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Plantlet formation from internode bases of carnation (Dianthus caryophyllus L.) in vivo - useful to mutation breeding or not?

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Summary

After decapitation of the main shoot and subsequently the axillary shoots of carnation plants, annular zones extending between axils of opposite leaves produced numerous buds. Low temperatures (10-14 °C) were essential for this bud formation, which was restricted to young internodes. The season affected the time till bud formation, and determined whether the buds were formed from the main axis or the laterals. Indications were found that these buds were adventitious. The possibility to make use of these buds in mutation breeding, and the possible risks if they are formed during clonal micropropagation in vitro, are discussed.

Introduction

A major problem of improvement of carnation by means of mutation breeding is chimaera formation, particularly when it is the aim to induce changes in quantitative characters. The regeneration of adventitious buds, ultimately originating from single cells, can solve this problem.

In carnation, meristem culture is a common method to eliminate viruses (Hollings, 1965; Buys et al., 1966), and shoot tip culture is used for clonal multiplication (Hackett & Anderson, 1967; Petru & Landa, 1974; Earle & Langhans, 1975; Davis et al., 1977). Attempts, however, to induce the formation of adventitious buds or embryoids from young stem internodes gave negative results (Debergh, 1972). This was also the case when callus or cell suspension cultures derived from internode tissue (Engvild, 1972; Debergh, 1973) or from shoot tip cultures (Kakehi, 1972) were used. Hauzinska (1974, 1975) did report plantlet formation from callus obtained from shoot tips, but she did not make sure that the calluses after subculture did not contain remnants of the apical meristem or the axillary meristems of the shoot tip she started from.

In spite of these disappointing reports, carnation tissue has on occasions shown some regenerative potential. Petru & Landa (1974) obtained adventitious bud formation from callus isolated from hypocotyl tissue, and Hauzinska (1974) from callus

isolated from young leaf explants. More remarkable, however, was the ability of mature plants in vivo to form new plantlets after decapitation, as described by Dommergues & Gillot (1973). Dense crowds of plantlets developed both on a level with the cut surface and in the axils of the leaf pair at the base of the internode which was cut. These authors successfully used these plantlets to isolate non-chimaeral plants from the periclinal chimaera cv. 'White Sim'. We did not succeed in obtaining plantlets by applying this method during spring and summer under carnation culture conditions as used in our country. However, since this method might have practical usefulness for mutation breeding, it seemed appropriate to pay attention to the various factors which might affect this type of plantlet formation. The work now described concerns the effects of the temperature after decapitation, of the age of the internode which was cut, of the ontogenetical stage of the stock plants, and of the tissue which is involved in the origin of the buds.

Material and methods

Most experiments were done with cv. 'White Sim'. Plants obtained as rooted cuttings from commercial sources were potted in a soil-peat mixture and grown under normal greenhouse conditions until the beginning of the experiments. To check the experience acquired with 'White Sim', a number of other genotypes were used, viz cv. 'Danilo' and the IVT clones 72020-6, 72161-1, 73120-1 and 74212-3.

In different seasons stock plants at various stages of ontogenesis were decapitated at different lengths. During the subsequent weeks the lateral shoots developing after this treatment, were pruned leaving only half or one and a half internodes.

Temperature experiments were accomplished by placing stock plants in the glass-houses of the IVT phytotron at 10, 14, 17, 20 and 23 °C under natural daylength conditions. To prevent direct solar radiation cheesecloth was used to shade the plants.

The stages of plant ontogenesis at the time of decapitation were the vegetative stage (plants with short internodes), the bolting stage (plants with elongating internodes), and the reproductive stage (plants bearing a terminal flower bud 3-5 mm in diameter). The age of the cut internode was defined by its position as numbered from the base of the stem to the top, and by its length.

Per experiment only one of the above-mentioned factors was varied. Each experiment usually comprised 10 stock plants per treatment and was repeated at least twice except the experiment on stock plant ontogenesis which was carried out only once.

After decapitation the plants were examined regularly for the appearance of meristem-like protuberances surrounding an internode base. The degree of bud formation was evaluated as the percentage of stock plants which had formed buds from such protuberances. When the buds showed good extension growth, the number of those which reached at least 5 mm was determined, and the mean per whorl per treatment calculated. The significance at $P = 0.05$ of differences between means was assessed by Student's t test.

Results

All the genotypes used showed the same reaction pattern. Therefore, detailed results of only cv. 'White Sim' are dealt with.

Temperature

In an autumn experiment bolting plants about 45 cm in length and having 8 visible internodes were decapitated and placed at the different temperatures. The cut was made in the middle of the 1.5 cm long 9th internode, which was still enveloped by young leaves. The results of this experiment are summarized in Table 1.

At the low temperatures only a few axillary buds developed into shoots, always only one shoot in a pair of axils. After pruning these shoots almost none of the stock plants did show new development of axillary buds, but instead formed an annular protuberant zone at the base of internode 9, in some cases also at the base of internode 8. Numerous buds were formed from each protuberant zone. At 14 °C these buds became macroscopically visible about 8 weeks after decapitation of the main axis, and at 10 °C about 11 weeks after decapitation. The annular zone of buds intersected the place where the second axillary bud of a pair of axils might be situated. However, such an axillary bud could not be detected amongst the buds of the whorl (see Fig. 1).

At the high temperatures, axillary bud development did not stop after decapitation of the first series of laterals. Repeated decapitation resulted in second and third order laterals. Frequently, 2, 3 or more shoots seemed to originate directly from the internode base, but their insertion revealed that they originated from the axils of the stipules borne at the base of the lateral shoots. At these temperatures annular protuberant zones were never observed.

A similar experiment with identical material, carried out at 10, 17 and 23 °C and started in winter, showed the same difference in response to the low and the high temperature.

A third experiment with such material started in early spring. In contrast to the first two experiments, more laterals developed after release from apical dominance. At the low temperatures the process of lateral formation did not stop after decapi-

Table 1. The effect of temperature on the formation of annular zones of plantlets from main axis internode bases after decapitation of carnation plants. The number of plants per treatment was 10. Mean values designated by the same letter are not significantly different from each other at P = 0.05.

Temperature (°C)	Number of plants with annular zone formation	Number of annular zones per treatment	Mean number of plantlets which reached at least 5 mm per annular zone
10	9	10	17.3 b
14	8	10	19.4 b
17	5	5	13.3 b
20	0	0	0 a
23	0	0	0 a

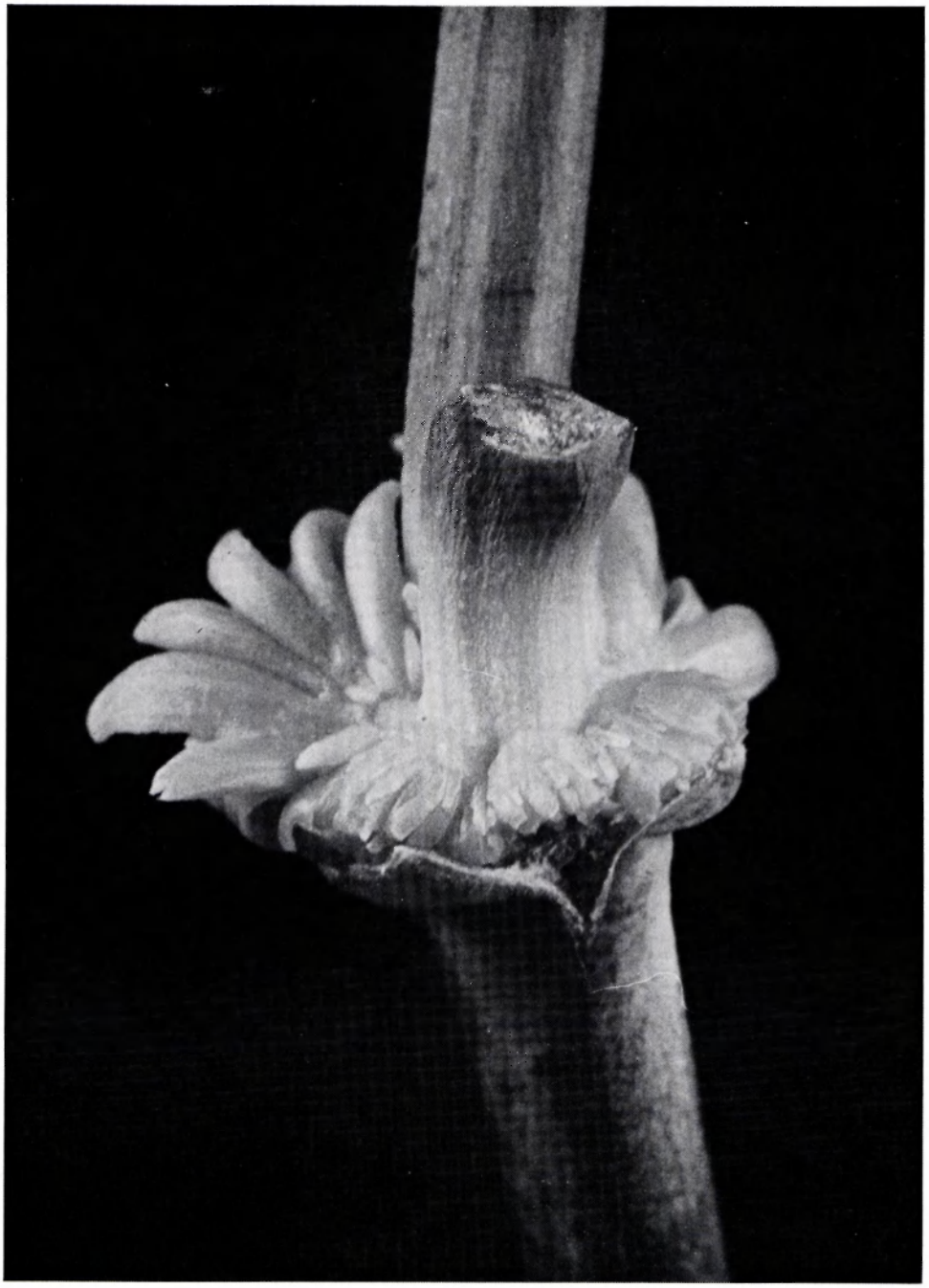

Fig. 1. Plantlet formation from the base of a young cut internode of the main stem. In the background the decapitated axillary shoot from the same pair of axils.

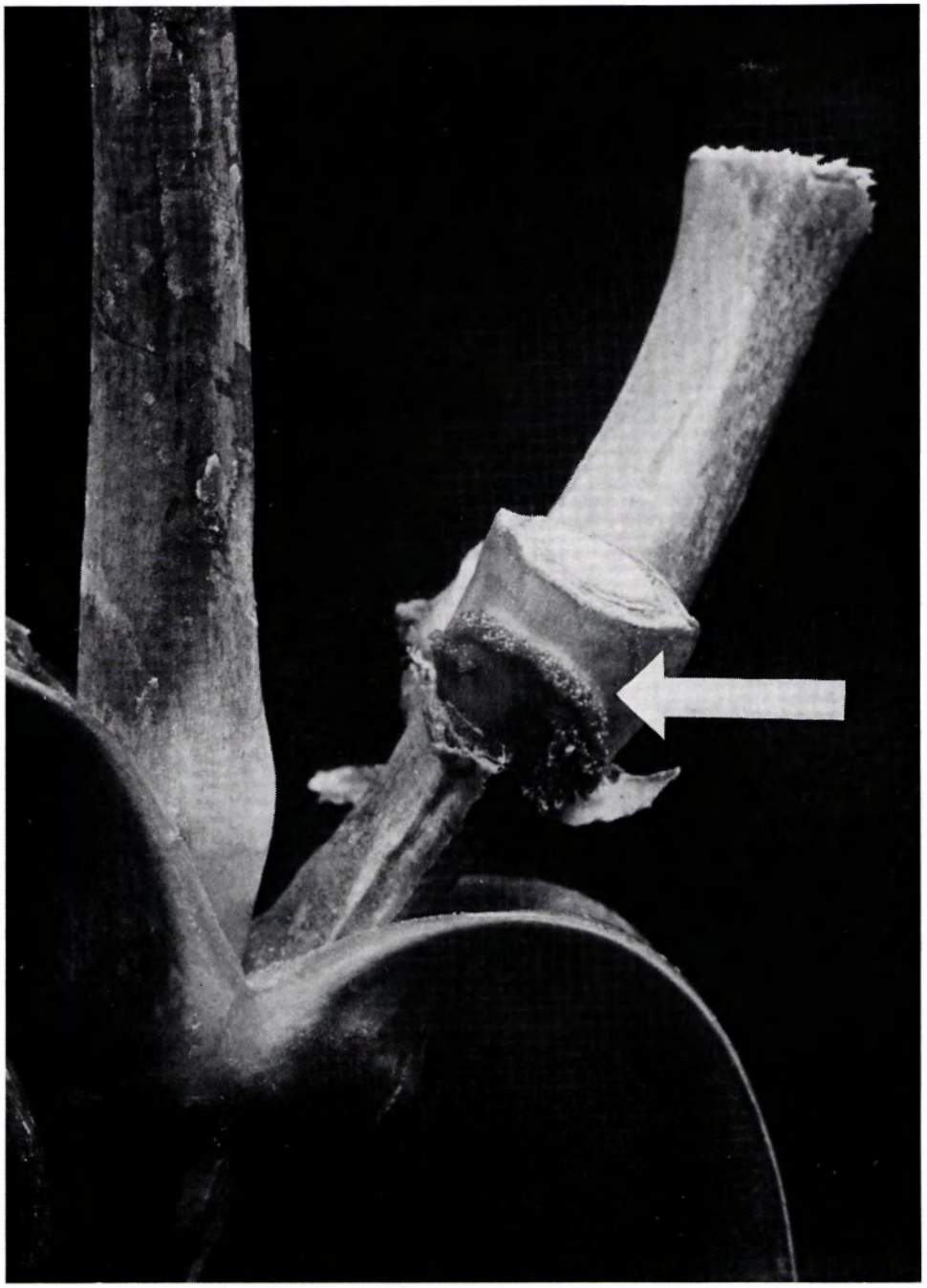

Fig. 2. Plantlet formation from the base of a second-order lateral. The annular zone described an arc over the axil (see arrow).

tation of the first series of laterals, resulting in many stumps of decapitated laterals at the internode bases of the main stems. About 4 months after the beginning of the experiment the internode bases of these stumps started to form annular zones of buds (see Fig. 2), whereas those of the main stem hardly did so. As in the previous experiments, plantlet formation occurred only at the low temperatures.

Internode age

In an autumn experiment bolting plants were pruned to different lengths and then kept at 10 °C. The cut was made in the middle of internodes at different positions along the main stem. The transition from lateral shoot development to annular zone activity soon appeared, and only the main stem internode bases formed annular protuberant zones as occurred in the first two temperature experiments. Table 2 shows that the frequency of annular zone formation and the number of plantlets per annular zone increased with decreasing age of the cut internode. It appears from Table 2, that when younger internodes were cut, we increase the chance that 2 internode bases produce an annular zone. The full-grown internodes hardly formed any protuberant zone at their bases.

In a second experiment, started in early spring, the transition from lateral shoot development to annular zone activity was delayed for a long time, and hardly any plantlet formation from main stems occurred.

Stage of stock plant ontogenesis

In a late winter experiment, decapitation was carried out of vegetative, bolting, and reproductive plants. In the first two groups a young internode which was still enveloped by young leaves, was cut, and in the third group the visible internode bearing the flower bud. The plants which were kept at 10 °C, were rather late to reach the transition from lateral shoot development to annular protuberant zone formation. Probably as a consequence of this the frequency of zone formation from the main stems was much lower than in the previous autumn experiments, viz 33, 63, and 45 % for the respective groups. No significant differences were found be-

Table 2. The effect of the age of the cut internode on the formation of annular zones of plantlets from main axis internode bases. After decapitation the stock plants were kept at 10 °C. The number of plants per treatment was 10. Mean values designated bij the same letter are not significantly different from each other at P = 0.05.

Internode cut		Number of plants with annular zone formation	Number of annular zones per treatment	Mean number of plantlets which reached at least 5 mm, per annular zone
position number	mean length at the time of decapitation (cm)			
10	0.6	10	15	19.8 d
9	1.7	9	10	17.7 cd
8	4.1	7	7	11.4 bc
7	6.3	2	2	4.5 b
6	6.1	0	0	0 a

Fig. 3. Shoots reproducing the original stage of ontogenesis of the stock plant top. The shoots developed from the uppermost internodes of a stock plant which was bolting at the time of decapitation.

tween the mean numbers of plantlets per annular zone.

In the bolting and reproductive plants it was observed, that the plantlets formed from the uppermost main stem internodes, when left at the stock plant, in most cases already produced a flower bud at the end of their third internode (see Fig. 3). The plantlets from the vegetative stock plants and those from laterals remained vegetative.

Experiments to induce bud formation from other places

As mentioned before, the annular zone of buds intersected the place where the second axillary bud of a node might be situated. In some cases, however, the annular zone described an arc over the axil (see Fig. 2). This was the case when a remnant of the leaf base was in close contact with the internode base and the annular zone was formed from internode tissue along the margin of that leaf remnant. To get more information about the origin of the new plantlets attempts were made to induce bud formation from other places too.

In a first approach very young internodes of bolting plants were pruned to different length to examine whether bud formation from the cut surface would be possible. The results were negative. When more than 20 % of the internode length was left, only annular zone formation from the internode bases was observed. A cut just above the node, however, fully inhibited annular zone formation.

Girdling or incision of a cut internode was carried out to examine whether plantlets would regenerate along the internode stump. These operations, however, did not induce bud formation from the treated tissue.

To determine the regeneration ability of leaf cuttings, five genotypes each contributed 75 such cuttings, which after root formation were placed at 10 °C. Only 2 cuttings of clone 72161-1 formed in total 12 buds from their bases. All the other leaf cuttings failed to regenerate buds, presumably because approximately 1 cm of the leaf base tissue died very soon after isolation from the stock plant or later on after root formation.

Discussion

The formation of annular zones of plantlets appeared to be dependent on the temperature. The physiological background of this temperature effect is not clear, but it may be mediated by endogenous growth regulating substances as is supposed also for the temperature effects on the adventitious bud formation in *Begonia* × *cheimantha* (Heide, 1964; 1965) and *Streptocarpus* × *hybridus* (Appelgren & Heide, 1972). As observed by Garrod & Harris (1974) in carnation a low temperature also seems to favour the formation of secondary growing centres originating around the gynoecium.

Different seasons also influenced the pattern of plantlet formation. Annular zone formation from the main axis internode bases only occurred during autumn and winter. The changing daylight conditions during spring and summer may have caused the continued development of lateral shoots during those seasons, reducing the chances of getting plantlets from main axis internode bases.

As in earlier experiments (Hengst, 1959; Pierik, 1967; Broertjes & Leffring, 1972), the ability to form plantlets increased with decreasing chronological age of the treated tissue. No significant effect was found, however, of the ontogenetical age. Probably this was mainly due to the extent of the internode chronological age effect and on the other hand to the interference of the development of laterals with the annular zone formation from the main axis internode bases in that experiment.

The results of the present experiments have shown under which conditions plantlet formation as reported by Dommergues & Gillot (1973) can be obtained. For the employment of this method for mutation breeding, however, it is important to know, if also axillary meristems are involved in this plantlet formation. The plantlets regenerating from leaf cuttings, and those in the annular zones, which described an arc over an axil, are most likely of adventitious origin. When the annular zone intersected the axil, an axillary bud was not detected during the time of swelling of the internode base tissue, and all the shoots of the whorl were similar. Champagnat & Berthier (1957) stated that in Caryophyllaceae a part of the axils did not contain

any axillary bud. The empty axils we observed macroscopically are very likely similar to those studied microscopically by these authors. The fact that Dommergues & Gillot (1973) isolated genetically homogeneous plants from the periclinal chimaera cv. 'White Sim' by means of this type of plantlets is also indicative of an adventitious origin. The question remains, however, whether this adventitious bud formation is a one cell event. Champagnat (1955) observed microscopically that adventitious buds in the empty axils of *Linaria chalepensis* originated from single epidermal cells. Champagnat & Berthier (1957) wondered whether the same might occur in carnation and other plants with empty axils.

Provided that the regeneration of adventitious plantlets and their origin from single cells can be confirmed, then our technique will be of particular interest in mutation breeding to improve quantitative characters of carnation.

It may also be worth trying to use internode base tissue for adventitious bud formation in vitro. In fact, it may well be that unintentional adventitious bud formation is already common in clonal propagation of carnation in vitro by shoot tip culture. Since this technique is based on the same physiological process as used in our experiments, viz suppression of the effect of the apical dominance, a part of the plantlets formed may originate from internode bases. It is known that among plantlets grown from adventitious buds the mutation frequency is relatively high, even if the parent plant has not been irradiated. Therefore, adventitious bud formation may be responsible for the relatively high mutation frequency observed in carnations propagated in vitro. Mueller et al. (1976), for instance, found a higher number of obvious mutations in plants propagated in vitro than in those obtained by conventional propagation.

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