DIFFERENTIATION IN POTATOES : HIDDEN-SPOTTED AND SPECTACLED

H. W. HOWARD

Plant Breeding Institute, Trumpington, Cambridge

Received 14.v.66.

1. INTRODUCTION

REMOVAL of eyes, or X-ray treatment, of potato (*Solanum tuberosum* L.) tubers, which have splashed colour in the periderm and full colour in the phelloderm may result in plants which have spectacled tubers (Howard, 1964a). It is now shown that the treatment of full-colour tubers of plants, which have one E gene for full colour in the periderm and another E gene coupled with M for splashed colour in the periderm, may produce plants with spectacled tubers. This shows clearly, as has already been pointed out by Simmonds (1965a), that the explanation, given in fig. 2 of Howard (1964a), for the origin of spectacled, is false.

2. MATERIAL

The clones used were produced from seedlings of family Q78. This family was grown from the cross W10B $\times$ C.P.C. 304S.

Clone W10B, from the cross Y30/8 (splashed purple tubers) $\times$ Ulster Knight (white tubers) has full purple tubers, the pigment being present in the periderm. The full colour of W10B originated from a meiotic crossing-over between genes E and M (Howard, 1962). Crossed with Ulster Knight (white tubers, pink sprouts), W10B gave family Q81 in which there were 109 seedlings with white tubers, 52 with full purple and 56 with full pink. Clone W10B is thus simplex for both the gene E^T for full pink periderm and the gene P which converts pink into purple (in this paper the basic gene D for tuber skin pigmentation need not be considered).

Clone C.P.C. 304S is an Andigena potato which was received from the John Innes Institute. It has pink tubers with spectacles (*i.e.* regions around the eyes without pigment) and the pigment is in the periderm. The spectacles are large and some tubers (fig. 1a) approach the intermediate type of Asseyeva (1931). Crossed with Ulster Knight, C.P.C. 304S gave family N18 which had 34 seedlings with white tubers, 36 with splashed pink, 27 with full pink, 22 with hidden spotted and five with spectacled. As will be shown later, scoring for hidden spotted *v.* full is not reliable and also, as is known from previous work, spectacled is related to hidden spotted. The segregation in family N18 can therefore be expressed as 34 white: 36 splashed: 54 full. This is what would be expected from $E^A E^S e_3 \times e_3$, where E^A produces full colour, E^S splashed colour, and $E^A E^S$ is full colour or spectacled, not splashed (*i.e.* E^A is dominant to E^S). In family N18, as in C.P.C. 304S itself, pigment was in the periderm and no pigmented tubers with colour in only the phelloderm were found.

Family Q78 (W10B $\times$ C.P.C. 304S) is therefore $E^T e_3 \times E^A E^S e_2$ (where E^T is the gene for fully pigmented periderm in Tuberosum potatoes, E^A being a similar gene in Andigena). In family Q78 there were nine seedlings with white tubers, 41 with full colour (21 full: 19 hidden-spotted: one spectacled), and 25 with splashed colour. The explanation which gives the best fit to this segregation involves E^A being dominant to E^S (as in family N18) but E^S being dominant to E^T (other

results support this suggestion). The factor designated E^S is presumably E^A in close coupling with the pigment-restricting gene M of Howard (1959).

Six seedlings of family Q78 were cloned to give material for the experiments. The tubers of three (Q78/5, Q78/6 and Q78/7) were scored as being full purple, of two (Q78/8 and Q78/9) as being purple hidden-spotted (full colour with deeper patches around the eyes) and of one (Q78/12) as being pink hidden-spotted. The six seedlings were chosen for the experiments because of their high yield of relatively large tubers.

TABLE 1
Results of eye-excision and X-ray treatments in 1963

Clone	Eyes excised		X-ray treated		Cuttings from X-ray treated plants. Total tubers
	No. of half-tubers treated	Results*	No. of rose-ends treated	Results*	
Q78/5	6	11f; 26f	3	14f; 16f; 14f	18of
Q78/6	10	12f; 13f	5	7f; 16f; 10f; 11f; 7f	13of
Q78/7	10	2f; 3f	5	15f; 5f; 32f; 12f; 3of	253f
Q78/8	10	1of; 1f+5sp	5	9f+3sp; 2f; 1of; 8f	4of+9sp
Q78/9	10	9f	5	5f; 3f; 4f; 3f; 7f	5of
Q78/12	10	2of; 19f; 14f; 2of; 27f	5	22f; 11f; 13f; 17f; 15f	336f

* f = full colour; sp = spectaclled. 1of; 1f+5sp means one plant had ten full coloured tubers and another plant had one full coloured and five spectaclled tubers.

3. EYE-EXCISION AND X-RAY EXPERIMENTS

With the exception of Q78/5 where only a few tubers were available, eyes were excised from five tubers, cut to give ten half-tubers, of each clone and five apical ends of tubers were given an X-ray dosage of 3500 r. As usual, only a few eye-excised tubers produced adventitious buds but nearly all X-rayed apical ends gave plants. The results

TABLE 2
Segregations in crosses of Q78 clones

Family no.	Parents	No. of seedlings with tubers				
		Full		Splashed		White
		Purple	Pink	Purple	Pink	
L29	Q78/5 (full, purple) × Cl. 650*	27	5	0	0	6
M113	Q78/6 (full, purple) × U. Knight*	3	5	0	0	2
M114	Q78/7 (full, purple) × U. Knight	30	22	0	0	35
L30	Q78/8 (hidden-spot, purple) × U. Knight	8	9	18	8	14
M115	Q78/12 (hidden-spot, pink) × U. Knight	0	99	0	0	13

* Cl. 650 has white tubers with purple sprouts; Ulster Knight has white tubers with pink sprouts.

are shown in table 1. It had been expected that clones with tubers classified as full colour (*E*) would show no effect and that clones with hidden-spotted tubers (*EE^S*) would give some plants with spectacled tubers. The first expectation was realised, but only one clone (Q78/8) with tubers classified as hidden-spotted gave plants with spectacled tubers (fig. 1c). The reason for this is discussed in the next section.

4. BREEDING BEHAVIOUR OF Q78 CLONES

Families have been grown from five of the Q78 clones (table 2). Only family L30 from Q78/8, the clone which produced the spectacled tubers in the eye-excision experiments and the X-ray treatments, showed a segregation for full *v.* splashed colour. Q78/12, which was scored as having hidden-spotted tubers, behaved like the clones with full coloured tubers (Q78/5, Q78/6 and Q78/7). It thus appears, as has also been evident in other work, that the gene *E* alone can give hidden-spotted phenotypes and it may be that Q78/9, which has deformed flowers and which did not produce berries in attempted crosses, also has only an *E* gene and not the pigment-restricting gene *M*. If Q78/8 is the only clone with an *E* gene coupled with *M* (*i.e.* factor *E^S*), then the results of eye-excision and X-ray treatment are what were expected.

TABLE 3

Constancy of hidden-spot and spectacled Q78/8

<i>A. Control series, hidden spotted</i>	
1962—	12 h.sp.
1963—	11 h.sp.; 10 h.sp.*
1964—	7 h.sp.; 8 h.sp.; 8 h.sp.; 6 h.sp.; 5 h.sp.
1965—	14 h.sp.; 9 h.sp.
<i>B. Spectacled series (from eye-excision)</i>	
1964—	3 h.sp.+8 sp.; 10 h.sp.; 13 h.sp.; 11 h.sp.
1965—	3 h.sp.+4 sp.; 7 h.sp.+2 sp.; 8 h.sp.
<i>C. Spectacled series (from X-ray treatment)</i>	
1964—	6 sp.; 3 h.sp.+3 sp.; 6 h.sp.+2 sp.; 7 h.sp.
1965—	11 sp.; 8 sp.; 12 sp.; 7 sp.

* h.sp. = hidden-spotted; sp. = spectacled. 11 h.sp.; 10 h.sp. means one plant produced 11 hidden-spotted tubers and another plant 10 hidden-spotted tubers.

5. CONSTANCY OF SPECTACLED Q78-8

Previous work (Howard, 1962 and 1964a; Simmonds, 1965a and b) has shown that the change from splashed to white and from hidden-spotted to spectacled brought about by either eye-excision or X-ray treatment is not always permanent and that there are in clones different

degrees of reversion to splashed and hidden-spotted respectively. Results for Q78/8 are given in table 3.

FIG. 1.—Tubers of (a) C.P.C. 304S (spectacled), (b) Q78/8 (hidden-spotted) and (c) Q78/8 (spectacled; X-ray series). Areas of pigmented phelloderm hatched.

Hidden-spotted Q78/8, as shown in table 3, produced no plants with spectacled tubers. There is a high frequency of reversion from spectacled to hidden-spotted, but it may be that a line has been obtained in the spectacled series from the X-ray treatment which is stable. The five plants of this line grown in 1965, which were obtained from the six tubers of a plant producing only spectacled in 1964, had a total of 38 spectacled and no hidden-spotted tubers. These tubers, fig. 1c, have spectacles which are nearly as large as those in C.P.C. 304S.

6. DISCUSSION

The change from hidden-spotted to spectacled depends upon the pigment-restricting gene *M* being present. In full-colour tubers, of either the periderm-pigmented type (gene *E*) or the phelloderm-pigmented type (gene *R*), neither eye-excision nor X-ray treatment changes tuber phenotypes in the absence of *M*. That both eye-excision and X-ray treatment produce the change from hidden-spotted to spectacled can be explained by the fact that both treatments lead to new meristems being formed from either single cells or very small numbers of cells (Howard, 1964b).

The production of spectacled tubers from hidden-spotted of the type with full pigment in the phelloderm and splashed pigment in the periderm was explained by Howard (1964a, fig. 2) by assuming that *M* inhibited pigment formation in areas of phelloderm about the eyes. In hidden-spotted this lack of pigment in the phelloderm was assumed to be masked by the splashed pigment in the periderm. The eye-

excision and X-ray treatments led to the disappearance of the splashed pigment in the periderm (cf. the change from splashed to white), thus revealing a spectacled condition. There was, however, no evidence that *M* did restrict pigment formation in the phelloderm and, as Simmonds (1964*a*) has pointed out and has been found in the work reported in this paper, spectacled tubers can be obtained from hidden-spotted tubers which have full colour in the periderm. Another explanation must therefore be sought for the change from hidden-spotted to spectacled. In order to find such an explanation it is necessary to consider all that is known about the action of the gene *M*.

Coupled with either genes *E^T* or *E^A* for full pigmentation in the periderm, *M* inhibits pigment formation in most of the periderm but allows it in areas around the eyes so that splashed tubers are produced by the genotype *EM*. Such splashed tubers, after either eye-excision or X-ray treatment, may produce plants with white tubers (Asseyeva, 1931; Howard, 1962; Simmonds, 1965*b*). The recombination frequency for genes *E* and *M* is about 0.6 per cent. in embryo-sac mother cells (Howard, 1966).

The genotype *REM* usually produces hidden-spotted tubers with full pigment in the phelloderm but splashed in the periderm. The gene *M*, when coupled with *E*, would thus appear to have no action on *R* which determines pigment production in the phelloderm. But certain *Andigena* clones with the genotype *REM* are spectacled (Asseyeva, 1931; Howard, 1964*a* and unpublished), and both eye-excision and X-ray treatment lead to hidden-spotted of the *REM* type producing spectacled tubers (Asseyeva, 1931; Howard, 1964*a*). These spectacled tubers have no pigment in either the phelloderm or periderm in areas around the eyes; *M* has, therefore, in some conditions an effect on the action of *R*.

The genotype *E^AE^AM* usually produces hidden-spotted tubers with full pigment in all areas of the periderm, the pigmentation being more intense in areas about the eyes (*e.g.* Q78/8 of this paper); occasionally it may produce spectacled (*e.g.* C.P.C. 304S). More intense pigmentation about the eyes may, however, be found when *M* is absent and either *E^T* or *E^A* is present (*e.g.* Q78/12). Eye-excision or X-ray treatment of *E^AE^AM* may lead to plants with spectacled tubers (cf. similar effect on *RE^TM*).

The genotype *E^TE^TM* produces splashed tubers, and not hidden-spotted (Howard, unpublished); this shows that *M* can have an effect on the action of an *E* gene not in the same chromosome. The different dominance relations between *M* and the two *E* genes (*i.e.* *E^T* and *E^A*) when they are duplex suggests that the balance, between a system in which the cells of the periderm produce pigment and a system in which pigment is not produced, may be easily altered. It should also be noted that *R* is probably allelomorphic with *E*.

Varieties, such as King Edward or Gladstone, which normally have splashed tubers, may occasionally produce tubers of the spectacled

type (see fig. 3*c* of Howard, 1962). Plants from these spectacled tubers give rise to plants which have normal, splashed tubers as is expected from the genotype E^TM . This suggests that the spectacled tubers are caused by changes in intracellular conditions, connected with differentiation phenomena determined by proximity or not to an eye, and not to any changes in the genes for pigment production.

FIG. 2.—(a) and (b) Single tuber of an *Andigena* × *Tuberosum* clone, showing both hidden-spotted and spectacled.
 (c) Area around a single eye of a tuber of an *Andigena* × *Tuberosum* clone.
 (d) Area around a single eye of a tuber of King Edward (splashed colour).
 Areas of pigmented phelloderm hatched.

Similarly in *Andigena* potatoes clones are found which may produce plants which have both hidden-spotted and spectacled tubers. It is even possible (figs. 2*a* and *b*) to find hidden-spotted and spectacled on the same tuber. This again shows that the balance between producing and not producing pigment is easily changed. Simmonds (1965*a*) has shown that it is possible in *Andigena* clones to select for and against the expression of spectacled. It may be that this selection is also for some intracellular state of the cells rather than directly for production or not of pigment. The difference found by Simmonds between cuttings from upper and lower nodes of the same stem give additional support for this suggestion.

Finally it should be noted that areas around the single eyes of hidden-spotted tubers of *Andigena* potatoes or even around eyes of splashed tubers of King Edward may show some development of spectacled (figs. 2*c* and *d*).

Although both Howard (1964*a*) and Simmonds (1965*a*) draw attention to the analogy between the change from hidden-spotted to spectacled and certain proved cytoplasmic variants of *Aspergillus* (Jinks, 1957; Mather and Jinks, 1958), it cannot be concluded that the change from hidden-spotted to spectacled is a cytoplasmic effect. The pos-

sibility that the change is due to some change in the intracellular conditions, rather than to a direct change of the pigment-producing system, would, however, fit a cytoplasmic rather than a chromosomal change.

It can be tentatively concluded that the change from hidden-spotted to spectacled tubers resulting from either eye-excision or X-ray treatment is due to some change, connected with differentiation, which occurs in the cytoplasm of the cells and which somehow affects the pigment-producing enzyme system. It is not surprising that more definite and detailed conclusions cannot be made because there is at present little knowledge of how differentiation of cells in higher plants is determined. It would, however, be of interest to see whether X-ray treatment or adventitious bud formation would produce changes in the phenotypes of other plants which have patterns of pigmentation.

7. SUMMARY

1. Both eye-excision and X-ray treatment of hidden-spotted tubers ($E^A E^A M$), which have full colour in the periderm, the colour being deeper around the eyes, produce some plants with spectacled tubers (*i.e.* no colour around the eyes).
2. Eye-excision and X-ray treatment of fully-pigmented tubers ($E^A E^T$, E^A or E^T), and of $E^A E^T$ tubers scored as hidden-spotted, produced no plants with spectacled tubers.
3. The change from hidden-spotted to spectacled therefore depends upon some action of the pigment-restricting gene M .
4. The spectacled tubers produced some plants which again had spectacled tubers but there was also reversion to hidden-spotted.
5. The action of the genes M , E^A , E^T and R in producing splashed, hidden-spotted and spectacled tubers is considered.
6. It is suggested that the change from hidden-spotted to spectacled tubers is due to some change, connected with differentiation, which occurs in the cytoplasm of cells and which somehow affects the pigment-producing enzyme system.

8. REFERENCES

- ASSEYEVA, T. 1931. Bud mutations in the potato. *Bull. appl. Bot., Leningrad*, 27, 135-217. (Russian, with long English summary.)
- HOWARD, H. W. 1959. Experiments with a potato periclinal chimera. *Genetica*, 30, 278-291.
- HOWARD, H. W. 1962. Experiments with potatoes on the effect of the pigment-restricting gene, M . *Heredity*, 17, 145-156.
- HOWARD, H. W. 1964a. Further effects of the pigment-restricting gene, M , in potatoes: hidden spotted and spectacled. *Heredity*, 19, 349-356.
- HOWARD, H. W. 1964b. The use of X-rays in investigating potato chimeras. *Radiat. Bot.*, 4, 361-371.

- HOWARD, H. W. 1966. The recombination value for genes *E* and *M* in potatoes. *Heredity*, 21, 313-316.
- JINKS, J. L. 1957. Selection for cytoplasmic differences. *Proc. Roy. Soc. B.*, 146, 527-540.
- MATHER, K., AND JINKS, J. L. 1958. Cytoplasm in sexual reproduction. *Nature, Lond.*, 182, 1188-1190.
- SIMMONDS, N. W. 1965a. Somatic segregation of the spectacle pattern of potato tubers. *Heredity*, 20, 277-288.
- SIMMONDS, N. W. 1965b. *Rep. John Innes hort. Instn.*, 1964, 44-46.