Studies on Photoperiodic Responses of Salvinia natans (VI) *On the Effects of Nucleic Acid Precursors on Sporocarp Formation*

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Since it was reported that floral fomation was affected by nucleic acid metabolism, a number of evidences confirming the fact have been obtained in various plants[2, 4, 6, 10]. As inhibited floral formation by some analogues of the nucleic acid precursors was reversed by supplied nucleic acid precursors in the same time, many workers [1, 4, 5] have suggested that nucleic acid metabolism, specifically that of ribonucleic acid, is involved in the processes of floral formation. MARUSHIGE and MARUSGHIGE[3], with *Pharbitis* plant, have observed increased flowers by some RNA precursors.

This paper presents an accelerated sporocarp formation of *Salvinia natans* by RNA precursors together with its interpretation from a view point of metabolism of sporocarp forming substances.

Materials and Methods

Salvinia natans was cultured under a continuous illumination for materials[7]. Photoperiodic treatment for sporocarp formation, consisting of 8 hour light and 16 hour dark periods, was thrice given. Photoperiodic effects were measured fifteen days after the beginning of inductive treatment.

A mixture of RNA nucleotides was manufactured by Nihonzôki pharmaceutical Co. Ltd., Osaka. It consists of equal weight of Na-salts of four acids such as adenylic, guanilic, cytidilic and uridilic. The hydrolyzed products of DNA were produced by destruction of DNA in hydrochlolic acid in our laboratory. Accordingly, DNA precursors are contained with a native ratio of DNA components. In other experiments, any one of the three, cytidine, guanosine or uridine was supplied to culture media respectively.

The experiment was repeated more than three times, and more than six plants were used for one experiment.

Results

I. *Effects of the hydrolyzed products of DNA.*

The hydrolyzed products of DNA were supplied to the culture media to contain DNA in 1–0.001 mg/cc.

In higher concentration, the number of sporocarps was less than in control plants, but it was recovered to attain the control level with decreased concentration.

II. *Effects of mixture of RNA nucleotides.*

The plants supplied with 1 or 2 mg of the nucleotides in 1 cc of culture media could not be measured for photoinductive effects owing to the fact that in such media leaves were disjointed from the others during the photoperiodic treatment. With an experiment, in which a few plants remained intact in 1 mg mixture/cc of culture media, 5.1 sporocarps and 80% induced plants were obtained.

A relation between the mixture concentrations and the photoinductive effects was shown in table 1. The induced plants were decreased in higher concentration of the nucleotides mixture, but more sporocarps were found than in control plants. The most sporocarps were found in the concentration of 0.01 mg of nucleotide mixture per 1 cc of the culture media, and the number of sporocarps gradually decreased with a decrease of concentration in the mixture. Generally speaking, so far the writer observed, the mixture applied in all concentrations increased the sporocarps in comparison with that of control plants.

Table 1. Effects of equal weight mixture of RNA nucleotides on sporocarp formation of *Salvinia natans*.

Mixture of RNA nucleotides (mg/cc)	Number of sporocarps	Induced plants (%)
1	.....	.....
0.1	6.5	100
0.01	8	100
0.001	6.4	100
0 (control)	4	100

III. *Effects of RNA nucleosides.*

From the experimental results obtained, it is presumed that the components which accelerated sporocarp formation can be detected among RNA precursors. So, uridine, a specific nucleoside in RNA, as well as guanosine and cytidine was studied in affecting on the sporocarp formation. A relation between the concentrations of guanosine or uridine and the number of sporocarps was shown in figure 1.

The plants treated with uridine in the highest concentration produced more sporocarps than in the controls, but the number of induced plants was less than in the controls. The most sporocarps were induced in 10^{-4}M, and as much sporocarps were induced in 10^{-5}M as in control plants. While sporocarps in the controls show the primordial stage of development, the plants which were supplied with uridine in any concentration tested developed the sporocarps of mature form. The growth of their vegetative organs was also especially vigorous in the plants supplied with uridine without any difference in growth by the concentration.

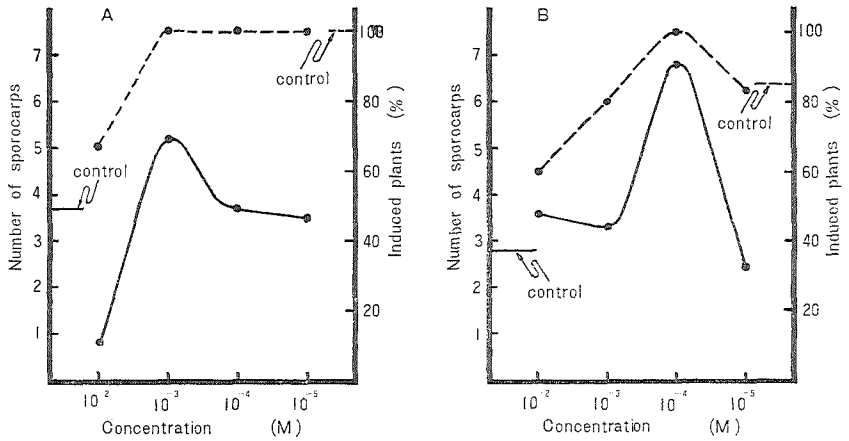

Fig. 1. A relation between the number of sporocarps and the concentration of guanosine or uridine. (A) guanosine, (B) uridine. Solid and broken lines illustrate the number of sporocarps and percentage of the induced plants respectively.

Guanosine in higher concentration (10^{-2}M) showed less photoinduction than those of control plants. In. 10^{-3}M, however, the substance induced more induction than in the control plants. In lower concentrations it was difficult to find any acceleration in comparison with that in control plants.

The number of sporocarps and the percentage of the induced plants fluctuated with the concentration of cytidine, but there was no significant difference from the condition in control plants.

IV. *Effects of RNA nucleotides mixture supplied during inhibitory or non-inhibitory dark period.*

In order to know the reason why specific RNA precursor accelerated the sporocarp formation, the experiments shown in table 2 were undertaken. In these experimental series, the mixture of RNA nucleotides was used at a concentration of 0.01 mg/cc of culture media. A light-interruption of 10 minutes was applied at the middle of 16 hour dark period.

Table 2. Influence of RNA nucleotides mixture on sporocarp formation with various conditions in dark period.

Expl. no.	1st and 2nd dark periods		3rd dark period		Inductive effects	
	RNA nucleotides	light interruption	RNA nucleotides	light interruption	number of sporocarps	induced plants
1	−	+	−	−	1.2	80
2	−	+	+	−	3.2	100
3	+	+	−	−	4.2	100

The mixture increased the sporocarps independent of the light-interruption. The plants supplied with the mixture only in the first two dark periods which were light-interrupted produced more sporocarps than those supplied with the mixture only in the third dark period without light-interruption.

Discussion

Recently, it has been reported by many workers that nucleic acid metabolsim is involved in the processes of photoperiodic induction. COLLINS et al.[2] have claimed that nucleic acid metabolism is an important factor for floral formation for the reason that antimetabolites of nucleic acid inhibtited the floral formation in cocklebur, and that nucleic acid precursors could reversed such inhibition. BONNER et al. [1, 6] observed an inhibitory floral formation in *Xanthium* which is inhibited in nucleic acid metabolism by 5-fluorodeoxyuridine or 5-fluorouridine, and suggested a relation between floral formation and RNA synthesis. It was reported by MARUSHIGE et al.[3] that *Pharbitis* plants which had been inhibited in floral formation by some analogues of RNA precursors were recovered in the activity by supplied RNA precursors, and that the activity of the plants was increased by RNA precursors alone.

In the present studies, RNA precursors were clearly shown to enhance photoperiodic effects, and uridine was found to be most effective among the precursors used. These facts were consistent with the results obtained by MARUSHIGE et al.[3] with *Pharbitis* plants.

The plants supplied with the mixture of RNA nucleotides or with some RNA nucleosides were induced not only to increase the number of sporocarps but to cause the sufficient development of them. The growth of the vegetative organs was also vigorous in all plants supplied with RNA precursors. Such vegetative growth may be understood by the fact that, in general, RNA has a serious responsibility to the protein synthesis of organisms. A better development of sporocarps appears to be interpreted similarly from a view point of cellular proliferation for sporocarp formation.

In the experiment 4, the different conditions in RNA precursors in the first

two light-interrupted dark periods brought a great difference in the number of sporocarp independent of the condition in the third dark period. This fact may suggest the possibility that RNA precursors affected the formation of the photoinductive stimulus in a period prior to that of sporocarp-forming substances. In the condition such as a light-interrupted dark period, it has been reported by the author [8] that a metabolic system for the production of sporocarp-forming substances was adaptively activated though it was not actually realized. Subsequently, with the present studies, it is suggested that the RNA precursors activate a metabolic system for the production of sporocarp-forming substances. This conjecture may be supported by the preliminary expereimental result that most functions to form sporocarp were especially found during 8 hours prior to the inductive dark period among different photoinductive periods.

The existence of the RNA precursors during the third dark period following the two dark periods of the same condition gave a remarkable increase in the number of sporocarps. The author can not say with certainty whether such increase was caused from exclusively the activation of the metabolic system as was discussed above. As TOMITA [9] reported on the role of uridine for floral formation in vernalization, probably, the RNA precursors may also have a role of constructive materials for the sporocarp-forming substances because, especially, uridine among the precursors accelerated the sporocarp formation.

Summary

1. The effects of nucleic acid precursors on sporocarp formation were investigated with *Salvinia natans* under short day condition (8 hour).
2. An active sporocarp formation was obtained with RNA precursors, but not with DNA precursors.
3. Guanosine and uridine among RNA precursors, especially the latter, exhibited the enhanced formation of sporocarp.
4. From the results obtained here, it is conjectured that RNA precursors such as uridine active a metabolic system for the synthesis of sporocarp-forming substances. Another possible interpretation may be made that the RNA precursors are utilized as the materials of the sporocarp-forming substances.

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