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EFFECT OF 2-THIOURACIL AND GIBBERELLIC ACID ON FLOWER FORMATION IN WEDGWOOD IRIS

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ABSTRACT

1) 2-Thiouracil (2-TU) applied during low temperature treatment, inhibited flower formation in excised stem discs of Wedgwood iris. The inhibition was overcome by gibberellic acid (GA) and orotic acid but not by uracil or thymidine.

2) The first signs both of an inhibitive effect on flower initiation by 2-TU and of GA promotion occurred at the beginning of the reproductive stage. Increase or decrease of the rate of development of the flower primordium could be established only two weeks later, during differentiation of stamens or tepals.

3) The peak in respiration rate occurring in the bud at the beginning of the reproductive stage was entirely repressed by 2-TU and not restored on a medium containing 2-TU and GA.

4) It is concluded that a specific RNA takes part in the earlier stages of flower formation in Wedgwood iris.

5) The role of gibberellin-like substances in flower formation of the Wedgwood iris is discussed in the light of possible interaction with nucleic acid metabolism.

INTRODUCTION

Expression of the floral stimulus in photoperiodically sensitive plants is affected by compounds that are known to hamper nucleic acid synthesis (COLLINS, SALISBURY and ROSS, 1963). The inhibition of flowering in *Streptocarpus* by 2-thiouracil (2-TU) (HESS, 1959), in *Pharbitis nil* by 8-azaguanine (8-Az) and 2-TU (MARUSHIGE and MARUSHIGE, 1962), in *Cannabis sativa* by 2-TU (HESLOP-HARRISON, 1960) and in *Xanthium* by 5-fluorouracil (5-FU) (SALISBURY and BONNER, 1960; BONNER and ZEEVAART, 1962) seems to involve inhibition of synthesis of ribonucleic acid (RNA). According to ZEEVAART (1962) inhibition of flowering in *Pharbitis nil* by 5-FU and 5-fluorodeoxyuridine involves inhibition of synthesis of deoxyribonucleic acid (DNA) and of cell multiplication in the apex. BROWN (1962) found promotion of flower formation after application of iododeoxyuridine to the axils of *Arabidopsis* seedlings (which is a facultative LDP). It may therefore be asked whether flower formation in photoperiodically non-sensitive plants can also be influenced by these antimetabolites. Stem disc cultures of Wedgwood iris bulbs seem suitable objects for study of this point, especially since, according to ZEEVAART (1962) it is the realization of the flowering stimulus in the apex and not its production in the leaf that seems to depend on nucleic acid metabolism.

Gibberellic acid (GA) is known to promote flower formation in LDP's and in cold requiring plants (LANG and REINHARD, 1961). In Wedgwood iris, induction and differentiation of flower primordia

takes place at 13°, and both in the bulb and the excised bud this process is promoted by GA (HALEVY and SHOUB, 1964; RODRIGUES PEREIRA, 1962, 1965). ZEEVAART (1962) found that inhibition of flower formation in *Pharbitis nil* by 5-FU could not be reversed by application of GA.

In the present series of experiments the antimetabolites 8-azaguanine, 5-fluorouracil or 2-thiouracil, alone or combined with the metabolites guanine, uracil, kinetin, orotic acid, thymidine or gibberellic acid were applied to excised stem discs of Wedgwood iris cultured on a nutrient medium and their influence on flower formation in the bud studied.

MATERIAL AND METHODS

All experiments were carried out with buds taken from large bulbs of *Wedgwood iris* possessing 100%flowering capacity. Methods for isolating and culturing explants were described earlier (RODRIGUES PEREIRA 1962, 1964), but may be briefly summarized here.

Before isolating the stem discs, bulbs were partially induced at 13°, with the duration of this induction (= pretreatment) depending on time of the year. Standard nutrient medium consisted of Knop's solution half strength, Heller's micronutrient solution, sucrose and agar. The explants were either cultured on the standard medium to which the compounds to be tested were added before autoclaving, or they were dipped into autoclaved aqueous solutions of these compounds and afterwards cultured on the standard medium. The amount of solution being brought on the explants by the latter procedure was 38.8 ± 4.2 mg. Although this method has the advantage of greater simplicity, incorporation of the chemical into the medium was more effective and the results were less variable. The 5-fluorouracil was kindly supplied by Hoffmann – La Roche, Basel, Switzerland; the gibberellic acid by Plant Protection Ltd., Yalding, Kent, England.

Respiration measurements were carried out using a standard Warburg apparatus following procedures described earlier (RODRIGUES PEREIRA 1962). This part of the experiments was conducted by Mr. O. Th. Schönherr.

Most of the experiments were carried out at least three times; there was an average of at least 20 explants per treatment.

RESULTS

1. *Preliminary experiments*

In experiments that were not followed up further the activity of 8-Az and 5-FU as inhibitors of flower formation and the ability of guanine and kinetin, and of thymidine, orotic acid and gibberellic acid to counteract this inhibition were tested.

Depending on concentration, both compounds had an adverse effect on flower formation. Although also at lower concentrations a small effect was noted, inhibition only became evident at 10^{-4} M, which concentration, however, was already a little toxic. Therefore, it does not seem probable that the two compounds are acting as specific inhibitors of flower formation in iris.

In concentrations rangeing from 10^{-6} to 10^{-4} M, neither of the four metabolites applied showed any consistent effect.

There were small indications that inhibition by 8-Az was counteracted by kinetin and guanine, and 5-FU inhibition by orotic acid and thymidine. Inhibition by 5-FU was in one experiment partly and in another one entirely overcome by gibberellic acid (Table 1).

In direct mutual comparison of the activities of orotic acid and GA as combined with the antimetabolite, it was established that reversal by GA was more pronounced.

TABLE 1

Ability of different metabolites to antidote 5-FU inhibition of flower formation in excised stem discs of Wedgwood iris

chemicals applied	flower formation					
	series I			series II		
	n_t	n_r	score	n_t	n_r	score
control	48	34	5	43	31	5
5-FU 3×10^{-5} M	22	0	—	24	14	5
5-FU 3×10^{-5} M + orotic acid 10^{-4} M	24	10	3	24	10	3
5-FU 3×10^{-5} M + thymidine 10^{-4} M	20	5	3	24	3	2
5-FU 3×10^{-5} M + GA 10^{-4} M	19	14	3	24	19	5

Chemicals incorporated into the medium. Pretreatment of bulbs of series I 2 weeks at 13°, of series II 10 days at 13°. Duration of experiment (pretreatment of bulbs plus incubation of buds) 7 weeks. n_t = number of stem discs incubated; n_r = number of apices found reproductive at control; score = average stage of flower formation in reproductive apices expressed in equal units ranging from 0–14.

2. *2-Thiouracil and gibberellic acid*

In our experiments the antimetabolite 2-TU was a more effective inhibiting agent than 5-FU. In one series of experiments 2-TU was tested over a range of concentrations, up to 10^{-3} M in trials with nutrient media containing pre-added 2-TU and up to 10^{-2} M in dipping experiments. In four out of six experiments, one of which is presented in Table 2, inhibition increased generally with concentration. In one dipping experiment in which the buds were only slightly activated at the outset and, by consequence, score in the flower forming controls was low, the lowest concentration applied caused an increase instead of a decrease; in another experiment the

TABLE 2

Effect of 2-thiouracil on flower formation in excised buds of Wedgwood iris

concentration of inhibitor	flower formation		
	n_t	n_r	score
control	21	21	9
2-thiouracil 10^{-4} M .	23	21	8
5×10^{-4} M	20	18	6
10^{-3} M	23	20	4
2×10^{-3} M	44	13	4
4×10^{-3} M	22	0	—

Dipping experiment; pretreatment of bulbs 2 weeks at 13°; duration of experiment 6 weeks. Other data as in Table 1.

lowest inhibiting concentration was as high as 2×10^{-3} M. In four out of six experiments in which several 2-TU concentrations were used, and in six out of ten experiments with one concentration, 2-TU inhibition of flower formation could be proved statistically.

Inhibition by 2-TU was not reversed by uracil or thymidine; it was, however, by orotic acid and GA (Tables 3 and 4). In several experiments the effect of 2-TU doses capable of depressing both n_r and score was reversed by equal (or slightly higher) concentrations of GA or orotic acid. In four out of four experiments, using various concentrations, number of reproductive apices was greater at combinations of 2-TU and GA, than when the inhibitor alone was applied. In three experiments with weekly sampling, using selected concentrations of 2-TU and GA (see next paragraph) n_r was twice and score once found higher with 2-TU and GA together than with the single inhibitor. With orotic acid no linear dependency on concentration

TABLE 3

Ability of orotic acid and gibberellic acid to antidote 2-thiouracil inhibition of flower formation in excised buds of Wedgwood iris

exp. nr.	chemicals applied	flower formation		
		n_t	n_r	score
1	control	34	27	5
	2-TU 10^{-3} M	18	9	3
	2-TU 10^{-3} M + orotic acid 10^{-3} M	18	17	5
2	control	14	11	5
	2-TU 2×10^{-3} M	47	10	3
	2-TU 2×10^{-3} M + GA 10^{-3} M	24	14	4

Dipping experiments. Exp. nr. 1: pretreatment 3 weeks at 13°; exp. nr. 2: pretreatment 1 week at 13°. Other data as in Table 1.

TABLE 4

Influence of 2-thiouracil and gibberellic acid on flower formation in excised stem discs of Wedgwood iris

concentration of gibberellic acid	flower formation					
	gibberellic acid			3×10^{-5} M 2-TU + gibberellic acid		
	n_t	n_r	score	n_t	n_r	score
	48	34	5	24	1	3
3×10^{-6} M	22	16	4	23	6	3
10^{-5} M	23	16	5	24	4	2
3×10^{-5} M	24	19	5	24	6	3
10^{-4} M	24	21	7	23	12	4
3×10^{-4} M	23	22	8	24	0	—

Chemicals incorporated into the medium. Pretreatment 2 weeks at 13°. Duration of experiment 7 weeks. Other data as in Table 1.

could be established. Very high amounts of GA sometimes did not reverse 2-TU inhibition at all, whereas in the same experiment low amounts did. Gibberellic acid applied alone never became inhibitory, even at high concentration.

By investigating several samples at intervals of 1 or 2 weeks following the start of the incubation, it was tried to find out whether susceptibility of stem discs to the activities of 2-TU, GA or the two together is dependent on time (Table 4). For example, the first sign of inhibition by 2-TU was found 4 weeks after the beginning of the experiments (i.e. after one week of bulb pretreatment and three weeks incubation). At that time the controls had just entered the first stage of the reproductive primordium (stage II, according to BEYER, 1942). Also after four weeks on the medium containing GA, n_r was slightly increased. One week later there was still no difference in score between the controls and the 2-TU and GA treated stem discs respectively. Six weeks after the beginning of the experiments score in the 2-TU treated stem discs was clearly depressed, whereas on the GA containing medium it was as clearly increased.

TABLE 5

Influence of 2-thiouracil and gibberellic acid on the flower formation in excised buds of Wedgwood iris

chemicals applied	flower formation								
	4 weeks			5 weeks			6 weeks		
	n_t	n_r	score	n_t	n_r	score	n_t	n_r	score
control	15	7	3	14	8	3	14	11	5
GA 10^{-4} M	15	11	3	13	11	4	14	12	10
2-TU 3×10^{-5} M	13	0	—	12	2	3	13	7	5
2-TU 3×10^{-5} M + GA 10^{-4} M	13	2	3	13	3	4	12	6	10

Pretreatment of bulbs 1 week at 13°. Chemicals incorporated into the medium. Three weeks after the beginning of the experiment the buds were still vegetative. Other data as in Table 1.

3. *Respiration measurements*

They were carried out with standard Warburg apparatus, using the methods described earlier. Excised stem discs were incubated on standard nutrient agar to which 2-TU, GA or both were added. At one-week intervals oxygen uptake of 5 of them together was measured in triplicate at 20°, in a solution containing the anorganic macronutrients of the standard medium. There were 0.2 ml 10 % NaOH and a wick in the side arm. After the manometric measurements were completed, developmental stages of the apices were determined (Table 4) and the explants discarded. Bulbs were pretreated by holding them at 13° for one week. The first measurements were carried out one week after the beginning of the incubation period, at which time all buds were still vegetative. Figure 1 gives the time course of respiration intensities over the experimental period.

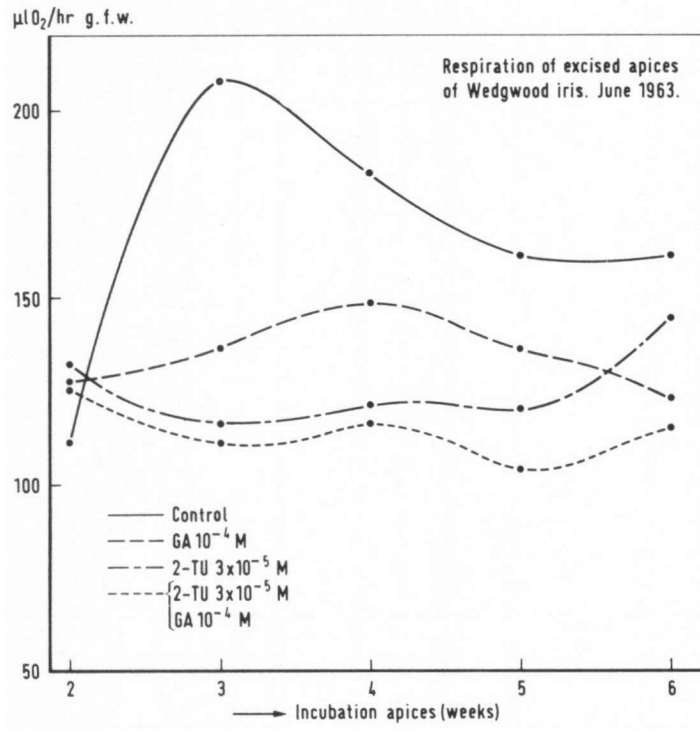

Fig. 1. Influence of 2-thiouracil and gibberellic acid on time course of respiration rate of excised stem discs during flower formation at 13°. Same buds as in Table 5. See data.

Plotting respiration rates of buds of iris bulbs incubated at flower forming temperature against time gives a characteristic maximum at the beginning of the reproductive stage (RODRIGUES PEREIRA, 1962), whether measurements are carried out at this same temperature or at 20°. A similar peak is seen when developmental stage (time) is plotted against respiration rate of excised stem discs incubated on a nutrient medium, both with and without exogenous GA. GA lowers respiration rate and 2-TU depresses it even more, and moreover 2-TU treatment results in the disappearence of the peak at the transition to the reproductive stage. Although 2-TU inhibition of flower formation is to a considerable extent reversed by GA, on the medium containing both 2-TU and GA respiration rate is about equal to that on the 2-TU medium, nor has the peak been restored.

DISCUSSION

There are some differences between the object of our investigations and those of the pertinent experiments described in the literature. The latter, *Cannabis*, *Streptocarpus*, *Xanthium*, *Pharbitis*, are all photoperiodically sensitive plants; antimetabolites were applied to the

leaf or the bud during or after induction in order to analyse their effect on induction or on realization of the flowering stimulus in the apex. Flower formation in Wedgwood iris bulbs is temperature induced, in the excised stem disc the leaves are absent and on media without any flowering agent added there is no matter of transport. In isolated scale fragments incubated on a nutrient medium, the contents of certain growth substances increase at the temperature which is optimal for flower formation, and we are probably entitled to make the same assumption for the bud. It follows that increase of these compounds in the bud of the normal bulb, as it was established in an earlier publication (RODRIGUES PEREIRA, 1964) may not be entirely caused by transport from the scales; on the contrary it is shown here that the whole process of flower formation can take place in the single apex.

The present results are in agreement with those reported by SALISBURY and BONNER (1960) and BONNER and ZEEVAART (1962) who showed that floral induction in Xanthium can be suppressed by 5-FU and that this suppression is reversible by orotic acid. From the present study it is evident that thymidine and GA are also capable of suppressing 5-FU induced inhibition.

In Wedgwood iris combinations of 2-TU with orotic acid or with gibberellic acid reveal much more about floral inhibition and its reversal. As is the case with 5-FU, 2-TU may in principle be supposed to interfere with DNA and/or RNA synthesis. The research of HESS (1961) has established that 2-TU inhibition of flowering in *Streptocarpus wendlandii* may be attributed to interference with synthesis of a specific flower-forming RNA. However, it does not seem to be established that 2-TU, as applied in his experiments, did not have an effect on vegetative growth. MARUSHIGE and MARUSHIGE (1962) have even pointed out that in their experiments on *Pharbitis nil* vegetative and flowering effects are unseparable. In our experiments with various antimetabolites the higher concentrations appeared to be toxic and even lethal, which might suggest that the harmful processes concerned also occur to some extent at lower concentrations.

The results of our respiration measurements, however, clearly suggest a specific action. The respiration rate in stem discs incubated on a 2-TU containing medium being depressed during the whole of the experimental period may be due to a general metabolic effect; but the total disappearance of the peak at the commencement of the reproductive stage suggests that at this time a much greater part of the respiration rate is inhibited than at any other time during the course of flower formation. Therefore, it may be deduced that at least at this developmental stage a specific 2-TU inhibitable nucleic acid activity is expressed as an increase in rate of respiration. Although the accompanying deceleration in flower formation is nullified by GA, the respiratory activity of this specific nucleic acid is evidently not restored. So perhaps we may conclude that there is only a very indirect relationship between the transition to the reproductive stage and the rise in respiration rate occurring at the same time.

Although the activity of 2-TU seemed to be more readily on the transition to the reproductive stage, whereas the effect of GA was more pronounced in the rate of development of the flower primordium, yet in either case it was impossible to differentiate between the two effects. On the other hand it does not seem warranted to measure progress in flower formation by one parameter, being a combination of n_r and score, because at the succeeding stages different mechanisms may be at work.

In examining the results of the present series of experiments it appears that also in cold requiring species such as Wedgwood iris it is possible to interfere with flower formation by way of inhibitors of nucleic acid metabolism. Although inhibition of flower formation by these substances and its reversal by GA has been established, we might not suggest that there is any direct interaction of GA with the metabolites concerned. The same inference can be drawn from the work by ZEEVAART (1962) and BROWN (1962) on interaction of GA and DNA metabolism in flower formation of *Pharbitis* and *Arabidopsis*. Evidence is accumulating, however, that several growth promoting activities of GA are exerted by way of its more or less directly interfering with nucleic acid metabolism. Activities of a variety of enzymes (amylases, protease, catalase, cellulase, phosphatase) are shown to be stimulated by gibberellic acid (DAHLSTRØM and SFAT, 1961). These authors suggested that the mechanism of the stimulation of these various enzyme systems occurs on the level of enzyme synthesis. SAHAI SRIVASTAVA and MEREDITH (1962) found that in germinating barley kernels amylase activity is depressed under the influence of chloramphenicol sodium succinate (CAL) which is thought to interfere with the function of the RNA component concerned in amino acid incorporation into proteins. In a solution containing CAL and GA this activity is not only restored but it is much greater than in water and almost as great as in GA alone. In our laboratory similar results were obtained by Schönherr (unpublished) with growth of lettuce seedling hypocotyls, which is known to be specifically increased by GA (FRANKLAND and WAREING, 1960). Schönherr found that growth of lettuce seedling hypocotyls was inhibited by CAL and by 2-TU and that in solutions of GA and any of the inhibitors, depending on the concentration ratio growth exceeded that in water.

Lastly, from recent investigations by MASUDA and coworkers (MASUDA and YANAGISHIMA, 1965) it also appears that GA exerts its growth promoting activities in Jerusalem artichoke, the *Avena* coleoptile and certain yeast strains, by way of a specific RNA.

Therefore, although no interaction could be established between GA and any of the metabolites used so far in the various investigations discussed, it seems justified to conclude that there are definite and even several points of contact between GA metabolism and nucleic acid metabolism. By way of these points of contact endogenous and exogenous gibberellin-like growth substances may influence such diverse processes as germination, elongation and flower formation. Added to this in the case of flower formation, and probably in all cases, is the

time dimension through which successive partial processes are controlled by different growth substances that in turn might be different forms of the same basic substance.

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