and 1974 on whole trees were not successful. The reasons for this are not known.

Discussion. The external tissues of citrus peel (flavedo) are known to continue their growth during the whole period of fruit development and maturation (1); their growth involves cell division and expansion. This is needed as fruit size in continuously increasing and peripheral layers must keep pace with the expansion of the endocarp. All known growth promotors participate in peel growth: endogenous auxin-like substances are found in the flavedo even at maturity (4). High endogenous gibberellins and cytokinins are responsible for excessive peel growth in rough oranges (2) and their effect can be counteracted by applied growth retardants (Erner, Goren and Monselise, unpublished). It is also well known that peel viability is enhanced by applied gibberellin (5, 8), and excessive senescence of peel, conducive to creasing, can be inhibited by applied gibberellin (9).

The prevention of corky spots by applied promotors of different classes is another case of growth regulation in citrus peel (6). When 2,4-D treatments are used to affect either set or size of early grapefruits in accordance with their exact timing (7), as in the hot interior valleys of Israel, an alleviation of the corky spot situation will ensue without further costs. As in other cases, negative results of applied regulators can be obtained, as satisfactory control of the disorder is linked with fine adjustment of the internal balance, and can be defeated by improper timing or doses and unusual climatic conditions; this does not disprove the possibility of peel conditioning against corky spots.

Our results fall in line with the hypothesis that tissue splits conducive to spots are due to lack of sufficient growth in the external flavedo layers. Different classes of regulators may cooperate in correcting this situation by enhancing either cell divisions (BA) or extension (GA, auxin). It is also of interest that applications of GA_{4+7} (identical with native gibberellins found in apples) to apple trees early after petal fall, have been recently shown to reduce the occurrence of russeting (12). In this particular case, however, BA seemed to act in the opposite direction.

Literature Cited

1. Bain, J. M. 1958. Morphological, anatomical and physiological changes in the developing fruit of Valencia orange. *Austr. J. Bot.* 6:1-24.
2. Erner, Y., R. Goren, and S. P. Monselise. 1976. Reduction of Peel Roughness of 'Shamouti' Orange with Growth Regulators. *J. Hort. Sci.* 101(5):513-515.
3. Faust, M. and C. B. Shear. 1972. Russeting of apples, an interpretive review. *HortScience* 7:233-235.
4. Goren, R. and E. E. Goldschmidt. 1970. Regulative systems in the developing citrus fruit. I. The hormonal balance in orange fruit tissues. *Physiol. Plant.* 23: 937-947.
5. Lewis, L. N., C. W. Coggins, Jr., C. K. Labanauskas, and V. M. Dugger, Jr. 1967. Biochemical changes associated with natural and gibberellin A_3 delayed senescence in navel orange rind. *Plant & Cell Physiol.* 8:151-160.
6. Monselise, S. P. 1973. Fruit quality in citrus and the effects of growth regulators. *Acta Hort.* 34(1):457-468.
7. ———— . Growth regulators used to extend the picking season of grapefruits. I. Congreso Mundial de Citricultura 1973, Vol. 2, in press.
8. ———— and R. Goren. 1965. Changes in composition and enzymatic activity in flavedo of Shamouti oranges during color break period as influenced by application of gibberellin and CCC. *Phyton* 22:61-66.
9. ———— , M. Weiser, N. Shafir, R. Goren, and E. E. Goldschmidt. Creasing of orange peel – physiology and control. *J. Hort. Sci.*, (in press.)
10. Safran, C. 1973. Corky and silvery spots on orange peel (in Hebrew). *Alon Hanotea* 38:90-97.
11. Tal, D. and S. P. Monselise. 1965. Corky (silvery) spots on rind of citrus fruits. *Israel J. Agr. Res.* 15:73-81.
12. Taylor, B. K. 1975. Reduction of apple skin russeting by gibberellin A_{4+7}. *J. Hort. Sci.* 50:169-172.

HortScience 11(4):422–423. 1976.

Tissue Culture and Differentiation of Garlic[1]

A. E. Kehr and G. W. Schaeffer[2]

Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705

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Abstract. **Growing points from cloves of *Allium sativum* L. were excised from mature air-dried corms and cultured *in vitro*. Media for vegetative increase of tissues in the undifferentiated form should contain (2,4-dichlorophenoxy) acetic acid, indole-3-acetic acid (IAA), 6-(furfurylamino) purine (kinetin), and coconut milk. The vegetative tissues differentiate on Murashige-Skoog medium supplemented with IAA and kinetin.**

The purpose of this study was to determine the tissue culture requirements for the increase of garlic tissue and for the differentiation of the callus tissue into plants.

Tissues from 'Rose de Lautrec' (RL), 'California Early' (CE), and 'California Late' (CL) garlic were excised, surface sterilized with Clorox[3] diluted 1:5 v/v, and rinsed with distilled water. Meristems were removed and reduced to 3 mm at the base. The tissues were then cultured on the Murashige-Skoog (MS) (2) or Nitsch (N) (3) media with the following hormonal amendments per liter:

1. MS including 1 mg IAA and 1 mg kinetin.
2. MS including 1 mg IAA, 1 mg 2.4-D and 25 ml of deproteinized coconut milk (Grand Island Biological Company, Grand Island, New York 14072).[3]
3. MS including 1 mg kinetin, 1 mg 2,4-D, 0.5 mg -napthaleneacetic acid (NAA).
4. MS including 3 mg 2,4-D.
5. Nitsch's medium without hormones.

Successive transfers were made in 25 mm test tubes or 125 ml Erlenmeyer flasks with 4 mm pieces of undifferentiated tissue at 4-8 week intervals. The tissues were cultured in an incubator at 970 lux with a 16 hr photoperiod at 25°C.

Garlic can be initially cultured as callus, mostly undifferentiated, on an MS medium supplemented with 2,4-D and organic constitutents (Fig. 1). Our best medium for tissue increase is medium 2, particularly for the RL cultivar. MS medium with 1 mg/liter of IAA and kinetin does not permit extensive callus growth but shifts growth dramatically toward differentiation. Thus, with garlic it is possible to control the growth form by altering the hormonal constitutents of the

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[2]Staff Scientist, National Program Staff, and Research Plant Physiologist, respectively.

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[3]Clorox = 5.25% sodium hypochlorite. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

media. The subculture of cells started on 2,4-D differentiate when placed on medium 1 (Fig. 2), however, tissues started on the standard MS medium 1 will not differentiate if subcultured on media 2 or 3, both of which have 2,4-D. If the initial explant is cultured on medium 1 (1 mg/liter of IAA and kinetin) and then subcultured on Nitsch's basic medium without hormones, the cells not only differentiate but the plant base has a bulb-like shape resembling the mature garlic bulb.

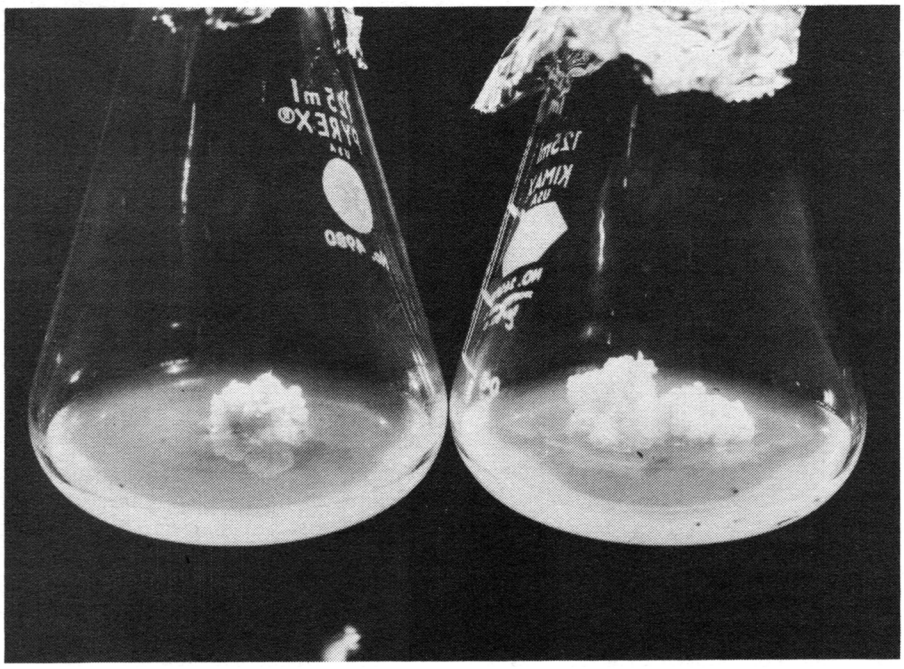

Fig. 1. Growth of apex isolated from cloves of 'California Late' (left) and 'Rose de Lautrec' (right) garlic 45 days after the first subculture. Both the original culture and first subculture were on medium 2.

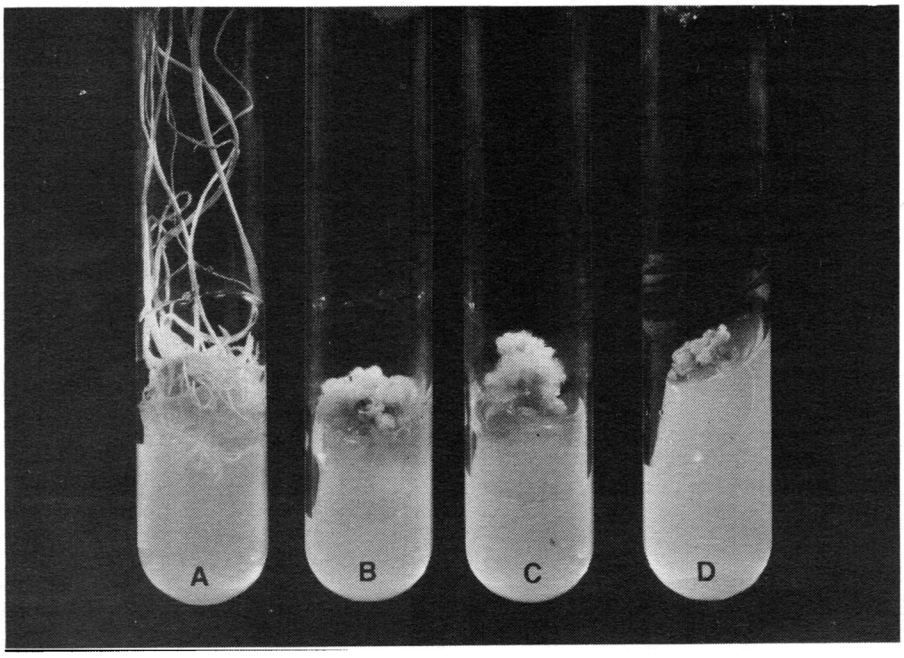

Fig. 2. Growth and development of 'California Late' garlic on different media. Apices were excised and cultured on media 1 or 4 (shown in parentheses below) for 45 days and then transferred to a second culture medium (culture number following arrow) for 60 days. Tissues in tubes from left to right are: A) medium 4⇒1; B) medium 1⇒2; C) medium 1⇒3; D) medium 4⇒4.

Observations of interest are: 1) the culture of garlic cells in liquid suspension was unsuccessful with media that produced callus growth; 2) some calluses kept for long periods without transfer became "waterlogged" and then would no longer subculture; 3) tissue that had been subcultured up to 6 times on media 2, 3, or 4 lost some vigor and did not survive subsequent transfer to medium 1. Liquid culture preparations for cells in suspension were done with media 2, 3, and 4 described above in 125 ml Erlenmeyer flasks, containing 20 ml of media on a gyrotory shaker at 86 cycles/min at room temp.

One of the major problems in commercial growth of garlic is the reduction in yield due to systemic viruses. Two prerequisites for the elimination of viruses by tissue culture method are the capability to increase the apical tissue and vegetative propagation, along with the recovery of differentiated plants (1,4). Even though the virus elimination was not the primary objective of this work, preliminary observations of electron micrographic preparations suggest that the tissues cultured as described above were virus-free (R. Lawson, Beltsville, MD-personal communication). We report here the tissue culture requirements for the successful growth and increase of callus tissues and the recovery of intact plants following *in vitro* culture.

For the purpose of garlic tissue culture and differentiation, our recommendation is to excise 3 mm apices, culture them initially on medium 2 containing IAA, kinetin, 2,4-D, and coconut milk. Subculturing on medium 1 or on Nitsch's medium then produces differentiated plantlets useful for virus elimination and genetic or physiological studies.

Literature Cited

1. Hollings, M. 1965. Disease control through virus-free stock. *Annu. Rev. Phytopath.* 3:367-396.
2. Murashige, T. and F. Skoog. 1962. A revised medium for the rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 914:473-97.
3. Nitsch, J. P. and C. Nitsch. 1969. Haploid plants from pollen grains. *Science* 163:85-87.
4. Pillai, S. K. and A. C. Hildebrandt. 1969. Induced differentiation of geranium plants from undifferentiated callus *in vitro*. *Amer. J. Bot.* 56:52-58.