ON THE INFLUENCE OF GIBBERELLIC ACID AND KINETIN ON THE GERMINATION OF TURIONS OF SPIRODELA POLYRHIZA (L.) SCHLEIDEN

M. A. M. LACOR

Botanisch Laboratorium, Utrecht

SUMMARY

The germination rate of turions produced in a medium without gibberellic acid depends on the time the turions spend in this medium. Gibberellic acid in light enhances the germination rate of turions harvested when turion production has just started. Kinetin enhances the germination of such turions in light and in darkness. The highest germination rate in light is obtained when both kinetin and gibberellic acid are added to the medium.

Turions produced in a medium with gibberellic acid do not require a cold treatment for rapid germination in light and darkness.

1. INTRODUCTION

At circumstances less favourable to the vegetative multiplication *Spirodela polyrhiza* fronds (plants) tend to produce turions instead of fronds in the reproductive pockets (CZOPEK 1963). A turion is much smaller than a frond and somewhat asymmetrical. Its colour is dark-green or violet-green. Roots are lacking although some clearly visible root primordia are present. Unlike floating fronds turions sink to the bottom when they are set free from the mother fronds. More detailed descriptions of the turion are given by HEGELMAIER (1868), JACOBS (1947), CZOPEK (1959), and HILLMAN (1961).

According to Jacobs turions that are produced at a temperature of 25 °C do not germinate at this temperature without a cold treatment. However, turions produced at low temperature (10°–15 °C) germinate quickly when they are placed in conditions of a high temperature. According to HENSSEN (1954) and CZOPEK (1959) turions produced at a higher temperature in sterile conditions with sucrose in the medium will germinate when they are placed in circumstances in which multiplication of fronds normally proceeds quickly, but the rate of germination is low. Vernalisation or storing in water at a temperature of 16°–20 °C both increase the germination rate (CZOPEK 1959).

For the experiments on the influence on germination of light, kinetin and gibberellic acid, CZOPEK (1962, 1964) used turions that had been stored at 0°–3 °C in the dark for a month. She found that white or red light enhances the germination rate. Gibberellic acid causes a drop in the germination rate in red light and in the dark. In white light gibberellic acid causes only a slight drop in the germination rate. Kinetin raises the germination rate in red light and in the dark. According to Czopek the influence of kinetin and red light is synergistic.

In this paper the results are given of experiments on the influence of gibberellic acid and kinetin in light and darkness on the germination of turions that had not been stored at low temperatures. We found that with these turions gibberellic acid stimulates the germination of turions at some specified experimental conditions.

2. MATERIAL AND METHODS

2.1. Medium and light

Erlenmeyer flasks of 300 ml were filled with 100 ml of the basic medium as used before (LACOR 1968) + 1% sucrose and with or without gibberellic acid 5.10^{-6} g/ml and sterilised for 60 minutes at 100° C. In the medium a mother frond with one visible daughter frond was inoculated. The flasks were placed in continuous light (Philips TL 55/40 W, 20 W m^{-2}) and at a temperature of 26 °C ± 1°. After three to four weeks there were enough turions available for the experiments. They were germinated in basic medium without sucrose with or without kinetin 5.10^{-7} g/ml, and/or gibberellic acid 5.10^{-6} g/ml, and placed in continuous white or red light or in the dark, in 300 ml flasks filled with 100 ml medium. The temperature during germination was 25° ± 1 °C. For each treatment we used about 80 turions distributed over 4 flasks. Kinetin (Kin.) was obtained from the Nutritional Biochemicals Corporation, Cleveland, U.S.A. Gibberellic acid (GA_3), "Berelex Powder", was obtained from the Imperial Chemical Industries Ltd, Yalding, England.

The following light sources were used during germination of the turions:

1. White light from fluorescent tubes (Philips TL 55/40 W), with an intensity of 15 W m^{-2} at the level of the turions.
2. Red light from a Philips red fluorescent tube 15/40 W, filtered by a layer of 4 mm plexiglass (red 501, Röhm & Haas, Darmstadt). The intensity at the level of the turions was about 1 W m^{-2}.

2.2. Germination of the turions

During germination the young fronds grow from the reproductive pockets of the turions. The small rootlets on the turion grow longer and the turion floates upward to the surface of the nutrient solution. A description of the germination is given by CZOPEK (1959) and GUERN (1965).

Sometimes the young fronds in our experiments could be seen very clearly when the germinating turions were still at the bottom of the flasks. We also found that the turions had arrived at the water surface before the young fronds and roots were visible. When such turions rose to the surface the young fronds grew out within a couple of hours. The turions were considered to have germinated when they had reached the solution surface. When the turions stayed too long in the medium they were formed in, they produced young fronds when they were transferred to a fresh medium, but they did not float up to the surface of the water. For this reason we made the experiments with turions that had not been too long in the medium they were formed in.

The course of germination was determined by registering the germination percentages. The data represent the means of 4 flasks with their standard deviations (standard deviations are not indicated in the figures when they are less than 2%).

The germination in the dark was followed by our inspection of the flasks in green light with an intensity of 0,0015 W m^{-2}, obtained from an incandescent lamp with three filters, namely a calflex filter (Balzer, Liechtenstein), a 519 PIL filter (Schott & Gen., Mainz) and a 519 nm DEPAL double band filter (Schott & Gen., Mainz).

3. RESULTS

As GA_3 influences the production of turions (LACOR 1968) it seemed of interest to use turions produced in a medium without GA_3 as well as turions produced in a medium with GA_3.

In the experiment on the influence of GA_3 on the germination rate of turions produced in a medium without GA_3 two groups of turions were used. Twenty-six days after inoculation of the fronds, a part of a series of erlenmeyer flasks already contained sufficient turions to provide for the first group. The remaining flasks supplied the turions of the second group 34 days after inoculation of the fronds. In the first group most of the turions had been set free from their mother fronds a few days only. In the second group a great part of the turions had been set free for 8 to 10 days.

In white light turions of the first group had a very low germination rate. However, they could germinate after they had been transferred to a fresh medium; after 26 days 95% of the turions had germinated (*fig. 1*). The germination rate was much higher if GA_3 had been added to the medium (*fig. 1*).

If turions had been left in the medium they were produced in for a longer period (second group) the germination rate in medium without GA_3 increased. The germination rates in a medium with or without GA_3 were the same in this part of the experiment (*fig. 2*), and were the same as the germination rate of turions in a medium with GA_3 of the first group (compare *figs. 1* and *2*).

Also in continuous red light the germination of freshly produced turions (first group) took place faster in a medium with GA_3 than in a medium without GA_3 (*fig. 3*). The germination rate in red light was lower than in white light (compare *figs. 1* and *3*).

The germination rate of turions of the second group was also lower in red light than it was in white light, though part of these turions did not rise to the surface.

In the dark turions of the first group did not germinate within 20 days, neither in fresh medium with GA_3 nor in a medium without GA_3. Had the turions remained in the old medium for a longer time (second group) then in a medium with or without GA_3 the germination percentages attained irregular values from 10% up to 70% within 14 days.

The experiments concerning the influence of Kin. on the germination of

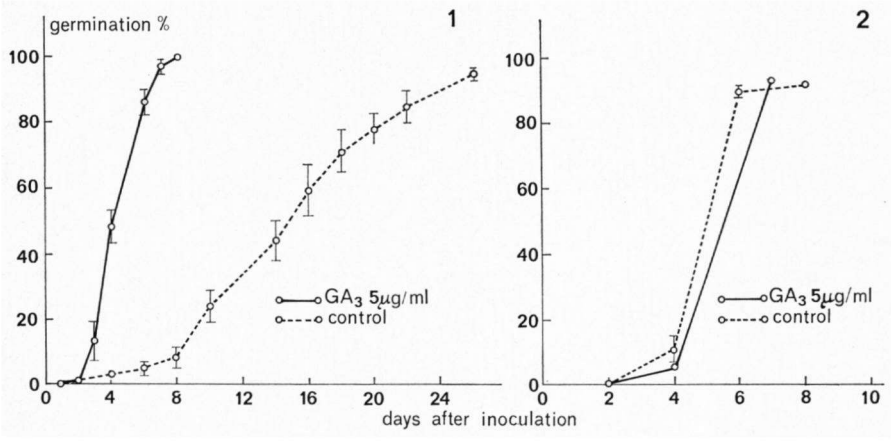

Fig. 1. The influence of GA_3 on the germination in white light of turions produced in a medium without GA_3 and harvested 26 days after inoculation of the fronds (first group).

Fig. 2. The influence of GA_3 on the germination in white light of turions produced in a medium without GA_3 and harvested 34 days after inoculation of the fronds (second group).

turions were performed with turions harvested 26 days after inoculation of the fronds (first group of this experiment) and with turions harvested 34 days after inoculation of the fronds (second group).

In white light the germination rate of the turions of the first group was higher in a medium with Kin. than with GA_3 (*fig. 4*), in the second group it was the same with Kin. or GA_3 or without these substances (*fig. 5*).

In the dark the germination of turions was stimulated by Kin. but not by GA_3. Newly produced turions (first group) had germinated within 9 days if the medium contained Kin. Only a part of the turions harvested later (second group) rose to the surface.

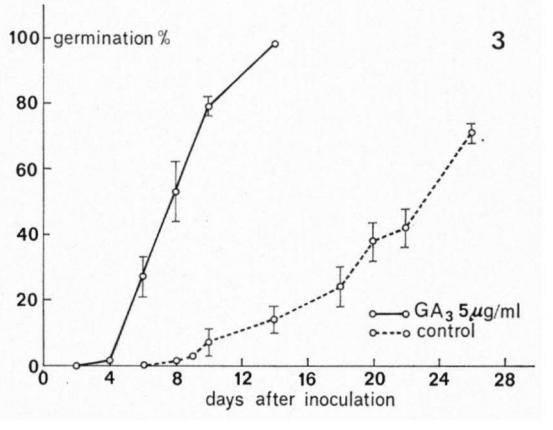

Fig. 3. The influence of GA_3 on the germination in red light of turion produced in a medium without GA_3 and harvested 26 days after inoculation of the fronds.

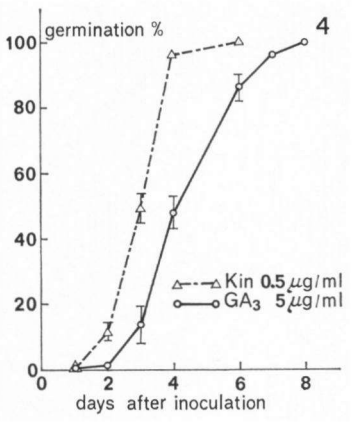

Fig. 4. The influence of GA_3 and Kin. on the germination in white light of turions produced in a medium without GA_3 and harvested 26 days after inoculation of the fronds.

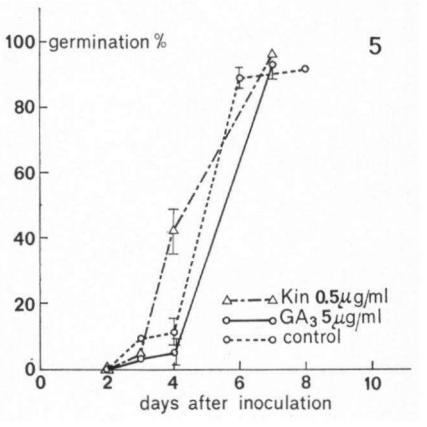

Fig. 5. The influence of GA_3 and Kin. on the germination in white light of turions produced in a medium without GA_3 and harvested 34 days after inoculation of the fronds.

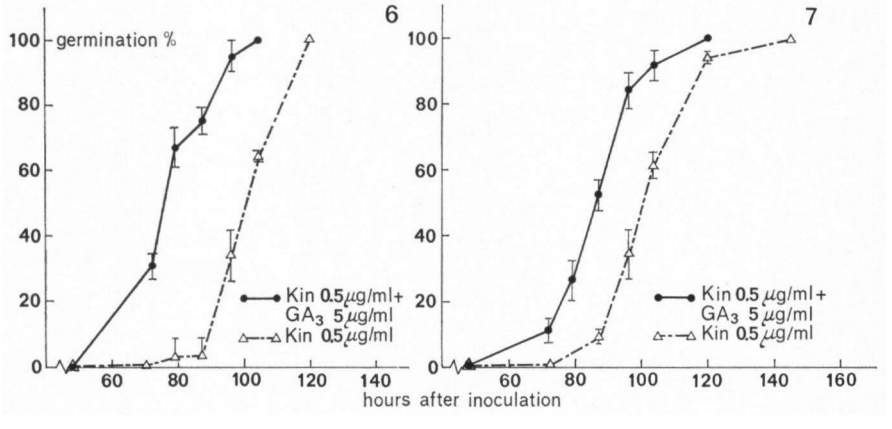

Fig. 6. The influence of Kin. + GA_3 and Kin. alone on the germination in white light of turions produced in a medium without GA_3 and harvested 26 days after inoculation of the fronds.

Fig. 7. The influence of Kin. + GA_3 and Kin. alone on the germination in red light of turions produced in a medium without GA_3 and harvested 26 days after inoculation of the fronds.

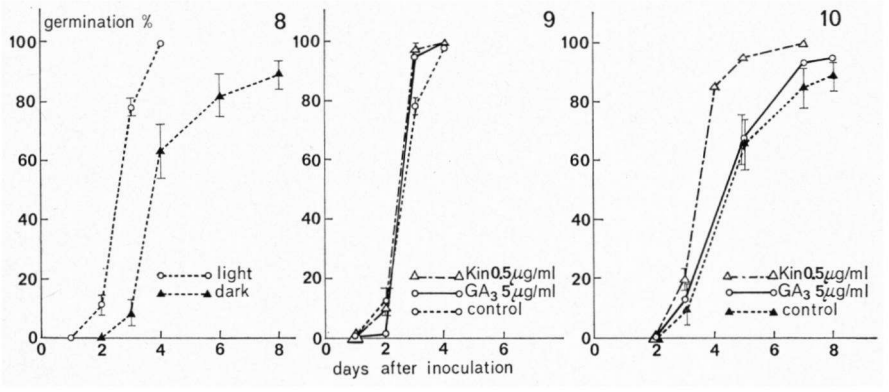

Fig. 8. The germination in white light and in the dark of turions, produced in a medium with GA_3 and harvested 22 days after inoculation of the fronds.
Fig. 9. The influence of Kin. and GA_3 on the germination in white light of turions produced in a medium with GA_3 and harvested 22 days after inoculation of the fronds.
Fig. 10. The influence of Kin. and GA_3 on the germination in the dark of turions produced in a medium with GA_3 and harvested 22 days after inoculation of the fronds.

Finally we studied the combined influence of Kin. and GA_3 on the germination of turions harvested 26 days after inoculation of the fronds. Both in white (*fig. 6*) and in red light (*fig. 7*) the germination rate was higher in a medium with GA_3 and Kin. than in a medium with only one of these plant hormones. In the dark the germination rate in a medium with Kin. and GA_3 was the same as in a medium with Kin. alone.

In the experiments on the germination of turions produced in a medium with GA_3 5.10^{-6} g/ml, turions were used that had been formed 22 days after inoculation of the fronds. In this medium turion production started at an earlier stage than in a medium without GA_3. From the curves presented in *fig. 8* it is clear that the turions germinated very rapidly in white light.

Also in the dark the germination rate was high, though lower than in white light (*fig. 8*).

In red light the germination rate was intermediate between the rate in white light and the rate in darkness. Neither in white nor in red light did GA_3 or Kin. in the medium increase the germination rates (*fig. 9*). In dark, however, only Kin. stimulated the germination rate and GA_3 did not (*fig. 10*). When the turions remained in the old medium for a longer period they germinated but lost the capacity to rise to the surface. None of the treatments used restored this capacity. After some time the plants died.

4. DISCUSSION

The results in *figs. 1* and *3* show that turions produced in a medium without GA_3 and remaining there for a couple of days only had low germination rates in light. In darkness the germination rate was even lower than in light. Also

CZOPEK (1959) found that such turions have a low germination rate. Turions set free from the mother fronds for a couple of days only, needed 24 days in white light to reach a germination percentage of 90%. Turions isolated 8 days later needed about 7 days to reach this germination percentage; that makes 15 days between their formation and their germination. So we may conclude that the rate of germination depends on the time the turions spend in the medium they were formed in.

The results in *figs. 1* and *3* demonstrate that GA_3 enhanced in light the germination rate of turions isolated immediately after turion formation had started, but GA_3 did not enhance the germination rate of turions harvested 8 days later (*fig. 2*). Also PERRY (1968) found that GA_3 increases the germination rate of unchilled turions of some *Spirodela* clones. However, the germination of cold treated turions is not influenced or even inhibited by GA_3 in light as well as in darkness (CZOPEK 1964). We may conclude that both a cold treatment and a longer stay in the old medium can act as substitutes of GA_3 for germination.

Kin. stimulated much more than GA_3 did the germination of turions produced in a medium without GA_3 and harvested immediately after turion production had started (*fig. 4*). The effect of Kin. and GA_3 together on germination of these turions was still greater than the effect of each of these individual substances (*figs. 6* and *7*). In the dark GA_3 was of no influence on the germination of these turions, but according to CZOPEK (1964), it inhibits the germination of cold treated turions. Kin., however, stimulated in the dark both chilled (CZOPEK 1964) and unchilled turions. So we may conclude that GA_3 and Kin. act in different ways on the germination of turions.

Turions produced in a medium with GA_3 did behave as cold treated turions. Neither Kin. nor GA_3 could in light accelerate the germination of these turions (*fig. 9*). Only Kin. could raise the germination rate in darkness (*fig. 10*).

ACKNOWLEDGEMENT

The author wishes to express his thanks to Prof. Dr. R. van der Veen for his stimulating interest and to Miss M. Houweling for her technical assistance.

REFERENCES

CZOPEK, M. (1959): Researches on the physiology of formation and germination of turions in Spirodela polyrhiza (L.) Schleiden. *Acta Biol. Cracov. Ser. Bot.* **2**: 75–90.

— (1962): The oligodynamic action of light on the germination of turions of Spirodela polyrhiza (L.) Schleiden. *Acta Soc. Bot. Polon.* **31**: 703–722.

— (1963): Studies on the external factors inducing the formation of turions in Spirodela polyrhiza (L.) Schleiden. *Acta. Soc. Bot. Polon.* **32**: 199–211.

— (1964): The action of kinetin, gibberellic acid and red light on the germination of turions of Spirodela polyrhiza. *Bul. Acad. Polon. Sc.* **12**: 177–182.

GUERN, J. (1965): Corrélations de croissance entre frondes chez les Lemnacées. *Ann. Sc. Nat. Bot.* Sér. **12, 6**: 1–156.

HEGELMAIER, F. (1868): *Die Lemnaceen. Eine Monographische Untersuchung.* Engelmann, Leipzig.

HENSSEN, A. (1954): Die Dauerorgane von Spirodela polyrhiza (L.) Schleid. in physiologischer Betrachtung. *Flora* **141**: 523–566.

HILLMAN, W. S. (1961): The Lemnaceae or Duckweeds. A review of the descriptive and experimental literature. *Bot. Rev.* **27**: 221–287.

JACOBS, D. L. (1947): An Ecological life-history of Spirodela polyrhiza (greater duckweed) with emphasis on the turion phase. *Ecol. Monographs* **17**: 437–469.

LACOR, M. A. M. (1968): Flowering of Spirodela polyrhiza (L.) Schleiden. *Acta Bot. Neerl.* **17**: 357–359.

PERRY, T. O. (1968): Dormancy, turion formation, and germination by different clones of Spirodela polyrhiza. *Plant. Physiol.* **43**:1866–1869.