アブサイシン酸とカイネチンを用いた二段階培養法によるイネカルスからの茎葉形成の促進

誌名	日本作物學會紀事
ISSN	00111848
著者	井上, 貢 前田, 英三
巻/号	50巻3号
掲載ページ	p. 318-322
発行年月	1981年9月

Japan. Jour. Crop Sci. 50(3) : 318-322 (1981)

Stimulation of Shoot Bud and Plantlet Formation in Rice Callus Cultures by Two-step Culture Method using Abscisic Acid and Kinetin

Mitsugu INOUE and Eizo MAEDA
(*Institute for Biochemical Regulation, Faculty of Agriculture, Nagoya University, Nagoya 464, Japan*)
Received October 17, 1980

Since the elegant demonstration by SKOOG and MILLER that the relative ratio of cytokinin to auxin determined the mode of organ formation in tobacco pith tissue[13], effects of auxins and cytokinins on organ formation have been examined in a number of other plant tissue systems[7]. In rice callus cultures, culture medium without auxin[8] or with a low concentration of auxin[6] has been seen to be suitable for shoot formation, which is further enhanced by the addition of cytokinin[11,14]. However, we obtained a low frequency of shoot formation in rice callus by the methods reported earlier[6,8,11,14].

Our previous report[4] indicated that composition of preculture medium plays an important role in the determination of organ formation in rice callus cultures.

We describe here some possible effects of abscisic acid (ABA), gibberellic acid (GA) and kinetin (K) on organ formation. Also, the role of ABA on organ formation in rice callus cultures has been examined. Our two-step culture method has a biological implication and shows a high frequency of shoot formation, especially by using ABA in the preculture medium and kinetin in the last culture medium.

Materials and Methods

Initial callus was obtained from rice (*Oryza sativa* var. Aichi Asahi) seeds cultured for about a month on modified LINSMAIER and SKOOG's medium containing 10^{-5}M 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg/*l* thiamine, 200 mg/*l* inositol, 3 g/*l* casein hydrolysate (N.B.C Co., U.S.A.), 30 g/*l* sucrose and 9g/*l* agar, as described previously[8]. This medium was also used as a standard preculture medium (PM) and to examine the effect of various plant hormones, they were added exogenously. The initial callus was subcultured on the preculture medium for about a month. The precultured callus was subcultured on the last culture medium (LM, i.e. PM minus 2,4-D) with or without hormones (ABA, GA and K) as the case may be, and in the presence of a low level of thiamine (0.1 mg/*l*). Two pieces of callus (each about 100 mg fresh weight) served as inoculum. Erlenmeyer flasks containing 50 ml of the medium were used. Callus tissues on the preculture and last culture media were maintained under about 200 lx light intensity at 29±1°C and under about 4,000 lx light intensity at 26± 1.5°C, respectively. The experiments were repeated twice.

Results and Discussion

Results are shown in Fig. 1 and Table 1. Shoot bud* and plantlet** formation was stimulated by kinetin in the last culture medium whereas they were repressed by it in the preculture medium. ABA in the preculture medium and kinetin in the last culture medium promoted shoot bud and plantlet formation and several fold increase in the yield resulted. However the absence of kinetin in last culture medium failed to promote shoot bud formation even in the presence of ABA in the preculture medium. On the other hand, ABA in last culture medium inhibited shoot bud formation even

* Only leafy structures.

** Plants with well developed shoots, leaves and roots.

Table 1. Effect of growth regulators on organ- and plantlet- formation in rice callus by two-step culture method (preculture and last culture)

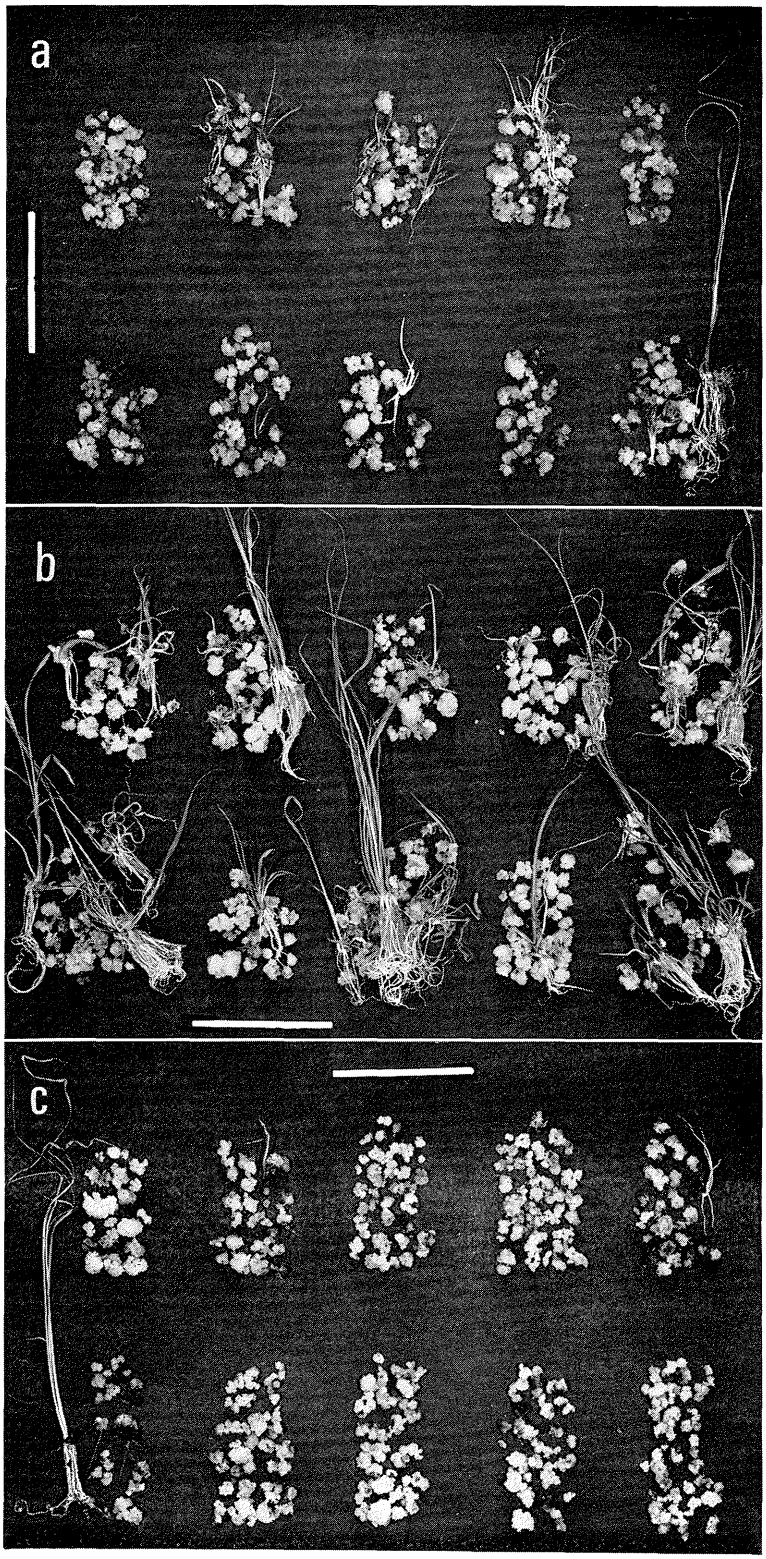
Experimental system	Treatment		Days after last inoculation								
			9 days			16 days			23 days		
	Preculture	Last culture	S	P	R	S	P	R	S	P	R
No. 1	PM	LM	1	0	0	2	0	7	0	0	7
	PM	LM+ABA 10^{-5}M	0	0	0	0	0	1	0	0	1
	PM	LM+K	0	0	0	9	1	2	6	2	3
	PM	LM+GA	0	0	0	0	0	0	0	0	0
No. 2	PM	LM	0	0	1	1	0	7	1	0	11
	PM	LM+K	0	0	0	4	1	3	9	4	4
	PM+ABA 10^{-4}M	LM	0	0	0	0	0	0	1	0	7
	PM+ABA 10^{-4}M	LM+K	1	0	0	11	6	0	27	22	1
	PM+ABA 10^{-4}M	LM+ABA 10^{-4}M	0	0	0	0	0	0	0	0	0
	PM+ABA 10^{-5}M	LM+K	0	0	0	6	4	0	12	9	0
	PM+ABA 10^{-6}M	LM+K	0	0	0	1	0	1	1	0	1
	PM+K	LM	0	0	0	0	0	0	0	0	1
	PM+K	LM+K	2	0	1	3	0	1	2	1	0
	PM+GA	LM	0	0	8	0	0	9	0	0	7
	PM+GA	LM+K	0	0	3	0	0	3	0	0	3
No. 3	PM	LM+ABA 10^{-4}M+K	0	0	0	0	0	0	0	0	0
	PM	LM+ABA 10^{-5}M+K	0	0	0	0	0	1	0	0	1
	PM	LM+ABA 10^{-6}M+K	0	0	0	0	0	0	0	0	0
	PM	LM+ABA 10^{-7}M+K	0	0	1	0	0	3	1	0	1
	PM	LM+ABA 10^{-8}M+K	0	0	1	0	0	1	1	0	3
	PM	LM+K	0	0	3	3	1	2	5	2	1

From preliminary experiments, kinetin (K) at 5×10^{-5}M in last culture was optimal for shoot bud formation and gibberellic acid (GA) at 10^{-5}M in last culture repressed almost shoot bud- and root -formation in rice callus. Then these concentrations were used here. Figures in each column indicate the number of regions forming organs and plantlet observed in 10 flasks. S: shoot bud, P: plantlet, R: root.

in the presence of kinetin. Thus, ABA seems to be effective during the preceding stages of organ formation and kinetin in the later stages of growth and development. Therefore, in order to promote shoot formation in rice callus cultures, ABA and kinetin should be added at particular stages in the preculture or last culture medium, respectively. Presence of GA in the preculture medium as well as in the last culture medium, resulted in suppression of both shoot bud and plantlet formation. Thus, ABA in the preculture medium could not be replaced by the other plant hormones like GA and kinetin.

Stimulation of shoot bud formation by ABA has already been reported in detached leaves of *Begonia*[2], discs of sweet potato tuberous roots[16,17] and callus tissues derived from potato protoplasts[12]. In addition to auxin and cytokinin which are known to induce organ formation[13], we propose here ABA to be an important factor which controls organ formation in rice callus.

Table 1 shows that the development of plantlets from regenerated shoots was more pronounced in rice callus treated with ABA in the preculture medium and kinetin in the last culture medium than those treated with other hormones while preculturing. In somatic embryo formation from cultured cells of caraway[1] it has also been shown


a
b
c

that ABA promotes high frequency of normal embryos.

In rice plant it is known that the level of ABA is much higher in the early stages of ear development and gradually, during ripening its level decreases[9]. Also a high level of *cis*- and *trans*-ABA in the immature rice seeds (the stage of embryo formation and its early development) has been seen[10]. Therefore, the studies on the occurrence of high level of ABA at the early stages of embryo development correspond with our results on the stimulation of shoot bud and plantlet formation by ABA in the preculture medium, i.e., in the early stages of development. These results suggest a close relationship of ABA to shoot bud formation in rice plants.

ABA is known to counteract the effects of GA[15] and cytokinin[17] on organ formation. Also YAMAGUCHI and NAKAJIMA reported that sweet potato tuberous roots, containing high level of endogenous cytokinins, required high level of ABA in the culture medium for shoot formation[17]. Rice callus also has high level of endogenous cytokinins[5], therefore, in our present study, promotive effect of high level of ABA and repressive effect of high level of kinetin in the preculture medium on shoot bud and plantlet formation supports the work of YAMAGUCHI and NAKAJIMA[17]. This suggests an antagonistic relationship between ABA and cytokinins in regulating the organ formation in rice callus.

Application of two-step culture method using preculture and last culture media may be useful for organ formation and plant regeneration in the other crop plant systems whose totipotency seems to be poor such as wheat, corn and soybean. Even if addition of kinetin to the preculture medium is inhibitory for shoot bud and plantlet formation in plant tissues containing high levels of endogenous cytokinin like rice callus tissues, effect of kinetin in last culture medium must be examined because this addition stimulated shoot bud and plantlet formation in rice callus cultures.

Therefore, it will be interesting to test the relationship between ABA and the other growth regulators with respect to organ formation in the other plant systems by means of two-step culture method technique.

Summary

Two-step culture method technique has been established, leading to a high frequency of shoot bud formation and plant regeneration in rice callus cultures. The roles of ABA in the preculture medium and kinetin in the last culture medium have been emphasized in organ formation.

Acknowledgement

We thank professor, Dr. HIROSHI SUZUKI, Kagawa University for his advice and encouragement. We also thank Dr. MINAKSHI SETHI, Delhi University for reading and improving our manuscript.

References

1. AMIRATO, P. V. 1977. Hormonal control of somatic embryo development from cultured cells of Caraway. Interactions of abscisic acid, zeatin, and gibberellic acid. Plant Physiol. **59**: 579—586.
2. HEIDE, O. M. 1968. Stimulation of adventitious bud formation in *Begonia* leaves by abscisic acid. Nature **219**: 960—961.
3. INOUE, M. and E. MAEDA 1976. Effect of auxin concentrations on the callus induction from various organs of rice seedlings. Proc. Crop Sci. Soc. Japan **45**: 545—557.
4. ———— and ———— 1980. Thiamine as a factor of organ formation in rice callus cultures. Japan Jour. Crop Sci. **49**: 1—7.
5. ————, ————, R. YOSHIDA and T. ORITANI 1979. On the occurrence of a high content of cytokinins in rice callus tissues. Plant and Cell Physiol. **20**: 917—924.

Fig. 1. Nearly 30-day-old cultures showing plantlets regenerated from rice callus in the last culture medium [LM+kinetin (5×10^{-5}M)]. These calli were precultured for about a month on the following media: (a) PM, (b) PM+ABA (10^{-4}M), (c) PM+kinetin (5×10^{-5}M). Each bar in the photographs represents 5 cm length. The photographs show the calli obtained from 10 cultures.

6. MAEDA, E. 1968. Subculture and organ formation in the callus derived from rice embryo in vitro. Proc. Crop Sci. Soc. Japan **43**: 151—160.
7. NARAYANASWAMY, S. 1977. Regeneration of plants from tissue cultures. In Applied and fundamental aspects of plant cell, tissue and organ culture. (Ed.) J. Reinert and Y.P.S. Bajaj. Springer-Verlag, Berlin, Heidelberg. 179—248.
8. NISHI, T., Y. YAMADA and T. TAKAHASHI 1968. Organ redifferentiation and plant restoration in rice callus. Nature **219**: 508—509.
9. ORITANI, T. and R. YOSHIDA 1971. Studies on nitrogen metabolism in crop plants. XI. The changes of abscisic acid and cytokinin-like activity accompanying with growth and senescence in the crop plants. Proc. Crop Sci. Soc. Japan **40**: 325—331.
10. ———— and ———— 1975. Growth inhibitors in immature seeds of rice plant. ibid. **44**: 329—334.
11. SAKA, H. and E. MAEDA 1969. Effect of kinetin on organ formation in callus tissue derived from rice embryos. ibid. **38**: 668—674.
12. SHEPARD, J. F. 1980. Abscisic acid-enhanced shoot initiation in protoplast-derived calli of potato. Plant Sci. Lett. **18**: 327—333.
13. SKOOG, F. and C. O. MILLER 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Sym. Soc. Exp. Biol. **11**: 118—131.
14. TAMURA, S. 1968. Shoot formation in calli originated from rice embryo. Proc. Japan Acad. **44**: 544—548.
15. THORPE, T. A. and D. D. MEIER 1973. Effects of gibberellic acid on shoot formation in tobacco callus cultures. Physiol. Plant. **29**: 121—124.
16. YAMAGUCHI, T. and T. NAKAJIMA 1972. Effect of abscisic acid on adventitious bud formation from cultured tissue of sweet potato. Proc. Crop Sci. Soc. Japan **41**: 531—532.
17. ———— and ———— 1974. Hormonal regulation of organ formation in cultured tissue derived from root tuber of sweet potato. In Plant Growth Substances. Hirokawa publishing Co. Ltd. Tokyo. 1121—1127.

〔和 文 摘 要〕

アブサイシン酸とカイネチンを用いた二段階培養法によるイネカルスからの茎葉形成の促進

井上 貢・前田英三
（名古屋大学農学部生化学制御研究施設）

イネカルスにおける茎葉および幼苗の形成率を，従来の方法に比較し，数倍高める方法，すなわち二段階培養法を開発した．前培養と最終培養において各種の植物ホルモン作用を検討した結果，前培養にアブサイシン酸，最終培養にカイネチンを用いた組み合わせの二段階培養が，茎葉および幼苗の形成を最も著しく促進した．これらの植物ホルモンとイネカルスにおける器官形成との関連性について議論した．カルスからの茎葉形成が困難な他の作物に対する二段階培養法の適用は，これらの作物における幼苗の作出にも有望と思われる．