就是以使绝大多数M_2期以上的细胞分色清晰。

2.2 水解时间、温度和盐酸浓度对SCD效果的影响

实验结果见表2。表2中可看出，只有3.5 mol/L HCl在温度37℃、水解时间为120 min；5.0 mol/L HCl在温度25℃、水解120 min或温度37℃、水解80 min才能显示分色效果。其中以3.5 mol/L HCl、37℃水解120 min分色效果最佳。

表2 水解时间、温度和盐酸浓度对蚕豆根尖细胞SCD效果的影响[1)]

水解温度(℃)		18			25			37		
水解时间(min)		80±5	100±5	120±5	80±5	100±5	120±5	80±5	100±5	120±5
HCl浓度	3.5 mol/L	++	++	++	++	++	++	++	++	+
	5.0 mol/L	++	++	++	++	++	+−	+−	−−	−−

1) Brdu浓度为40μg/ml ++：两条染色单体深染 +−：两条染色单体分色 −−：两条染色单体浅染

2.3 Brdu掺入时间及其长短对SCD效果的影响

Brdu的掺入是显示SCD的重要基础。由于蚕豆种子在萌发过程中自身合成的胸腺嘧啶核苷与外来的Brdu发生竞争性矛盾，若处理不当往往会造成掺入不进而导致实验失败[7]。一定要掌握大多数侧根长至1—1.5 cm时开始掺入，此时侧根生长最旺盛，幼苗内胸腺嘧啶核苷供需矛盾加剧，Brdu容易掺入取代。Brdu掺入时间的长短，根据其细胞周期来决定。蚕豆根尖细胞周期为19.3 h，其中G_1期为4.9 h，S期为7.5 h，G_2期为4.9 h，M期为2 h。只有在S期Brdu才能代替胸腺嘧啶核苷掺入DNA，所以如果从第一个细胞周期的S期掺入Brdu到第二个细胞周期的M期，需31.7 h，所以掺入所需时间只需比31.7 h稍长即可，大约为33—35 h。

2.4 Brdu浓度对蚕豆SCEs频率的影响

Brdu浓度是否适当，不仅影响分染效果，也影响交换频率。浓度低，分染差，但SCEs频率较接近自然状态；浓度高，分染好，但会诱度SCEs频率增加。表3显示了不同浓度Brdu对蚕豆SCEs频率的影响。观察计数SCEs频率时，端部的一段染色体互换或着丝点部位互换计为1次互换，染色单体中间的一段互换计为2次互换。从表3中可看出，当Brdu浓度为10 μg/ml时，平均每条M染色体和每条S染色体的SCEs频率，分别为3.19和1.62，而当浓度为100 μg/ml时，分别为8.78和3.40，均增加

表3 不同浓度的Brdu对蚕豆根尖细胞SCEs频率的影响

组别	Brdu浓度(μg/ml)	平均每条染色体的SCE数($X+S$)		观察细胞数	平均每个细胞SCEs数
		M染色体(1对)	S染色体(5对)		
Ⅰ	10	3.19+1.33	1.62+0.57	26	22.58
Ⅱ	30	3.29+1.44	1.66+1.17	41	23.18
Ⅲ	40	4.02+1.72	2.10+1.02	50	29.04
Ⅳ	50	5.18+1.74	2.35+1.39	60	33.86
Ⅴ	100	8.78+2.45	3.40+1.36	55	51.56

2倍以上。

2.5 环境诱度剂对蚕豆SCEs频率的影响

诱变剂丝裂霉素C(MMC)和$K_2Cr_2O_7$对蚕豆SCEs频率的影响见表4和表5。实验结果用t检验进行统计学处理[8]，从表中可看出，即使浓度很低，也能使SCEs频率显著增加，并呈现明显的剂量效应关系，这说明蚕豆SCEs对诱变剂十分敏感(图1)。

表4　MMC对蚕豆根尖细胞M染色体SCEs频率的影响

组别	剂量(mol/L)	观察的染色体数	SCE总数	均值±S	t值	P值	t值	P值
1	对照	50	201	4.02±1.72				
2	5×10^{-6}	30	209	6.97±2.45	6.32	<0.001		
3	5×10^{-5}	30	302	10.07±2.30	13.39	<0.001	5.05	<0.001

表5　$K_2Cr_2O_7$对蚕豆根尖细胞M染色体SCEs频率的影响

组别	剂量(μg/ml)	观察的染色体数	SCE总数	均值±S	t值	P值	t值	P值	t值	P值
1	对照	50	201	4.02±1.72						
2	0.1	30	186	6.20±2.46	4.66	<0.001				
3	1.0	30	306	10.20±2.81	12.22	<0.001	5.87	<0.001		
4	10	30	371	12.37±1.87	19.99	<0.001	10.72	<0.001	3.47	<0.001

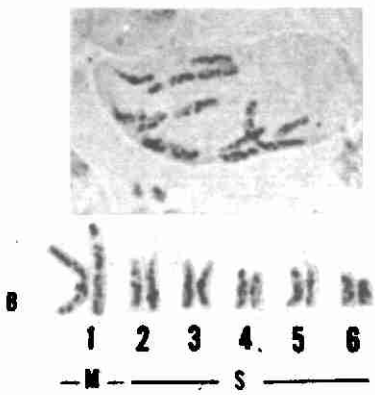

图1　MMC(5×10^{-5} mol/L)诱发的蚕豆根尖细胞SCEs(Brdu-Feulgen法)

A　显微摄影照片×1500

B　蚕豆根尖细胞染色体组型(示SCE交换部位)×1500

为了对本方法进行扩大验证，本实验将蚕豆SCE试验与蚕豆根尖微核试验进行了比较研究，实验结果见表6。实验结果表明，蚕豆根尖细胞微核率与SCEs频率之间的相关系数有显著性意义($r=0.9649$，$Y_{0.05(2)}=0.9500$，$P<0.05$)。说明2种试验方法在指示致突变效应上是一致的。

表6　$K_2Cr_2O_7$诱发蚕豆根尖细胞微核率与SCE频率的相关性

剂量(μg/ml)	微核率(‰)	每条染色体SCE频率 $X\pm S$
0	2.97±1.40	4.02±1.72
0.1	9.6±3.51	6.20±2.46
1.0	14.0±6.20	10.20±2.81
10	15.2±2.39	12.37±1.97

3　结语

综上所述，采用Brdu-Feulgen法进行蚕豆SCE试验时，如能掌握Brdu的浓度、掺入时间以及稀酸水解浓度、时间和温度等，就能获得最佳的实验结果。该方法与FPG法相比，具有程序简便、经济、易于推广等优点，在指示遗传毒性效应上与其他遗传毒理学方法相吻合，期望能得到推广与应用。

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protection. In addition, the environmental drag were used to explain the model's meaning as well as to discuss the measurement of the environmental drag.

Key words: social rate of return, private rate of return, elasticity of elasticity, environmental discount rate, environmental investment, environmenal improvement, environmental drag.

The Growth and Purification Function of *Eichhornia crassipes* Solms in Oil-refinery Wastewater. Tang Shuyu et al. (Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014): *Chin. J. Environ. Sci.*, **17**(1), 1996, pp. 44—46

The growth of *Eichhornia crassipes* Solms in oil-refinery wastewater has been described in this paper. An influence of COD, a comprehensive index of the pollultant concentration in the wastewater, on the growth of *Eichhornia crassipes* Solms was quantitatively studied. It was found that an optimum working condition for treating oil-refinery wastewater by *Eichhornia crassipes* Solms eco-engineering is established as follows: 65 mg/L<[COD]<131 mg/L; and 262. 6 mg/L of COD at effective critical point.

Key words: *Eichhornia crassipes*, oil-refinery wastewater, purification.

Study on Method of Sister Chromatid Exchange in *Vicia faba* to Detect Environment Mutagen. Kong Zhiming et al. (Dept. of Environ. Sci. and Eng., Nanjing University, Nanjing 210093): *Chin. J. Environ. Sci.*, **17**(1), 1996, pp. 47—49

The experimental conditions of the Brdu-Feulgen method of SCE in *Vicia faba* root which include the content of Brdu, labelling time of Brdu, the impacts on SCE of the content of hydrochloric acid and time and temperatrue for hydrolysis were studied and discussed in this paper. The best experiment conditions and procedure, which overcome the short-comings of FPG method that is complicated in procedure and, hence, difficult to be popularized, were obtained. In addition, such method was compared with other genotoxicology method in order to probe into the possibility of utilizing such technology to detect environment mutagen.

Key words: *Vicia faba*, SCE, Brdu-Feulgen method.

A Pulse-feed Upflow Anaerobic Sludge Blanket Reactor. Su Yumin et al. (Dep. of Environ. Eng., Taiyuan Univeristy of Technology, Taiyuan 030024): *Chin. J. Environ. Sci.*, **17**(1), 1996, pp. 50—53

The key parts of Upflow Anaerobic Sludge Blanket Ractor are gas-solids separator and feed system. The goals of this research, in which a conventional continuous feed system was replaced by an intermittent pulse-feed one, are to provide gently hydraulic mixing, to promote hydraulic selection, and to improve the contact between substrate and microorganisms. Pulse-feed method can raise the orgainc load rate as high as 27. 5 gCOD/(L. d), reduce HRT to nearly 3 hrs, and quickly develop granulated sludge in 47 days. It can not cause shock load and intermediates accumulation, as every pulse only releases a small amount of wastewater (1/56 reactor volume), which can not raise the substrate concentration in whole reactor. The pulse-feed also can not cause sever wash-out of sludge, because pulse-feed mi-xing can effectively separate sludge flocs and entrapped gas bubbles, and hence improve sludge settleability. The advantages of enrichment of *methanosarcina* species in the process of granulation are also discussed. At high load rate, *methanosarcina* species do appear in clumps on the granules.

Key words: anaerobic digestion, UASB, pulse-feed, mixing, granulation, *methanosarcina* species.

Study on Biological Pretreatment Method-bio-ceramic Reactor Treating Micro-pollution Source Water at Low Temperature and Low Turbidity. Hu Jiangyong et al. (Dept. of Environ. Eng., Tsinghua Univ., Beijing 100084): *Chin. J. Environ. Sci.*, **17**(1), 1996, pp. 54—56

One of biological pretreatment methods-bio-ceramic reactor (BCR) was used to treat a typical source water with micro-pollution at low temperature and low turbidity. By means of in-situ experiments with the bio-ceramic reactor, it was found that: the organic matter (OC or COD), ammonia, SS in the source water could be removed about 20%—30%, 60%—70% and 80%, respectively. Removl efficiency could be reduced at low temperature. Low turbidity and high concentration of organics in the source water would be benifical to BCR. In general, BCR would be a powerful way to purificate this kind of source water.

Key words: micro-pollution, source water, low temperature, low turbidity, organics, bio-ceramic pretreatment process.

Studies on the Leaching and Species of Aluminum in Soil. Huang Yanchu and Qu Changling (Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085): *Chin. J. Environ. Sci.*, **17**(1), 1996, pp. 57—59

The leaching and chemical forms of aluminum in soil by sequential fraction procedure were studied. Solutions used sequentially to extract Al are inorder of 1 mol/L KCl, 1 mol/L NH_4Ac, 1 mol/L HCl and 0. 5 mol/L NaOH. The spectrophotometric determination of leaching Al was performed with Eriochrom Cyamine RC. It has been found that the type of soil and the amounts of organic materials and total Al in soil have a siginificant effect on the amount of leaching Al. A certain amount of exchangeable Al can be leached from acid soil with 1 mol/L KCl extractant, however, it can not be leached from alkaline soil. The leaching Al extracted with 0. 5 mol/L NaOH is correlated at a high level of siginficance with the total Al in soil.

Key words: soil, leaching aluminum, chemical form.

Efficiency of Fluidized Biofilm Method for Treating Phenolic Wastewater. Yin Jun et al. (Jilin Architechtural and Civil Eng. Institute, Changchun 130021): *Chin. J. Environ. Sci.*, **17**(1), 1996, pp. 60—62

A dynamic experiment was conducted to examine the efficiency of the fluidized biofilm method with home-made carrier for treating phenolic wastewater. The experimental results have shown that COD and phenol were removed on an average over 80% and 90%—100%, respectively, while COD volumetric loading is 4. 0 kg/(m^3·d), and the final concentrations of COD and phenol in the effluent can meet Chinese Standard of Wastewater dischange permission. The biofilm can adhere quickly to the home-made carrier and the thickness of biofilm is suitable