*Potato Res. 15 (1972) 73–75* SHORT COMMUNICATION

# A suggested method for investigating L1 constitution in periclinal potato chimeras

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Accepted for publication: 8 September 1971

**Summary**

The most reliable way to study the nature of L1 in suspected periclinal chimeras is to investigate plants derived from L1 tissue only. Induction of adventitious buds and shoots from dedifferentiated epidermal tissue only, has proved to be easy in several ornamental plants such as *Saintpaulia* and *Streptocarpus*, but for potato until now only some incidental successes have been obtained using detached leaflets with different treatment. If this method could be developed to give adequate numbers of adventitious shoots, it is suggested that it could be used on a monochlamydeous (periclinal) chimera to prove the L1 origin of these shoots.

Howard (1969) described the main methods to investigate periclinal chimeras of potato. As both embryo-sac and pollen-mother cells are of L2 origin, studies of breeding behaviour (normally by crossing with another variety) reveal the L2 constitution. For L3 investigation either the eye-excision method described by Asseyeva (1927, 1931) or the induction of adventitious buds on roots according to the methods of either Howard (1964a) or Miedema (1967) can be used. Both cases represent an example of growing plants from one layer only, i.e. there is no or almost no influence of other layers of the plant.

Even when layer isolations have been performed with the utmost care one still has to keep in mind that the above methods have their limitations, especially when the number of plants studied is small. The plants obtained represent only a limited part of the cells present within one layer. Therefore spontaneous or induced chimerism within one layer may not be detected unless many plants are investigated. Those changes within one layer could be caused either by direct mutations or by replacement of tissue of the respective layer by cells from other layers, which in case of periclinal chimeras can be genetically different.

For L1 two methods of investigating its constitution can generally be applied. For certain visible characteristics the study of the appearance of the plants may be adequate. However in case of stem or tuber colour, for instance, deeper layers may influence the picture or there may be other causes for unreliable information, such as the question whether the periderm of potato tubers is of L1 or L2 origin (Howard, 1970).

Another way to investigate L1, is using X-rays, which for potato was described by

Howard (1964b, 1967). This method is useful, when both L2 and L3 are mutated and the constitution of L1 is unknown. After X-irradiation Howard obtains 5–10% of plants with at least L1 and L2 having the constitution of L1 of the original suspected periclinal chimera. L2 analysis then reveals L1 nature. Howard mentions that the method cannot be used, when L2 is mutant and L3 is normal, since replacement of L2 can originate from L3 as well as from L1, which in that case cannot be checked. Furthermore when using this indirect method one has to keep in mind that even when (L2 and L3) layers are isolated, results are not completely reliable. Using the above method for L1, therefore, may introduce even more sources of error. Hence it is very difficult to decide, especially with limited numbers of plants, whether one is really dealing with reduplication of L1.

A more reliable method to investigate L1 would therefore be most welcome, preferably a method which makes it possible to study L1 independently.

Considerable attention is being directed in mutation breeding work to the induction of adventitious buds and shoots on petioles or veins on leaves, leaflets, etc., which shoots develop in many cases from only one single epidermal cell (for an extensive literature survey see Broertjes et al., 1968).

In the Netherlands especially Broertjes has strongly advocated this method, the importance of which was not realized until Sparrow et al. (1960) found very high numbers of either solid mutants or completely normal plants in *Saintpaulia*, thus confirming the histological work of Naylor and Johnson (1937) who demonstrated the one-cell epidermal origin of adventitious buds.

The importance of the one-cell origin for mutation breeding is of course quite evident, but to investigate periclinal chimeras the development of the shoot from dedifferentiated epidermal tissue is even more interesting. If such a method were available for the potato it would become relatively easy to obtain L1 plants, provided that the potato follows the regular pattern of adventitious sprouting, i.e. from L1 tissue. A reliable way to check the epidermal origin of adventitious shoots would be to start from a monochlamydeous (L1) periclinal chimera. Due to the relatively stable nature of the different layers in the potato such types are available, e.g. the very stable B 165, a yellow red splashed tuber colour mutant in the red variety Burmania, obtained by Dr F. P. Ferwerda (now retired) of our Institute in 1962, or the less stable (L1) periclinal chimeras *Red Craigs Royal* and *Red King* mentioned by Howard (1971). As far as known nobody has ever tried to apply this method to prove the L1 nature of adventitious shoots. In our Institute large-scale experiments have been going on since 1969 to work out a reliable method to obtain large numbers of adventitious shoots, the work being concentrated on the induction of shoots at the petiole base of detached leaves or mainly leaflets.

Thousands of leaflets of various varieties and different ages have been treated with auxins and kinetins, apart or in combinations, with different day-lengths, different temperature regimes, different soil components. Leaflets floating on perforated tempex sheets in water, etc. have also been used. The results, however, have not been very encouraging.

Adventitious shoots or stolons, even with a small tuber at the stolon end, have been obtained, but no regularity could be observed. Sometimes adventitious shoots developed in the control material only. More cases however have previously been reported, the oldest of which by Knight, who in 1816, according to Kupfer (1907), obtained a tuber from the base of a rooted potato leaf. Rooting of potato leaves is, by contrast, easy. By using proper methods, even without growth hormones, about 90% of rooting can be obtained, the advantage of applying IAA being mainly a more regular rooting of the leaflets. Rooted leaflets can be kept alive and green for three months or longer. One may think of the possibility that the induced roots prevent adventitious shoot formation, however it has not been possible yet to obtain shoots before rooting takes place. Of course the application of in vitro techniques might be considered, but apart from other complications, the plantlets obtained will not be of epidermal origin only. There is no specific reason to expect that producing adventitious shoots in potato should be fundamentally different from that in e.g. *Streptocarpus* and it may just be a question of time till we will be able to produce L1 plants in sufficient quantities. This method should facilitate mutation work and would increase the reliability of chimera analyses, and it appears to merit further work to develop it.

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