THE EFFECTS OF SELECTION FOR REDUCED DORMANCY IN CHARLOCK (*SINAPIS ARVENSIS*)

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1. Introduction

Dormancy of seeds is a character of frequent occurrence amongst annual weed species of temperate and colder regions. It enables a species to overcome the hostile environment of winter and allows the seeds to germinate in more favourable environmental conditions. Dormancy is determined by many different physiological mechanisms (Wareing, 1969), and in charlock, Edwards (1968) showed that growth inhibiting substance is produced at low oxygen concentration in the interior of the seed. Purified extracts from the testa of charlock have also been shown to have inhibitory effects on germination (Witcombe, Hillman and Whittington, 1969).

That dormancy is under genetical control has been shown by many workers. For example, in subterranean clover (*Trifolium subterraneum* L.), Morley (1958) showed that it was at least partly dependent on the genotype of the embryo as distinct from the testa. Results from the analysis of an F_2 generation and a diallel cross showed that dormancy was highly heritable. Interspecific crosses in the genus *Papaver* have shown that dormancy was heritable and that the influence of the maternal parent was not predominant. Instead the behaviour of the hybrid seeds was often unlike the germination of the seeds of either parents (Harper and McNaughton, 1960). Further evidence of maternal and paternal effects with various levels of interaction can be found in work reported by Simmonds (1964) on potato, and by Battle and Whittington (1971) on sugar beet.

Environmental factors may modify germination behaviour and in particular the dormancy of charlock seeds may be broken by gibberellic acid (Edwards, 1968).

The present investigation examines the genetic control of dormancy in charlock and in particular demonstrates that dormancy and germination characteristics can be modified by selection.

2. Methods and materials

Charlock seeds were obtained from a natural population growing in Nottinghamshire.

Germination tests were carried out, except where otherwise stated, at 25° C. in continuous darkness in plastic petri dishes on moist filter paper. Germination was defined throughout the experiment as the emergence of the radicle through the testa. In certain of the selection experiments, gibberellic acid (GA_3) was used to break dormancy and the specific effects of GA on

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germination are described in the first part of the Results section. Gibberellic acid (GA_3) solutions were made up by dissolving the GA in the minimum quantity of 5 per cent Na_2HCO_3 solution and distilled water was added to make up the required volume.

Plants were grown in a greenhouse with artificial lighting and heating, in plastic pots filled with John Innes No. 1 potting compost. In most cases plants were sprayed with Metasystox to prevent aphid attack. Further details of the methods used are given with the results of the various experiments.

3. Results

(i) *The influence of seed-coat colour in determining dormancy*

Charlock seeds show variability in the colour of the testa from light brown to black. Ten replicates each with 50 brown or black seeds were germinated in water either with or without prior treatment for 24 hours with 2000 p.p.m. GA. When no treatment was given brown seeds still exhibited dormancy but showed a higher percentage germination than black seeds ($9{\cdot}0 \pm 1{\cdot}1$ and $1{\cdot}0 \pm 0{\cdot}3$ per cent. respectively). There was a uniformly high percentage germination for both brown and black seeds after treatment with GA ($97{\cdot}4 \pm 0{\cdot}5$ and $95{\cdot}2 \pm 1{\cdot}0$ per cent. respectively).

Brown and black seeds were also selected and planted after treatment with GA in a randomised block design. Twenty plants were raised in each block for the two selected lines and these were pollinated by members of the same line. Seeds were harvested and ten seeds from each plant were scored for colour. The percentages of brown seeds per block ranged from 23·0 to 24·5 and 11·5 to 26·5 from material derived from the brown and black seed respectively. This suggests that while variability in seed-coat colour may be important in determining the germination of the seeds, the heritability of the character is low and the colour is largely determined by the maturity of the seed. In all later experiments black seeds only were used.

(ii) *The effects of gibberellic acid on germination*

Charlock were germinated in concentrations of 0, 10, 20, 40, 100, 200, 500 and 1000 p.p.m. of GA. There were 100 seeds per treatment for each species in ten replicates of ten seeds per dish. Germination was recorded after 1, 2, 3, 4 and 7 days.

After imbibition for 1 day there was only a small response to GA since few seeds had germinated in any treatment (fig. 1). The effect of GA in increasing the percentage germination became apparent, in both species, after 2 days. At this time GA, at concentrations above 100 p.p.m. produced an increase in percentage germination. This concentration, therefore, can be described as the threshold value of GA concentration above which there is a marked increase in germination percentage. This precise demarcation of the point at which GA began to exert an effect was also shown on other days but the threshold concentration became lower as the time after imbibition increased. The threshold concentration of GA for charlock on day 4 was 20 p.p.m.; this contrasted to the threshold concentration of 100 p.p.m. GA on day 1. Thus, given sufficient time, the threshold effect disappears and all concentrations of GA were found to cause a response after 7 days. A specific time rather than a specific concentration is required before a response.

Thus 40 p.p.m. GA did not produce a response until 4 days after imbibition whereas 1000 p.p.m. GA produced a large response after 2 days.

A seed will not germinate until the products of enzyme controlled reactions, which are necessary for germination, have reached a sufficient level. Since GA may act by promoting enzyme activity (Chrispeels and Varner, 1967; Filner and Varner, 1967; Jacobsen and Varner, 1967) and the rate of enzyme controlled reactions are dependent upon concentration of substrate

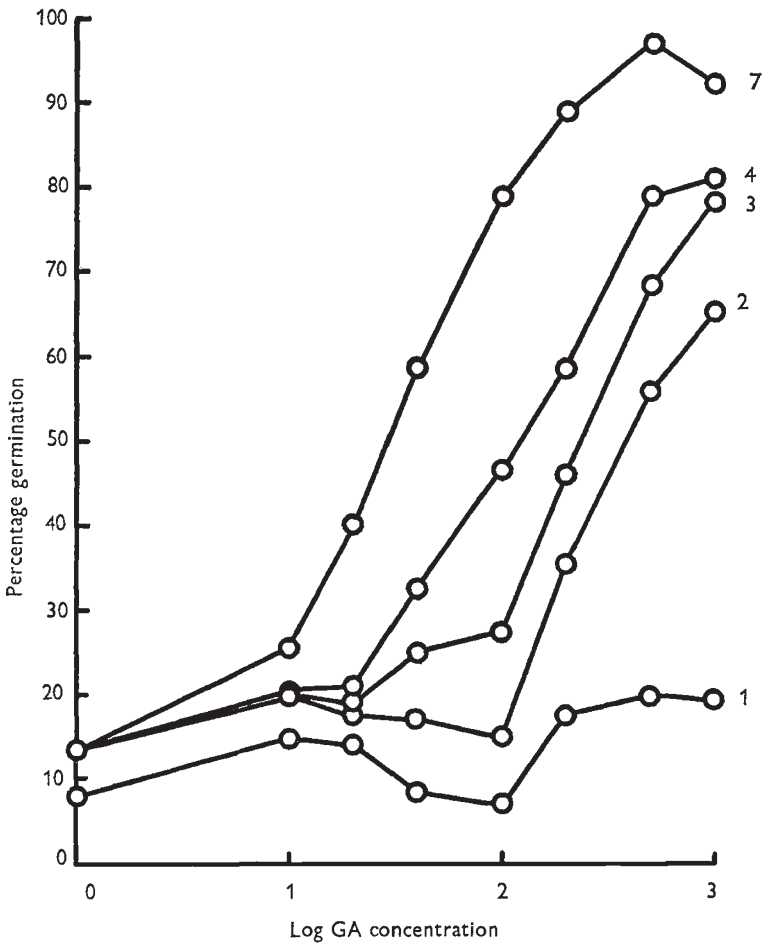

FIG. 1.—Percentage germination of charlock seeds after 1, 2, 3, 4 and 7 days in different concentrations of gibberellic acid.

and enzymes, then it is to be expected that low concentrations of GA take longer to produce a response.

When the concentrations, or time available were sufficient for the GA to produce a response then the relationship between the effect of GA and the log of its concentration was linear (fig. 2). This linear relationship between the effect of GA and its log concentration is a relationship common to many physiological responses to growth substances, *e.g.* Smith and Rappaport (1961).

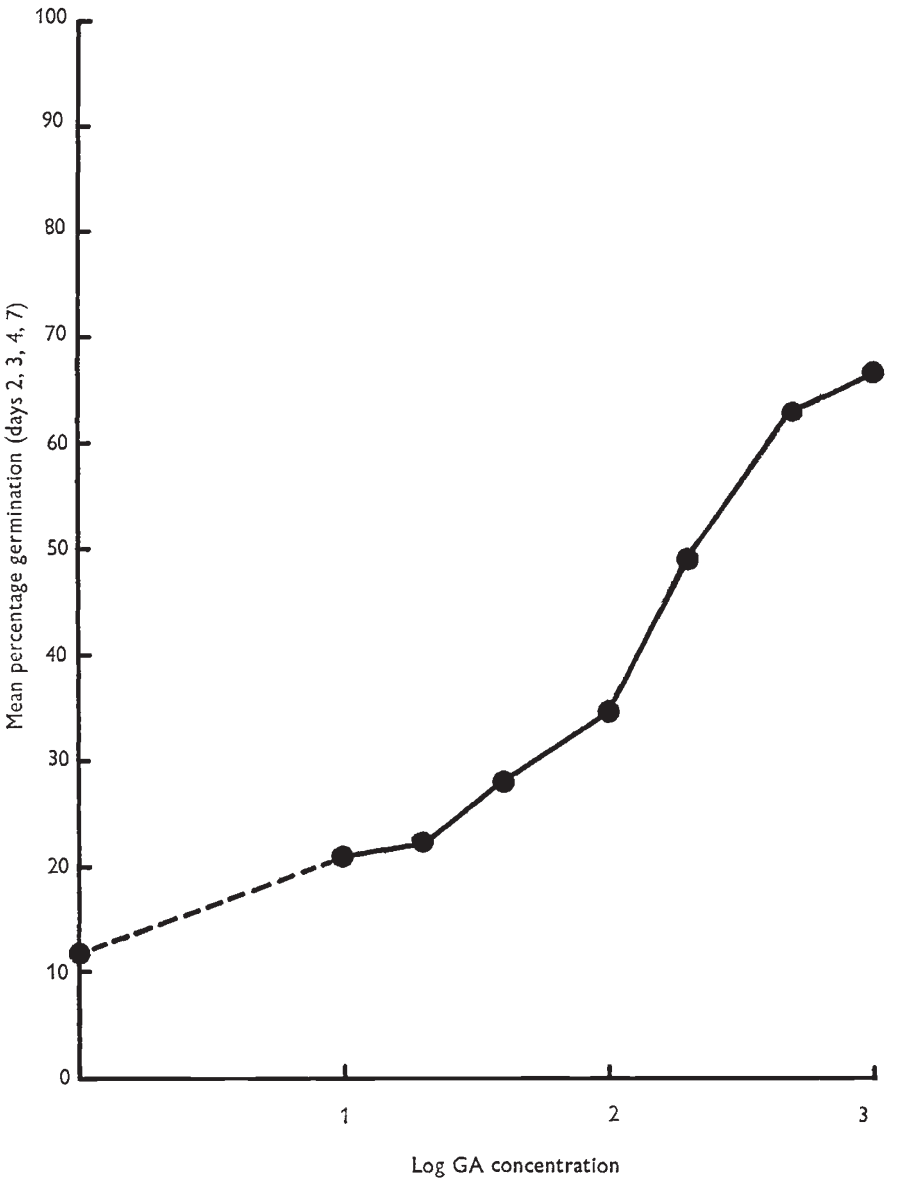

FIG. 2.—Mean percentage germination on days 2, 3, 4 and 7 of charlock seeds in different concentrations of gibberellic acid.

(iii) *The effects of selection on germination characteristics*

(a) *One generation of mass selection*

A selection line for non-dormancy was initiated from charlock seeds which had germinated within 60 hours of placing them in water. A control population was obtained by taking a random sample of seeds from the wild population. The dormancy of this sample was broken with 2000 p.p.m. GA.

The plants were grown in a randomised block design in two blocks with 20 plants per selected line. When in flower the plants were pollinated daily using a camel-hair brush and the selected lines were crossed within themselves. The lines were grown in pollination cages which were covered at the sides with non-woven terylene. These techniques for pollinating and isolating the selection lines were also used in later experiments where selection lines were grown. The seeds were harvested when mature and the percentage germination in water of 15 seeds was determined.

The line selected for non-dormancy showed a higher percentage germination in water than the control line (table 1). A heritability estimate was

TABLE 1

Percentage germination of charlock seeds after 9 days in selected lines and heritabilities of dormancy

	Percentage Germination		
	Non-dormant selection line	Control line	Heritability (%)
Block one	11·3	2·0	10·7
Block two	16·3	6·6	11·2

Mean heritability = 10·9 per cent.

made by comparing the observed response to the expected response. In order to allow for the fact that the parental seed of the control population had a final percentage germination in water of 13·4 per cent., the difference in percentage germination between the selected and control populations was compared against an expected response of 86·6 rather than 100 per cent. The mean heritability was 10.9 with good agreement between the two replicates.

This might be an underestimate if maternal effects, which are commonly found in experiments on seed germination, were present. Effectively this could mean that only the characteristics of one parent influence germination and the heritability estimate might be multiplied by a factor of 2 to allow for the maternal effects.

(b) *Selection for several generations*

In an experiment initially concerned with selection for early flowering records were also kept of the percentage germination of the seeds at each generation with the exception of the S_1 generation. The concentration of GA employed to break the dormancy of the seeds which gave rise to the next generation of plants was recorded.

The extent of any change in germination behaviour is obviously dependent on the selection pressure applied and no alteration occurred in the germination behaviour of progeny from parents arising from seeds treated with the high GA levels (fig. 3). These levels were used merely to produce large populations in which selection for flowering time could occur. The third generation arose from seeds treated with 500 p.p.m. GA and the progeny from these plants showed a reduction in dormancy because a concentration of GA was being used that was sufficiently low to exert a selection pressure (fig. 3). Thereafter the increase in percentage germination with continued selection was very marked apart from one generation (S_6) where

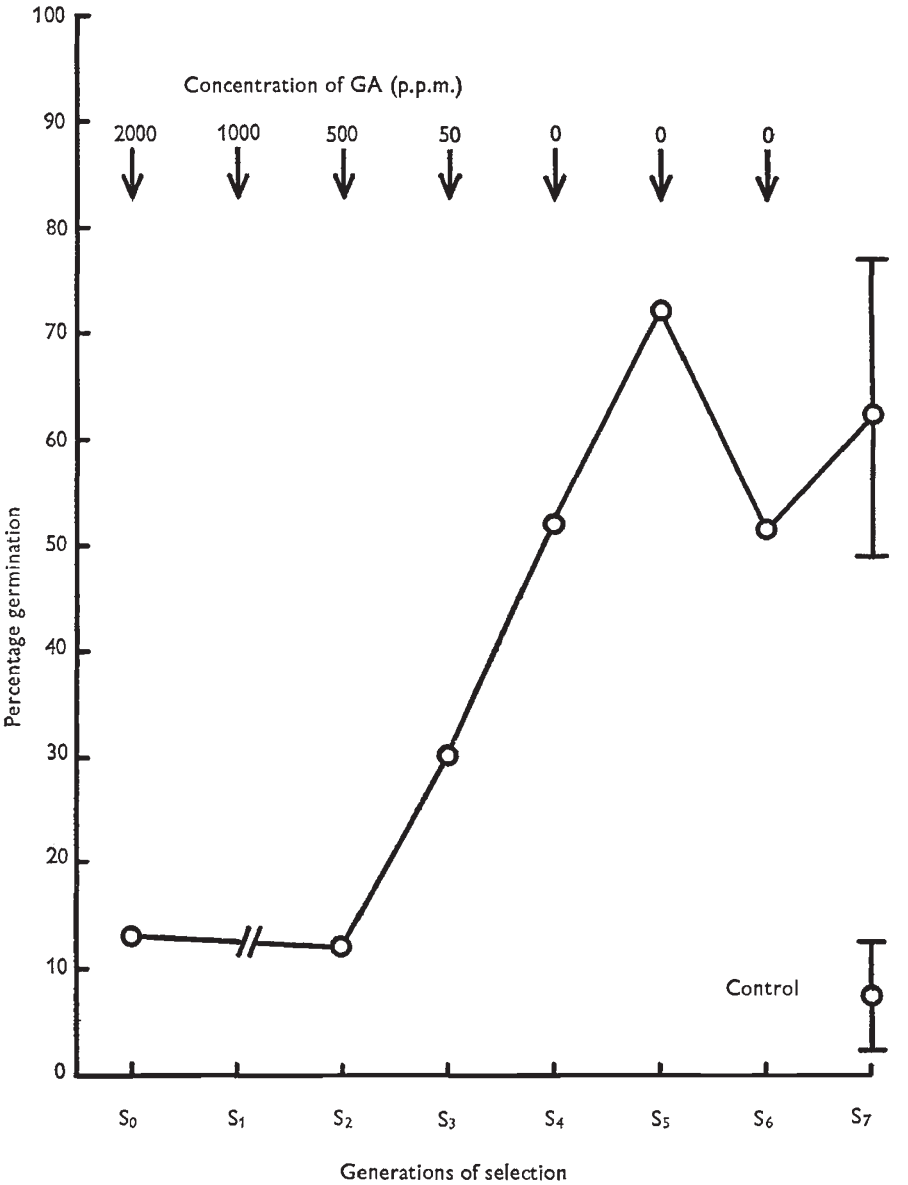

FIG. 3.—Effects of selection over several generations on the percentage germination of charlock seeds. The concentration of gibberellic acid used to break dormancy and initiate each generation are shown.

germination was lower than expected. By the S_7 generation a germination in water of 64 per cent. after 3 days was obtained. Control plants were grown in this generation from S_0 generation seeds and the seeds they produced showed a percentage germination of 7 per cent. Thus, over two generations of low selection intensity, and five with more intense selection, a species with dormant seeds was modified to produce seeds with a high degree of non-dormancy.

The differences between the selected seeds and the control seeds was confirmed in four different day lengths (table 2) in the S_7 generation. In

TABLE 2

Mean percentage germination and S.E. for charlock seeds of the S_7 and control population in water under four different environments

Environment	S_7 population	Control population
Laboratory	96±9	28±13
18-hr day	90±4	11±12
8-hr day	86±14	17±11
Dark	64±14	7±5

each instance the seeds of the selection line showed a higher percentage germination than seeds from the control population.

(c) *Selection for response to different levels of GA*

In this experiment the heritability of the response of the seeds to different GA levels is examined.

Charlock seeds were treated with 50 p.p.m. GA and the seeds that germinated at this concentration were selected as a " low GA " selection line.

TABLE 3

Percentage germination after 7 days under different conditions of the seeds of selected lines of charlock

	Low GA line	High GA line	
Percentage germination in H_2O			
Block one	3·0	0·5	
Block two	7·5	1·0	
Additional Percentage germination in 20 p.p.m. GA			
Block one + GA	8·0	4·0	
Block one control	0·0	0·0	
Block two + GA	13·0	2·0	
Block two control	0·0	1·0	
Total Percentage germination in H_2O *and 20 p.p.m. GA*			*Heritability*
Block one	11·0	4·5	6·5%
Block two	20·5	4·0	16·5%

Mean heritability = 11·5 per cent.

A " high GA " selection line was obtained from seed which failed to germinate at 50 p.p.m. GA concentration, but which later germinated in 2000 p.p.m. GA.

The low and high selection lines were planted out in a randomised block design with two blocks and 20 plants per selected line. The seeds were harvested and 10 seeds per plant were assessed for germination in water and those that failed to do so were either assessed after drying for germination in 20 p.p.m. GA or reimbibed in water as a control.

It was found that " low GA " selection line showed a higher germination percentage than the " high GA " line in both water and 20 p.p.m. GA

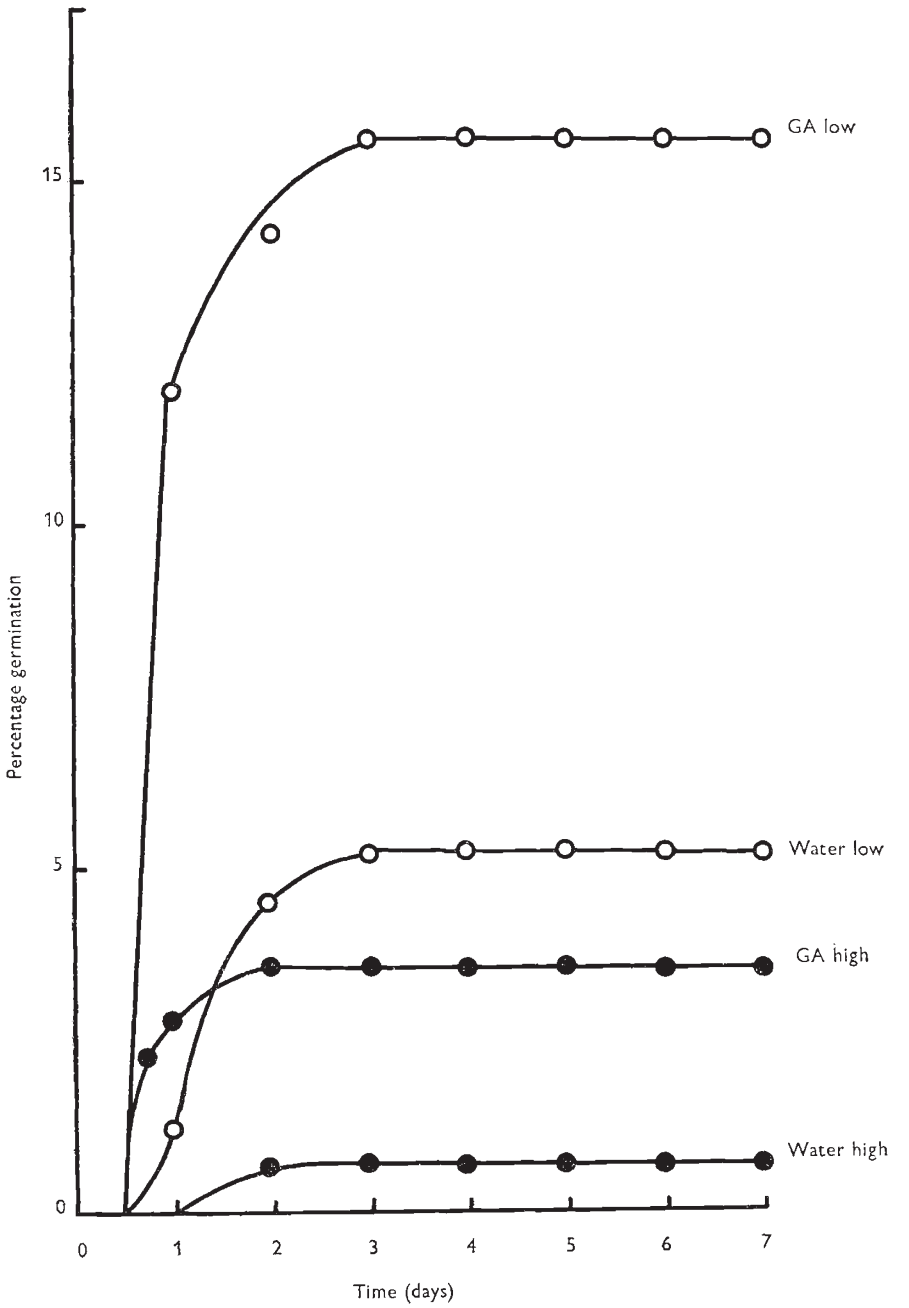

FIG. 4.—Percentage germination of low ○, and high ●, GA selection lines in water or after treatment with 20 p.p.m. GA.

fig. 4, table 3). Thus there was genetic variability for response to two different concentrations of GA and a heritability of 11·5-23·0 per cent., depending on the extent of the maternal effects, was found for this character.

When seeds are allowed to imbibe in water under suitable conditions for germination a proportion of those seeds may germinate. The population of seeds may then be divided into two classes; non-dormant seeds and dormant seeds and a discontinuous variation is thus assumed. However, dormancy should not be regarded as discontinuous since some dormant seeds are more dormant than others and the results of this experiment demonstrate this perhaps obvious but important point. The dormant seeds of the low GA selection line showed a greater response to a weak dormancy breaking agent (20 p.p.m. GA) than the dormant seeds of the high GA selection lines. Thus the dormant seeds of the low GA selection line were less dormant than those of the high GA selection line.

It can be concluded that each seed has a specific requirement for GA. A seed which germinates in water has a GA requirement of zero, whereas dormant seeds require larger but varying amounts of GA to germinate. The degree of dormancy of a seed can thus be quantified by its GA requirement to break its dormancy.

(d) *Selection for rapidity of germination after GA treatment*

Although high levels of GA broke the dormancy of all treated seeds it was observed that these germinated at different rates. In order to investigate this further, charlock seeds were germinated after treatment with 2000 p.p.m.

TABLE 4

Percentage germination and mean times to germination (M.G.T.) under different conditions of the seeds from selected lines of charlock

Parental M.G.T. of early line (hours)	24·0
Parental M.G.T. of late lines (hours)	79·2
Selection differential (hours)	55·2

	Early line percentage	M.G.T.	Late line percentage	M.G.T.	
Percentage germination and M.G.T.'s in H_2O after 7 days					
Block one	4·0	35·4	2·4	38·1	
Block two	8·4	35·14	5·2	40·2	
Percentage germination and M.G.T.'s in 2000 p.p.m. GA after three days					*Heritability*
Block one	79·0	35·6	60·0	40·4	18·2%
Block two	67·0	35·6	67·8	38·2	4·7%

Mean Heritability = 12·9 per cent.

GA and an early germinating line was selected from seed which germinated within 24 hours of placing the seeds in GA solution and a late germinating line was selected from seeds that germinated after 48 hours.

The selected seeds were planted out in a randomised block design with two blocks and 25 plants per selected line. The germination percentages and germination rates were determined from 10 seeds from each plant in 2000 p.p.m. of GA solution and water. Germination rates were calculated as mean germination times in hours, where this is the average time taken for germination by the seeds. Seeds which failed to germinate were omitted from the calculation.

The early germinating selection line had a higher percentage germination in water and a more rapid germination rate in both water and 2000 p.p.m.

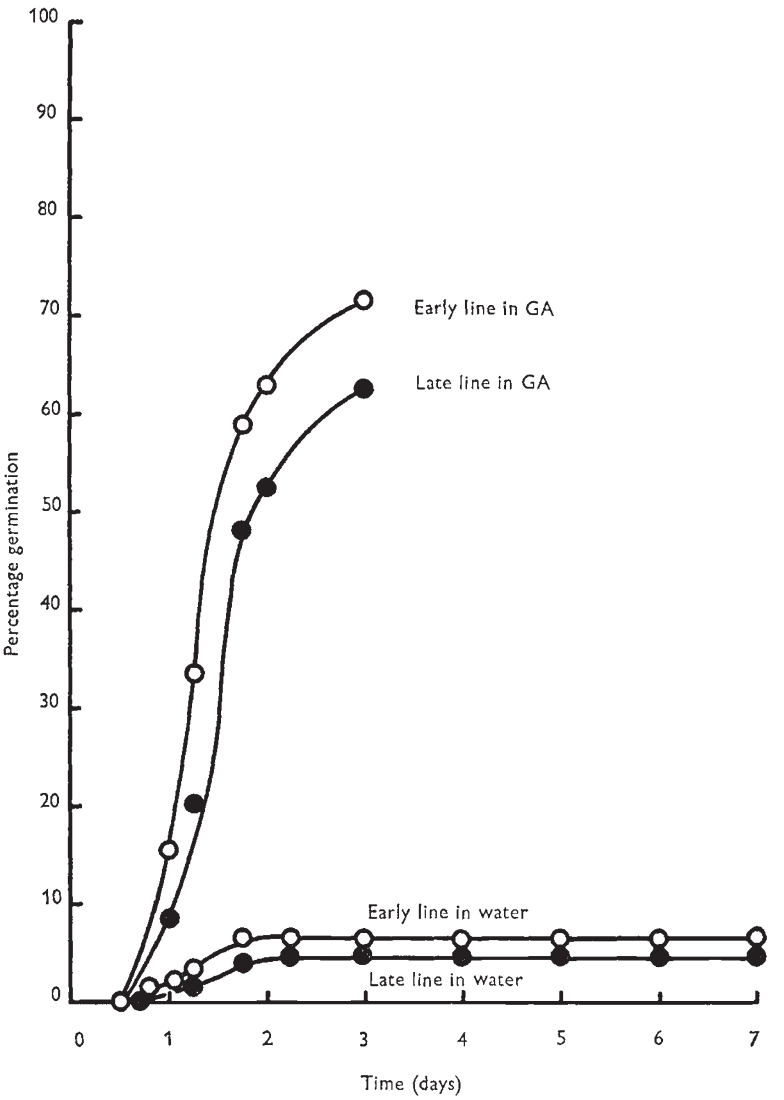

FIG. 5.—Percentage germination of early ○ and late ● GA selection lines in water, or after treatment with 20 p.p.m. GA.

GA (fig. 5, table 4). The data for germination in 2000 p.p.m. has not been recorded after 3 days although asymptotic values for germination had not been reached. The results show that seeds which germinated most rapidly after treatment with G.A. were also the more likely to germinate in water. It can be concluded from the previous experiment that seeds which germinate the most rapidly after GA treatment have a lower requirement for GA and the results again indicate that dormancy is a character which is inherited in a quantitative fashion.

The minimum heritability estimate, ignoring maternal effects, for mean time to germination was 12·9 per cent. and this agrees with the other

estimates from the response to different GA levels and for dormancy in water. It therefore seems likely that in all of the experiments concerning dormancy and response to GA the different expressions of the same gene system are being studied.

4. Discussion

In any species exhibiting seed dormancy it would be expected that the degree of dormancy would be related to the intensity of selection against individuals with non-dormant characteristics. The material studied was taken from natural population in the Midland Counties where winters are often mild enough for autumn germinated seeds to survive. Such plants are likely to have a competitive advantage in crowded populations relative to those from seeds which germinated in the spring and will produce larger plants with a greater number of seeds due to their longer growing season. The population is thus likely to retain genetic variability for seed germination with gene frequencies relative to the intensity of selection.

Inevitably a further source of variation arises from the effects of environmental factors, *e.g.* light, temperature and water availability during the maturation and subsequent germination of the seeds. One of the most obvious effects of environmental factors in charlock appears to be an effect of immaturity on the seeds which simultaneously results in a brown rather than a black seed-coat and a reduction in dormancy. This provides a source of variability for dormancy which is largely environmental since the experimental results indicated a low heritability for seed-coat colour. It is an environmental variability which may well have adaptive significance since plants which die prematurely will tend to leave non-dormant seeds. There is thus a possibility of a second generation arising from these plants when there is sufficient of the growing season remaining for successful maturation to occur.

Exposure to GA can hardly be regarded as a normal environment and thus the observed responses to selection for increased germination have to be carefully assessed to establish the belief that genetic variability for dormancy existed in the material studied and that natural selection can modify germination characters.

In the one generation mass selection experiment in fact GA was used only to provide the control population. The selected population was formed from seeds which had germinated in water and the improvement in germination over the control line of 9·5 per cent. is clear evidence that dormancy can be modified by selection. As would be expected, selection over several generations was at first ineffective until the intensity of selection was increased by lowering the concentration of GA used to break dormancy. There is of course no necessity to use GA except to increase to reasonable proportions the number of seedlings available. It is possible, however, that response to selection after germinating the seeds in water alone may be less effective than using a low GA concentration since some of the seeds germinating in water might do so for purely non-genetic causes. Stimulation of some seeds to germinate by GA treatment may help to ensure the inclusion of some genotypes with low dormancy characteristics and thus raise the heritability for germination characteristics. The relative efficiency of selection with and without GA treatment has not yet been examined but the results would be

much affected by the initial seed sample examined, and the environments in which subsequent generations were grown.

When selection for non-dormancy over several generations was examined the responses to selection only occurred if selection intensity was high, since there was no response where dormancy had been broken by high concentrations of GA. Low concentration of GA caused those seeds to germinate which would have germinated in water together with others only requiring exposure to low concentrations of GA. The initiation of germination with an environmental factor followed by selection and response under conditions of decreased stimulation or in the absence of stimulation resembles the results of Waddington on genetic assimilation (Waddington, 1959). The results of this experiment gave a convincing demonstration of how a wild species can lose its dormancy, a process which must occur during the evolution of crop plants. What is notable here was the extreme rapidity with which the process occurred and unconscious selection of crop plants by primitive man could obviously have had similar effects, particularly when it is realised that this unconscious selection pressure would have a high intensity. Where the site of sowing was not repeated, for example, dormant seeds would have an effectiveness of zero.

The experimental results support the view that the variability in germinability in the seeds from the population studied has a genetic basis. The variability is continuous in nature and this inevitably follows from the combination of environmental as well as genetic effects. It is clear that a population of freshly harvested charlock seeds is likely to be composed of some seeds which will germinate readily either for environmental or genetic reasons, together with others which are at first dormant. These germinate over a period of time in response to environmental factors. The precise characteristics of a population of seeds is likely to depend on geographical conditions, the relative maturity and size of the seeds, the variability of the environment and the selection intensities operating on the population.

5. Summary

1. Charlock seeds with brown seed-coats germinate more readily than black seeds but variation in seed-coat colour has a low heritability.

2. The dormancy of charlock seeds is broken by gibberellic acid. Response to low concentrations is less rapid than to high concentrations but the final percentage germination is independent of the concentration.

3. Germination was higher in the progeny of plants from seeds which initially germinated in water than in progeny from seeds which were artificially stimulated to germinate.

4. Selection for non-dormancy over several generations led to a marked response such that a species with a high degree of dormancy was modified to produce seeds with a high degree of non-dormancy.

5. Charlock seeds were shown to be variable in their response to the level of gibberellic acid used and this response was shown to be heritable. Plants from seeds which had germinated at low concentrations produced seeds which also germinated well at low gibberellic acid concentrations and also in water.

6. The response of charlock to selection for non-progeny is discussed in relation to changes in germination behaviour during crop plant evolution.

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