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# The eye-excision method of investigating potato chimeras

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**Summary**

Adventitious buds produced by eye-excised tubers are formed from tissues which trace back to layer L3 at the stem apex. Results suggesting that some adventitious buds may be formed by tissues tracing back to L2 are due to faulty experimentation, not all eyes being removed or an eye only partially removed.

Eye-excision (Augenblendung; Blendungsversuche) was first used by Asseyeva (1927) to show that some potato bud-sports were periclinal chimeras and since that time it has become a standard method for investigating the composition of aberrant types. In her comprehensive account of 1931, Asseyeva suggested that 'under the influence of removal of eyes the above three types of mutants behave in a different manner; the epidermal type becomes transformed into the initial form; the sub-epidermal one partly also reverts to the normal type, partly turns into dichlamydes. As regards the dichlamydes they also, similar to the epidermal mutants, revert to the normal type, though the percentage of reversion is less, In some of the dichlamydes (*Rode Star* $M_{I+II}$, *Institut de Beauvais* $M_{I+II}$) the removal of eyes up to now could induce no perceptible changes'. This statement of Assesyeva needs examining in the light of modern views on the structure of the stem apex and the tissues produced by it.

At the stem apex in potatoes there are three growing-point layers, L1, L2 and L3 (Howard, Wainwright and Fuller, 1963; Klopfer, 1965). From L1 is produced the epidermis, from L2 the outer cortex and from L3 the inner cortex, vascular tissue and pith (Sussex, 1955). When eyes are excised from tubers, adventitious buds are produced on callus tissue originating in the cortex or vascular regions. There is evidence (Howard, 1961) that the adventitious buds trace back to a single or a few cells. Hence, if the adventitious buds originate from a callus produced by the outer cortex they would have the constitution of L2 of the bud-sport, and if from a callus produced by the inner cortex the constitution of L3 of the bud-sport. This would appear to fit Asseyeva's statement to a large extent. The epidermal type (i.e. a mutation in L1 only) reverts to the normal whether the adventitious buds come from either the outer cortex or the inner cortex. The sub-epidermal type (i.e. a mutation in L2 only) would revert to normal if the adventitious bud comes from the inner cortex but would produce a trichlamyde for the mutation (usually similar in phenotype to the dichlamyde) if the bud comes from the outer cortex. Dichlamydes (i.e. mutations in L1 and L2, but not in L3)

would behave similarly to the sub-epidermal type, i.e. give a mixture of normals and trichlamydes. The *Rode Star* and *Institut de Beauvais* results, as Asseyeva herself pointed out, can be explained by suggesting that all three layers, L1 L2 and L3, are mutant in these sports (Howard, 1967).

Although other workers, e.g. Simmonds (1965), have obained results suggesting that the adventitious buds produced after eye-excision may arise in tissues which trace back to either L2 or L3, there are reasons for suggesting that these results are false and due to faulty experimentation in not removing all the eyes or in only partially removing an eye. For example, both Crane (1936) experimenting with *Golden Wonder* (mutation to russet skin in L1 only) and Howard (1959) experimenting with *Red King* (mutation from splashed pink to full pink in L1 only) did not obtain 100% reversion to normal after eye-excision. Howard (1959) did, however, show that his results were due to faulty experimentation; the mutant-type tubers were eye-excised in the following year and then gave reversion to normal.

Holly-leaf *Majestic* (Simmonds, 1965; Howard, 1967) is a striking bud-sport in which a mutation has occurred in L2 but not in L3. Simmonds found that of 15 plants from eye-excision only three had normal leaves and 12 were still holly-leaf whereas Howard found 16 plants with normal leaves and only one with holly leaves. In the following year two plants were obtained from eye-excision of four tubers of the non-reverted plant; both had normal leaves. It would appear therefore that Simmonds' results were due to faulty experimentation although there is another possible explanation.

In potatoes there is a tendency for L3 to be replaced at some growing points by cells from L2 (Howard, Wainwright and Fuller, 1963; Howard, 1967). Hence a bud-sport which was originally L1 normal – L2 mutant – L3 normal will tend to change to L1 normal – L2 mutant – L3 mutant. The latter will not give normal plants after eye-

Table 1. Effect of eye-excision on tubers of holly-leaf *Majestic*.

| Year | Material | Number eye-excised | Number of plants obtained | Number of plants | |
|---|---|---|---|---|---|
| | | | | normal | holly-leaf |
| 1967 | Whole tubers | 8 | 7 | 5 | 2 (plants A & B) |
| | Half tubers | 8 | 4 | 3 | 1 (plant C) |
| 1968 | Plant A, whole tubers | 1 | 0 | – | – |
| | Plant A, half tubers | 4 | 2 | 2 | 0 |
| | Plant B, whole tubers | 2 | 1 | 1 | 0 |
| | Plant B, half tubers | 4 | 2 | 1 | 1 (plant D) |
| | Plant C, half tubers | 6 | 4 | 4 | 0 |
| 1969 | Plant D, half tubers | 6 | 2 | 2 | 0 |

excision. It might be, therefore, that Simmonds' stock of holly-leaf *Majestic* was a mixture of L2 mutant – L3 normal and L2 mutant – L3 mutant. Accordingly further eye-excision experiments were carried out with holly-leaf *Majestic* (Table 1). Both holly-leaf and normal plants were obtained from eye-excised tubers of holly-leaf plants, but carrying out a second and third year of eye-excision treatments showed conclusively that the holly-leaf plants must have been due to faulty experimentation in not removing all the eyes.

It can be concluded therefore that eye-excision leads to adventitious buds being produced from tissues which trace back to L3 and that results suggesting that some buds may have come from tissues tracing back to L2 should be treated with caution. A second series of eye-excision should be carried out. The constitution of L2 in bud-sports cannot be determined by eye-excision experiments; it can, however, in many cases be determined by breeding experiments (Howard,1967; 1970).

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