Experientia 42 (1986), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Grape vine physiology: the contribution of culture in vitro

by R. Bessis

Laboratoire de Sciences de la Vigne, Université de Dijon, F-21000 Dijon (France)

Key words. Conformity of propagation; hormones, in vitro methods; juvenile forms; micropropagation; regeneration; somaclones; somatic embryos; tissue culture; grape vine improvement; grape vine physiology.

Introduction

The grape vine and its physiology constitute a vast and diffuse subject, including on the one hand general aspects of plant physiology and on the other hand the attributes particular to the vine. Progress of knowledge occurs in both these domains, with reciprocal and often unexpected benefits.

Plant physiology may be treated under various themes: nutrition, metabolism, growth, and development. In a review like this it is not possible to treat all these subjects. In choosing an orientation within these themes, for reasons evident later, I will concentrate upon certain aspects of development.

The fact that the vine is a perennial with a major and complex interaction between storage and mobilization of reserves marks the physiological responses of this plant. Moreover, development of the rooting system can be quite different according to genetic, soil and climatic factors which the scientist cannot control completely. For example, the vine's mineral nutrition is still little understood: it is not generally possible to demonstrate in the vineyard a requirement for phosphorus, although it is no doubt indispensable. Work needs to be done in this area, but there is little progress at present; laboratory models give conclusions which cannot be transposed to field conditions, whilst experimental methods in the vineyard are disappointing as their results are irregular. This is a typical example of an important area which remains vague, the diverse results producing no firm conclusions. Hence, one needs an experimental model enabling an analysis of the sources of variation, notably climate and soil, affecting the measured attributes. Twenty years ago such studies were run in phytotrons, especially in the F.R.G.[1-3] and in Australia[17,18]. Although the results, were interesting, they were limited because of the costs involved, because of the space required for a vine and the length of time needed for experiments with a perennial. Over the past 50 years more tractable experimental systems have been developed, using only parts of the vine (tissues) or whole plants maintained in vitro. As shown below, there are now attempts to use in vitro the smallest unit of living matter, the cell of the vine, to study the physiology (the functions and regulatory mechanisms) of the cell and of the plant.

This review will accordingly be centered on a technique, cultivation of the vine in vitro, which has enabled coherent and repeatable experimentation around the world. These studies seek to understand the functioning of the cell, of tissues, and of organs of the vine, and to understand the phases of development revealed or manipulated by this technique. An abundance of new knowledge has resulted.

What is the vine in vitro?

Once nurserymen were no longer satisfied to multiply the vine by mature cane cuttings (*août*é, or lignified and rich in reserves), they began to meet the problems which opened the route to an in vitro approach. Indeed, to reduce the time required to multiply a variety, one naturally adopts increasingly younger and smaller cuttings, such as green shoot cuttings[36,64], the use of which is relatively simple, except for two types of difficulty compared to classical cuttings. Firstly, the external protective tissues are less developed and the cutting is sensitive to attack by numerous fungi and bacteria normally non-pathogenic for the vine. Secondly, as a consequence of the low reserves and small size, a complete and balanced nutrition must promptly be supplied to green cuttings in contrast to the situation for large and mature cuttings with extensive reserves.

Of course as the size of cuttings is diminished such problems will be increasingly acute; the cut section – the most direct route of entry for microorganisms – will become more important relative to the volume and the nutritional demand will be more exacting. These two difficulties may be resolved by in vitro culture.

As long as the cutting includes a bud it remains possible to produce a plant by the classical methods of a specialized nursery, notably with mist propagation and accepting a high percentage of losses from small shoot cuttings. But with a bud-free cutting, termed a tissue explant, maintenance of the living state for the long or medium term requires adoption of in vitro methods, especially with regard to the two conditions mentioned already: protection from diverse microorganisms and supply of a medium covering nutritional requirements. For culture in vitro the *constraint of sterility* is absolute because, on the one hand, the cutting is very sensitive to competition and to toxins released by microorganisms and, on the other hand, the culture medium is a favorable substrate for numerous microorganisms.

The *nutritional constraint* is much less precise. Plants are effectively capable of extensive autotrophy, not only for energy substrates but also for growth regulators. However, certain growth regulators or effectors are produced by one part of the plant and act upon another; such chemical messengers may be lacking in a culture of tissue fragments – hence the need for an exogenous supply. Likewise for energy substrates it is possible to supply a sugar to the plant in sterile medium: this is an absolute requirement with non-chlorophyllian tissues. For a small cutting with one bud, sugars greatly stimulate the initial growth of the bud. But even for a whole plant in vitro, a significant part of sugars supplied to the root system is utilized.

Whenever living tissue is to be manipulated to obtain a novel response, a strategy of culture in vitro is generally warranted. In this respect the work of G. Debreaux[23] is notable. Her objective was to maintain leaves in a state of survival as long as possible: with the vine (Chasselas de Fontainebleau) she obtained survival times of 13 months with excised leaves, especially when with hormone treatments these leaves initiated roots. These results were obtained in non-sterile medium with distilled water as sole nutrient (accepting neither of the two constraints evoked above). However, these experiments reveal one of the key interests of the in vitro approach, to provide controlled rates of supply of various hormones through the sterile culture medium. To have maintained an isolated leaf in survival during 13 months under such conditions was a remarkable achievement then, whereas now living tissue may be indefinitely maintained in vitro without difficulty.

As for the contribution of in vitro studies to plant physiology, the method offers the possibility of controlling factors of the environment, both those of the atmosphere (light, temperature, humidity, gaseous environment) and of the culture medium. But the method is particularly useful as a means of studying a small group of cells from a single tissue or organ and for observing the diverse potentials of such a sample.

For example, a cell of leaf parenchyma is subject to a system of correlations, receiving messages from neighboring cells and from the various plant organs such that in the leaf that cell retains its essential function and chlorophyllian characteristic without ever dividing. If that cell is removed from its environment, and so from the intercellular messengers, it will be able to express other capacities of its genome. Given the totipotency of plant cells (a feature which is demonstrated and used daily), one may hope to obtain a complete plant with meristems and with sexual reproductive organs ensuring genetic continuity based upon the genotype of that cell. Except for the in vitro step, this cell would have finished its existence in the autumn like all the other cells of leaf parenchyma.

When a well-differentiated functional tissue is isolated, it does not generally retain its function for long in vitro. It dedifferentiates, returns progressively towards an undifferentiated state and recovers young cell characters.

The vine in vitro before 1975

In 1931 and after numerous attempts[40, 49, 69], Cracium summarized the situation as follows: 'All authors seem to agree that it is impossible to obtain true tissue cultures'. The first positive results arose from emphasis of the notion that all tissues do not have the same potential and in 1934 Gautheret[32] succeeded in making the first cultures of cambial tissue, which became permanent cultures in 1939. At this time White[81] produced cultures of tumoral tissue. In both cases the original explant possessed a remarkable aptitude for cell proliferation. This logic which produced these successes with tissue culture should have led to the relatively simple step of somatic embryogenesis with the vine. As is apparent below, despite the existence of model systems offering a basis for deduction, the practical achievements are sometimes surprisingly slow to follow.

Morel produced the first substantial and important in vitro work on the vine, beginning in 1943[53-58] and continuing to 1948 with a thesis showing the strong correlation between the proliferation activity of cells in the source tissue of the vine and their aptitude for dividing in vitro. Tissue sampled during vegetative quiescence would not yield tissue cultures. He also showed the importance of growth factors in this area, using 'heteroauxin' to affect cell proliferation. Morel described the different types of calli obtained with various auxin concentrations and in fact suggested this as a mean 'to produce cultures of isolated cells' from the vine[58] (p. 104). He noted the now common phenomenon of habituated cultures[33] *(anergie)* which corresponds to an evolution of the hormonal requirements of tissue cultures. He also studied cell differentiation in tissue cultures, especially those giving rise to organization into secondary tissues or to conductive vascular tissues. In passing, one must note the hand of a master in associating the culture of this tissue in vitro with an obligate parasite, downy mildew *(Plasmopara viticola)*.

It is known that auxins lead to intense rooting activity, which Morel observed but did not study. It is striking that at that period each time a tissue culture produced an organ it was treated as uninteresting, a mere reconstitution of part of the plant or of a whole plant as already known elsewhere!

Only in 1964 was the rooting process of vines studied in vitro. J. Fallot[24] analyzed in detail the responses of various *Vitis* species and varieties. Trials with numerous combinations of hormones, vitamins and nutrients led to a fine control of cell multiplication and root formation, but in contrast shoots never appeared. From this moment the difficulty of regenerating the vine in tissue culture was taken as proven and this plant, which had largely contributed to a general understanding of tissue culture, was temporarily abandoned as the reference model and usurped by other species, such as carrot[78, 79].

The choice of a tissue with readily obtainable cell multiplication (such as cambium) has remained a fruitless approach to the task of regenerating a shoot meristem. This approach is still adopted to investigate tissue differentiation in culture[45] but it is evidently deceiving to obtain only root meristems for such study.

From 1961, in parallel with such tissue culture, the technique of whole plant culture in vitro developed. Amongst the numerous reports those of R. Galzy[28-31], aimed primarily at the elimination of some virus diseases, will be cited. These cultures used an axillary bud as starting material and were also maintained through axillary buds, without any hormone in the culture medium. It is noted that, by good fortune, the vine develops without problems in a test tube typically 2 cm in diameter and 20 cm long. It was simply necessary to place the vine in vitro to observe its miniaturization, although the factors producing that effect are still little known. At the University of Dijon we have shown that the gaseous atmosphere is a primary factor affecting the degree of miniaturization[27] and the activity of the terminal meristem (Fournioux, unpublished results). The propensity for vegetative multiplication in vitro amongst virtually all *Vitis* species is most useful for studies on this plant.

The vine in vitro since 1975: Dual emphasis upon tissue competence and growth factor management

After this remarkable scientific activity with the vine in the middle of this century, other simpler material was used to continue such fundamental studies. The vine appeared difficult for manipulation in vitro as the governing idea was partially false: it was commonly considered that the plant cell was totipotent and that it would merely be necessary to establish a hormonal and nutritional equilibrium to regenerate, in this case, a vine. This was a poor entry to the problem and the numerous studies on that basis have always been unsuccessful. Although this approach may yet yield results a much simpler handling of the task would emphasize association of an appropriate hormonal stimulation with the cellular competence of a correctly chosen tissue, restoring the reasoning mentioned above which led to initial successes in tissue culture.

In 1976 Favre[25] in France and Mullins[59] in Australia obtained, simultaneously but by distinctive means, the regeneration of vine plants in tissue cultures.

Tissue competence. Two approaches have proven useful with many plants that otherwise only give shoot meristems or somatic embryos with much difficulty. The condition that cells should be very young or actively proliferating is not always adequate for subsequent regeneration: it is sometimes necessary to take *juvenile cells* from a young plantlet derived from seed[68]. Such juvenile cells, even though they are relatively differentiated (e.g. from leaf parenchyma tissue), conserve an excellent regeneration capacity. In the vine this possibility is of little practical interest since the starting material is then a zygotic embryo whose genetic characteristics are undefined, a handicap naturally carried into any plants regenerated therefrom. This difficulty has been overcome in two ways.

- It is possible to take an adult bud from a given stock and in vitro to return towards the juvenile forms[25,61] characterized by disappearance of tendrils and by a phyllotaxy 2/5. Concomitantly, the plant induced to a juvenile form exhibits the regenerative capacity characteristic of that development stage[19,20,26,63].
- Furthermore, having mastered the difficult step of getting somatic embryos in cultures, one can exploit the embryos' prolific regenerative capacity. They will readily display intense embryogenesis. Adventitious embryos will form naturally in agitated liquid suspensions or in solid media at the transition zone between the shoot and the root of these primary embryos[11,47]. Furthermore, sequential transfers of such embryonic tissues produces an intense embryogenesis both on solid and in agitated liquid media.

While these studies use the potential of juvenile cells for regeneration, another approach uses cells close to the reproductive apparatus, cells which in many species are particularly disposed to regeneration. For the vine, nucellar tissue and connective tissues of the stamen have this aptitude[13,16,42,43,67].

The attention of scientists was drawn to the nucellus because in certain plant species under natural conditions this tissue can develop adventitious embryos much like a sucker of the mother plant. In the young ovule of the vine, the nucellus develops about three weeks before flowering with rapid mitosis and growth. A surface cell produces the archeospore which becomes the embryo sac after several divisions and a meiosis. In several woody plants, and notably in Citrus[19,46,76], the nucellus gives rise to multiple embryos (polyembryony) from each seed; this feature has been extensively used with Citrus with or without in vitro manipulation. Natural polyembryony also seems to occur in the vine[14]. It is less apparent and rarer than in Citrus, but through in vitro methods Mullins[59,60,77] has obtained active expression of this trait.

The grape nucellus appears to feed the developing embryo sac and thereby degenerates and disappears rapidly after flowering[62,66]. Therefore the period during which tissue cultures may be established from the nucellus is about one week, during which cell multiplication is most active in this tissue.

Growth factor management in tissue culture. With the vine this field is on the one hand classic and on the other hand poorly mastered; techniques are often successful but results may be most irregular. Several general notions indicated below are used, although various elements of technique are still not understood despite systematic experimentation. One must admit that the technique is to some extent an art, which is not always easy to communicate between one laboratory or practitioner and another.

To demonstrate the imprecise nature of the method, despite its coherent and logical basis, I shall take the example of regeneration from nucellar culture as it is regularly performed at the University of Dijon[12]. The variability of results can be analyzed and to some extent mastered. This introduction is particularly intended to excuse a rather vague and goal-oriented description.

The nucellus is an integral part of the ovule, whose length is less than 1 mm upon excision. The starting material is thus the whole ovule as it is impossible to isolate the nucellus. Culture is in agitated liquid medium containing salts, sugars, vitamins, and hormones of the auxin group with a low rate of the cytokinin, 6-benzyl aminopurine (6 BAP). The latter hormone is thought to enable plant cells to conserve or to acquire young-cell character and in many species it stimulates shoot meristem formation in tissue cultures. However, during this first phase, the auxinic function is primordial even though poorly measurable. Indeed, auxins normally have two known roles, to stimulate cell proliferation as sought here and to initiate root meristems as needed in the subsequent phase.

The auxin used in this first medium is 2,4-dichlorophenoxyacetic acid (2,4-D) at a concentration in the order of mg/l. Of course, this molecule is used in agriculture as a herbicide. The vine is highly sensitive to 2,4-D with such rapid damage or destruction that in the Champagne for example its use is prohibited within 500 m of a vineyard. In the culture medium this auxin activates nucellar cell division. But whole ovules are used and cell division may originate from other ovule tissues (evident in microscopic sections); such calli never become embryogenic. For example, if the initial culture has a slightly increased 2,4-D concentration the external tegument of the ovule develops into callus. Secondly, if the auxin:cytokinin ratio is increased there is a spurt of division; cells became large, the callus is friable, cells differentiate and lose their embryogenic capacity.

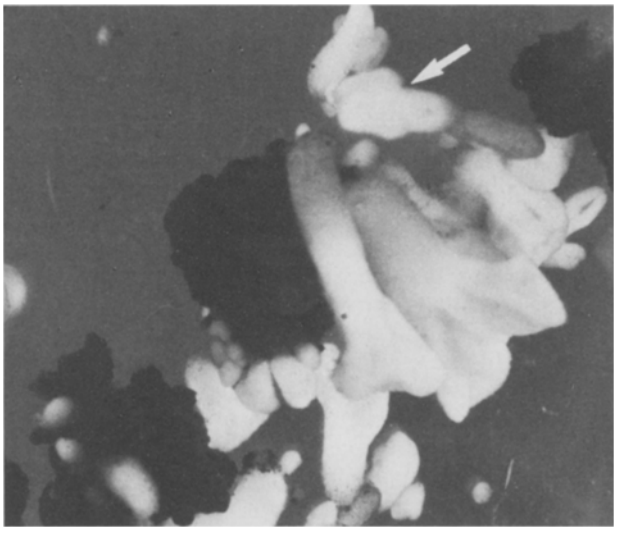
The first phase of culture is of 15–30 days. A second phase of about 30 days involves replacement of 2,4-D by another auxin hormone, 2-naphthoxyacetic acid (NOA). Why the change, and is it indispensable? The basic idea is that 2,4-D is a very active but reputedly mutagenic hormone, causing abnormal mitosis. 2,4-D is necessary to activate division but may be rapidly substituted by another auxin less aggressive for the genome, such as NOA. This deduction has little direct experimental support, and notably not from any studies with the vine.

During this month-long second phase there is always only slow growth of callus, and any attempt to hasten growth by hormone application only reduces the tendency for embryogenesis. The culture is in agitated liquid medium. Upon completion the largest calli are generally less than 10 mm in diameter and sometimes only 2 or 3 mm. The calli grow more slowly and become brown. This state marks a predisposition for the third phase, embryogenesis.

The calli are transferred to a hormone-free medium. Growth is only slight but after several weeks or months there are small cellular aggregates floating in the agitated liquid medium: these are glossy white embryos. Embryogenesis continues for several months; some calli may become completely black (figure). In the later part of this phase embryos form directly on the callus rather than in the medium.

The term embryos has been used in a broad sense. Certain authors would no doubt disapprove, but following the general studies of Street[79] and those of Krul on the vine[48] it is apparent that somatic embryos follow a development pattern close to that of zygotic embryos, even though aberrations are frequent.

One central notion in this methodology is as follows: initially, a few cells have marked embryogenic competence; these are multiplied under conditions favoring retention of that capacity, and then they are placed in a hormone-free medium where they may express this capacity. This implies balanced use of hormones adapted to each circumstance in the culture sequence according to relatively subjective observation. The hormone concentrations are measurable but the evolution of these target cells is difficult to assess, even though they determine success of the culture.



Embryogenesis on nucellar callus. We note the high embryonic activity despite the black coloration of the callus. The arrow indicates an embryo which closely resembles that found in the mature seed of the grapevine. This embryo is 1 mm long.

The methodology, for developing plants from these embryos will not be discussed; this process generally has a low success rate. As shall be seen, plants derived in such a manner are valuable and contribute to our understanding of vine physiology.

Growth factor management in apex culture. Management of the complex cultures just described highlights the contrasting simplicity of apex culture in which a cutting containing a shoot meristem is developed to an entire plant[5, 6, 29, 30, 31, 34, 36, 70]. Three classical groups of hormones are useful.

1) Auxins are generally supplied during about 15 days to stimulate root formation and so rapidly to transform the shoot cutting into a whole plant.

2) Gibberellins are also used in the first phase of culture to enable the internodes of the young shoot to begin elongation. This stimulation is especially necessary for the most reduced explants comprising a single meristem with the last two leaf initials, about 0.2 mm in all. (For the vine this is at present the closest approach to true meristem culture.)

3) Cytokinins are fundamental to the field of micropropagation. They suppress the correlative inhibition of axillary buds such that all the higher order axillary buds start growing. The original cutting therefore branches abundantly in a witches' broom form. Each ramification can be transferred to a medium without cytokinin to develop a whole plant. Chee et al.[21] calculated that from one apex they might obtain 75.10^6 vines in 330 days! The method could be most rewarding, provided that several serious problems discussed below can be solved.

An approach intermediate between tissue and apex culture, proposed by Skene[75], is to use a fragmented shoot apex. The culture includes axillary and non-axillary tissue such that the source of regeneration is uncertain[8–10]. The method is rapid and relatively easy. The initial culture includes cytokinins and once regeneration is evident the bud is normally transferred to a medium without cytokinin.

Practical utilization of the vine in vitro

Micropropagation. The objective here is to hasten vegetative propagation and this is altogether feasible. Two serious problems remain and are unlikely to be resolved for several years. The first arises from the fact that the large majority of cultivated vines are phylloxera-sensitive *Vitis vinifera* requiring grafting upon resistant rootstocks. The use of scions on their own roots as in many other horticultural crops such as the rose[51] is not generally applicable. Micropropagation should however enable simple multiplication of those rootstocks whose rooting in the nursery is difficult to obtain.

The second major problem is that for the vine an in vitro cycle can modify the resultant plant relative to its source material. Grenan[37–39, 80] demonstrated that a vine cultivated in vitro without any hormone during two months was modified when returned to field conditions, com-

pared to its stock. His report underlines the need for methods to assess and to control the conformity of vegetatively propagated material.

The matter remains to be elucidated but the vine, now that micropropagation is possible, should be a useful model to study conformity. For the comparison of two plants supposes precise knowledge of the phenotype, and the vine is probably the best known of species in this sense, it is the source of the science of ampelography: knowledge of the characteristics and aptitudes of different varieties and of clones derived therefrom. It is thus a particularly interesting material. Using ampelography, Grenan showed that an in vitro cycle always produced vines with more deeply cut leaves, increased pubescence and pigmentation of the cane relative to the original stock[38]. Fertility was also modified, sometimes more, sometimes less, depending upon the variety[37]. These modifications were essentially stable over time and throughout growth[35]. Given the strict obligation to ensure *trueness of type,* especially in classified wine-growing areas, vine micropropagation could not be extensively used in practice until these major questions are resolved.

Elimination of virus. It is well-known that in many instances viruses do not pass through the seed or into the meristem. The above technologies and notably meristem culture enable elimination of certain virus diseases such as leaf-roll, stem pitting and corky bark[4,6,7,15,44,65], diseases which classical heat-therapy cannot eliminate. Obviously the aforementioned problems associated with culture in vitro apply here too.

Variety improvement. As an in vitro cycle induces modifications the next step is to exploit such changes. Conventional genetic improvement have proved to be of limited use as the vine requires broad heterozygosity. When this is lessened by self-fertilization, inbreeding rapidly leads to loss of the plant through poor vigor[14]. Furthermore, hybrids have a very low reputation in the viticultural community since the economic catastrophe associated with their abuse in the first part of this centruy. In the traditional viticultural zones and especially in regions of *grands crus,* vine improvement must be based upon existing varieties which have assured the reputation of the areas' wines. Clonal selection through selection in vineyards has produced good results but it has limited potential. In contrast, use of the variability appearing in vitro opens fresh perspectives and will hopefully enlarge the scope of clonal selection.

Successes in this area have already been obtained with other plants such as sugarcane[41] and potato[71,72]. Somaclones derived by somatic embryogenesis arise from diploid cells and should therefore be very close to the original clone: they constitute (phenotypic or genotypic?) variants with potentially useful characters. Initially it is adequate to look for spontaneous variation and to assess embryos in the vineyard. A further strategy now being applied with respect to certain problems[12,15] involves primary selection of exceptional embryos on the basis of salt or toxin resistance[52], for example, following with field tests. Eventually, genetic manipulation may allow introduction of a single character into the genome, but in this area even the physiological tools are not yet ready. Regeneration of the vine from the isolated wall-less cell, the protoplast, has not yet been achieved[73,74].

Conclusion

In the area of cell and tissue physiology, the grape vine has been a major reference material. As with all woody perennials, in vitro technologies are relatively delicate. Nevertheless, remarkable progress has produced a situation where, during the past ten years, practical application of results from in vitro work has been envisaged. Reciprocal understanding has yet to be established between scientists developing micropropagation (multiplication with conformity to type) and those using in vitro methods for variety improvement (multiplication for novelty of type). I am convinced that the future should not see one of these objectives dominating the other. On the contrary, certain technologies should be developed to ensure a framework for conformity of propagation and others to obtain greater variability with the goal of plant improvement. This will render effective the two applications considered.

Acknowledgments. The author wishes to thank the Moet-Hennessy group and the Ministère de la Recherche et de la Technologie for their support of this work. He thanks also S. Brown for reviewing the manuscript.

1 Alleweldt, G., Die Wirkung des Störlichtes auf die photoperiodische Reaktion der Reben. Vitis *4* (1964) 357–364.
2 Alleweldt, G., Die Umweltabhängigkeit des vegetativen Wachstums, der Wachstumsruhe und der Blütenbildung von Reben *(Vitis species)*, III. Die Blütenbildung. Vitis *4* (1964) 240–261.
3 Alleweldt, G., and Radler, F., Interrelationship between the photoperiodic behavior of grapes and the growth of plant tissue culture. Pl. Physiol. *37* (1962) 376–379.
4 Ayuso, P., and Pena-Iglesias, A., Microinjerto de meristemos: una nueva y prometedora technica para regenerar vides enfermas por virus. Proc. 6th meeting ICVG (1976) 319–324.
5 Ayuso, P., and Pena-Iglesias, A., Shoot apex (meristem) grafting: a novel and promising technique for regeneration of virus infected grapevines Proc. 6th conf. virus of grapevine Monografias INIAS (1978) 1–11.
6 Ayuso, P., Villegas, T., and Pena-Iglesias, A., La micropropagation comme méthode derégénération sanitaire dans les raisins de table espagnols. Symp. Intern. 'Raisin de table – Raisin sec' Heraklion Grèce (1982) 497–501.
7 Barlass, M., Skene, K.G.M., Woodham, R.C., and Krake, L.R., Regeneration of virus free grapevines using in vitro apical culture. Ann. appl. Biol. *101* (1982) 291–295.
8 Barlass, M., and Skene, K.G.M., Studies on the fragmented shoot apex of grapevine. I The regenerative capacity of leaf primordial fragments in vitro. J. expl Bot. *31* (1980) 483–488.
9 Barlass, M., and Skene, K.G.M., Studies on the fragmented shoot apex of grapevine. II Factors affecting growth and differentiation in vitro. J. expl Bot. *31* (1980) 489–495.
10 Barlass, M., and Skene, K.G.M., In vitro propagation of grapevine (*Vitis vinifera* L.) from fragmented shoot apices. Vitis *17* (1978) 335–340.
11 Bessis, R., Callogenèse et régénération chez le Pinot, le Chardonnay et le Gamay. Colloque Amélioration de la Vigne et Culture in vitro – Paris, in press (1985).
12 Bessis, R., and Labroche, C., Obtention de somaclones chez la vigne: variabilité spontanée. Bull. Soc. bot. Fr., in press (1985).
13 Bigot, C., Multiplication végétative in vitro par néoformation de bourgeons et d'embryons somatiques, in: La multiplication végétative des plantes supérieures. Eds R. Chaussat and C. Bigot. Gauthier-Villars, Paris 1980.
14 Bouquet, A., La polyembryomie spontanée chez *Vitis vinifera* L. Intérêt pour la génétique et l'amélioration de la vigne, pp. 17–32. IIème Symp. int. Amérioration de la Vigne, Bordeaux. INRA 1978.
15 Bouquet, A., Interêt des techniques de culture in vitro pour la multiplication et l'amélioration génétique et sanitaire des variétés de vigne, pp. 38–43. 2ème Coll. Int. Multiplication de la Vigne, Bordeaux 1982.

16 Bouquet, A., Piganeau, B., and Lamaison, A.M., Influence du génotype sur la production de cals, d'embryoïdes et de plantes entières par cultures d'authères in vitro dans le genre *Vitis*. C.r. Acad. Sci. Paris *295* (1982) 569–574.
17 Bouzid, S., and Lasram, M., Utilisation de cultures in vitro pour l'obtention de clones sains de *Citrus* homogènes et en bon état sanitaire. 8th Cong. Intern. Agrum. méditer. Madrid *2* (1966) 1–6.
18 Buttrose, M.S., Fruitfulness in grapevines: effects of changes in temperature and light regimes. Bot. Gaz. *130* (1969) 173–179.
19 Buttrose, M.S., Vegetative growth of grapevine varieties under controlled temperature and light intensity. Vitis *8* (1969) 280–285.
20 Champagnat, M., Morel, G., Chabut, P., and Cognet, A.M., Recherches morphologiques et histologiques sur la multiplication végétative de quelques orchidées du genre Cymbidium. Revue gén. Bot. *73* (1966) 706–746.
21 Chee, R., Pool, R.M., and Bucher, D., A method for large scale in vitro propagation of *Vitis*. N.Y. Food Life Sci. Bull. *109* (1984) 1-9.
22 Cracium, E., La culture de tissus en biologie experimentale. Masson, Paris 1931.
23 Debraux, G., La feuille isolée en survie. Son application à l'étude de quelques problèmes de morphogenèse et d'histogenèse. Thèse, Poitiers 1951.
24 Fallot, J., Sur la prolifération des tissus de *Vitis* et d'autres végétaux. Rôle des bourgeons. Action des bactéries. Thèse, Toulouse 1964.
25 Favre, J.M., Influence de l'état physiologique de la plante et de la nature de l'organe sur l'obtention de néoformations caulinaires de vigne. 101ème Congr. Nat. Soc. Savantes, Lille *1* (1976) 465–474.
26 Favre, J.M., Premiers résultats concernant l'obtention in vitro de néoformations caulinaires chez la vigne. Annls Amél. Pl. *27* (1977) 151–169.
27 Fournioux, J.C., Influence de la teneur en CO_2 sur la morphogenèse de la vigne cultivée in vitro. Colloque Amélioration de la vigne et culture in vitro (Paris), in press (1985).
28 Galzy, R., Confirmation de la nature virale du court – noué de la vigne par des essais de thermothérapie sur des cultures in vitro. C.r. Acad. Sci. Paris *253* (1961) 706–708.
29 Galzy, R., Remarques sur la croissance de *Vitis rupestris* cultivée in vitro sur différents milieux nutritifs. Vitis *8* (1969) 191–205.
30 Galzy, R., Recherches sur la croissance de la vigne saine et court nouée cultivée in vitro. Thèse, Clermont 1970.
31 Galzy, R., La culture in vitro des apex de *Vitis rupestris*. C.r. Acad. Sci. Paris *274* (1972) 210–213.
32 Gautheret, R.J., Culture du tissu cambial. C.r. Acad. Sci. Paris *198* (1934) 2195–2197.
33 Gautheret, R.J., Sur la variabilité des propriétés physiologiques des cultures de tissus végétaux. Revue gén. Bot. *62* (1955) 5–110.
34 Goussard, P.G., Effects of cytokinins on elongation, proliferation and total mass of shoot derived from apices of grapevine cultured in vitro. Vitis *20* (1981) 228–234.
35 Grenan, S., Possibilités d'éliminer des modifications foliaires apparues sur la variété Grenache N après un passage prolongé en culture in vitro. Prog. Agric. Vitic. *7* (1979) 152–157.
36 Grenan, S., Rhizogenèse de bourgeons apicaux de boutures de vigne cultivées in vitro. Conn. Vigne Vin *2* (1979) 125-136.
37 Grenan, S., Implications fondamentales et appliquées de conséquences de la culture in vitro de *Vitis vinifera*. Thèse, Orsay 1982.
38 Grenan, S., Polymorphisme foliaire consécutif à la culture in vitro de *Vitis vinifera* L. Vitis *23* (1984) 159–174.
39 Grenan, S., and Truel, P., Réfléxions sur un aspect de la variabilité au cours de la multiplication de variétés de vignes issues de semis. Agronomie *3* (1983) 675–680.
40 Haberlandt, G., Kulturversuche mit isolierten Pflanzenzellen. Sber. Akad. Wiss. Wien *111* (1902) 145–172.
41 Heinz, D.J., Krishnamurthi, M., Nickell, L.G., and Maretzki, A., Cell, tissue and organ culture in sugarcane improvement, in: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture, pp. 3–17. Springer-Verlag, Berlin 1977.
42 Hirabayashi, T., and Akihama, T., In vitro embryogenesis and plant regeneration from the anther-derived callus of *Vitis*. Proc. 5th Cong. Plant tissue and cell culture. Pl. Tiss. Cult. (1982) 547–548.
43 Hirabayashi, T., Kozaki, I., and Akihama, T., In vitro differentiation of shoots from another and callus in *Vitis*. Hort. Sci. *11* (1976) 511–512.
44 Jako, N., Obtention de plantes ne présentant pas de symptômes à partir du méristème des pointes de pousses de pieds de vigne *Vitis vinifera* L. CV Pinot noir atteints par l'enroulement. Mitt. Klost. *33* (1983) 15–17.
45 Jordan, M., Brezeanu, A., and Rosu, A., The micropropagation of *Vitis vinifera*. II. Aspects of morphogenesis in callus culture. Revue roum. Biol. *26* (1981) 141–150.
46 Kochba, J., Spiegel-Roy, P., Neumann, R., and Saad, S., Stimulation of embryogenesis in *Citrus* ovular callus by ABA, ethephon, CCC and Alar and its suppression by GA_3. Z. PflPhysiol. *89* (1978) 427–433.
47 Konar, R.N., and Natajara, K., Experimental studies in *Ranunculus sceleratus* L. Development of embryos from the stem epidermis. Phytomorphology *15* (1965) 132–137.
48 Krul, W.R., and Worley, J.F., Formation of adventitions embryos in callus cultures of Seyval, a french hybrid grape. J. Am. Soc. hort. Sci. *102* (1977) 360–363.
49 Lamprecht, W., Über die Kultur und Transplantation kleiner Blattstücken. Beitr. allg. Bot. (1918) 353–398.
50 Larkin, P., and Scowcrofft, W.R., Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. appl. Genet. *60* (1981) 197–214.
51 Martin, C., Carre, M., and Vernoy, R., La multiplication végétative in vitro des végétaux ligneux: cas des rosiers. C.r. Acad. Sci. Paris *2* (1981) 175–177.
52 Meredith, C.P., Building better grapes through genetic engineering, pp. 1–8. 8th Wine Industry Technical Seminar. Santa Rosa, California 1982.
53 Morel, G., Sur le développement des tissus de vigne cultivée in vitro. C.r. Soc. Biol. *138* (1944) 62.
54 Morel, G., Action de l'acide indole B acétique sur la croissance des tissus de vigne. C.r. Soc. Biol. *138* (1944) 93.
55 Morel, G., Isolement de cultures pures de parenchyme de vigne. C.r. Acad. Sci. Paris *221* (1945) 78–80.
56 Morel, G., Caractères anatomiques des tissus de vigne cultivées in vitro. C.r. Soc. Biol. *139* (1945) 674.
57 Morel, G., Remarques sur l'action de l'acide naphtyle acétique sur le développement des tissus de vigne. C.r. Soc. Biol. *140* (1946) 269–270.
58 Morel, G., Recherches sur la culture associée de parasites obligatoires et de tissus végétaux. These Imp. Nat., Paris 1949.
59 Mullins, M.G., and Srinivasan, C., Somatic embryos and plantlets from an ancient clone of the grapevine (CV. Cabernet Sauvignon) by apomixis in vitro. J. expl Bot. *27* (1976) 1022–1030.
60 Mullins, M.G., and Srinivasan, C., Plantlets of "Cabernet Sauvignon" grapes by nucellar embryogenesis in vitro, pp. 11–15. IIème Symp. int. Amélioration de la Vigne, Bordeaux. INRA 1978.
61 Mullins, M.G., Nair, Y., and Sampet, P., Regeneration in vitro: induction of juvenile characters in an adulte clone of *Vitis vinifera* L. Ann. Bot. *44* (1979) 623–627.
62 Nitsch, J.P., Pratt, C., Nitsch, C., and Shaulis, N.J., Natural growth substances in Concord and Concord Seedless grapes in relation to berry development. Am. J. Bot. *47* (1960) 566–576.
63 Nozeran, R., Ducreux, G., and Rossignol-Bancilhon, L., Réflexions sur les problèmes de rajeunissement chez les végétaux. Bull. Soc. bot. Fr. 129, Lett. bot. *2* (1982) 107–130.
64 Ottenwaelter, M.M., Hevin, M., and Doazan, J.P., Amélioration du rendement du bouturage des extrémités après thermothérapie sur plantes en pots par l'utilisation de la culture sur milieu nutritif gélosé stérile. Vitis *12* (1973) 46–48.
65 Pena-Iglesias, A., and Ayuso, P., Shoot apex (meristem) micrografting and indexing of infected grapevine varieties at the same time. Proc. of 7th meeting of viruses and virus-like diseases of grapevine. Niagara Falls, Canada *8–12* (1980) 333–338.
66 Pratt, C., Reproductive anatomy in cultivated grapes. A review. Am. J. Enol. Vitic. *22* (1971) 92–109.
67 Rajasekaran, K., and Mullins, M.G., Embryos and plantlets from cultured anthers of hybrid grapevines. J. expl Bot. *30* (1979) 399–407.
68 Rajasekaran, K., and Mullins, M.G., Organogenesis in internode explants of grapevines. Vitis *3* (1981) 218–226.
69 Robbins, W.J., Cultivation of excised root tips and stem tips under sterile conditions. Bot. gaz. *73* (1922) 59–79.
70 Rosu, A., Brezeanu, A., and Jordan, M., Micropropagation in *Vitis vinifera* L. III. Studies regarding 'in vitro' stimulation of multiple axillary shoot development in some grapevine cultivars for clonal multiplication. Revue roum. Biol., Biol. végét. *28* (1983) 115–122.
71 Shepard, J.E., Protoplasts as sources of disease resistance in plant. A. Rev. Phytopath. *19* (1981) 145–166.
72 Shepard, J.E., Bidney, D., and Shahin, E., Potato protoplasts in crop improvement. Science *208* (1980) 17–24.
73 Skene, K.G.M., Culture of protoplasts from grape-vine pericarp callus. Aust. J. Pl. Physiol. *1* (1974) 371–376.

74 Skene, K.G.M., Production of callus from protoplasts of cultured grape pericarp. Vitis *14* (1975) 177–180.
75 Skene, K.G.M., and Barlass, M., Micropropagation of grapevine. Int. Pl. Propag. Soc. Combined Proc. *30* (1980) 564–570.
76 Spiegel-Roy, P., and Kochba, J., Stimulation of differentiation in orange *(Citrus sinensis)* ovular callus by gamma irradiation. Radiat. Bot. *13* (1973) 97–103.
77 Srinivasan, C., and Mullins, M.G., High-frequency somatic embryo production from unfertilized ovules of grapes. Scientia hort. *13* (1980) 245–252.
78 Stewart, F.C., Growth and organized development of cultured cells. III. Interpretation of the growth from free cell to carrot plant. Am. J. Bot. *45* (1958) 709–713.
79 Street, H.E., and Withers, L.A., The anatomy of embryogenesis in culture: Tissue Culture and Plant Science, pp. 70–100. Ed. H.E. Street. Academic Press, New York 1974.
80 Valat, C., and Rives, M., Information and comments on variations induced by thermotherapy. Proc. 5th meeting ICVG. Revue Path. vég. *4* (1973) 291–293.
81 White, P.R., Potentially unlimited growth of exised plant callus in an artificial nutrient. A. J. Bot. *26* (1939) 59–64.

0014-4754/86/080927-07$1.50 + 0.20/0

Nature and physiological effects of grapevine diseases

by G.P. Martelli, A. Graniti and G.L. Ercolani*

*Dipartimento di Patologia vegetale and *Istituto di Microbiologia agraria, University of Bari, Via G. Amendola 165/A, I-70126 Bari (Italy)*

Key words. Grapevine; fungi; bacteria; viruses; infectious diseases, physiopathology.

Several factors are known to have detrimental effects to the productivity and appearance of grapevines, through interference with various basic physiological processes. The causes of some adverse effects can be identified as abiotic factors such as climatic adversities, air pollution and nutritional imbalance. Others are infections by pathogens; fungi, bacteria and viruses are most often involved. The effects of infection by the most important of such pathogens will be outlined below.

1. Fungal diseases

A considerable number of fungi are known to cause diseases of grapevines, including species that in many areas are the most important pathogens for this crop. Apart from control costs, the economic importance of most fungal diseases of the grapevines is mainly related to a reduction in the quantity and quality of the yield, particularly of table grapes. For the wine industry, additional losses may arise from the undesirable effect on fermentation of substances produced during the plant-pathogen interaction processes. Only a limited number of fungi cause destructive diseases which may shorten the lifetime of the vines or cause them to die.

Because of the vastness of the pertinent literature, only some physiological aspects of two major diseases, downy mildew and grey mold, will be discussed in detail here.

1.1 Downy mildew

Spring symptoms of downy mildew appear on leaves, green shoots, inflorescences and young fruit clusters. Leaf spots are at first pale green and lightly translucent ('oily spots'), then yellowish – or reddish in some red-berried cultivars – and eventually turn brown and wither. On older leaves, the summer or autumn lesions of mildew are restricted to smaller, polyhedrical, interveinal areas ('mosaic spots'), the tissues of which usually harbor gametangia and oospores of the pathogen, *Plasmopara viticola* (Berk et Curt.) Berl. et de Toni. The asexual sporulation occurs on the lower surface of mildew spots, appearing as a whitish mold. Heavy infections may cause severe leaf fall. Attacks on flowers and developing clusters cause discoloration and distortion ('S'-shaped bending) of the rachis, 'grey-rot' and loss of young fruits, withering and dropping of part or all of the inflorescence. Later, mildew infections through the fruit peduncles and bunch axes may cause 'brown rot' of grapes. Shoots, green nodes of older canes, and buds are also subject to attack and may be severely damaged.

Infection occurs by stomatal penetration of germ tubes developed from encysted zoospores. Before encysting, the zoospores released from a zoosporangium head for open stomata by swimming in a thin film of rain or dew water on the plant surface. Attraction of zoospores by stomata is probably chemotactic with reference to concentration gradients of oxygen or exudates. Several zoospores (4–5) often congregate around a single stoma which is penetrated by as many thin germ tubes. These enlarge in the substomatal cavity to form vesicles from which the invading hyphae of the pathogen originate.

Infection of the grapevine by *P. viticola* is relatively rapid, the entire process, from the deposition of the sporangial inoculum on the leaf surface to the formation of the first haustorium being completed in about 3 h[55]. Colonization of the host tissue is due to the growth of branched coenocytic hyphae of the pathogen in the intercellular spaces. The biotrophic habit of *P. viticola* is shown by a profuse development of pyriform haustoria within the host cells. The bud-shaped hyphal outgrowths, from which the haustoria originate, pierce the plant cell wall mostly mechanically, although enzymic degradation of cell-wall components is supposed to be involved as well. In the early phase of haustorial penetration, the host plasmalemma is invaginated by the growing haustorium. Later, the continuity of plasmalemma around the fully developed haustorium is no longer detected in electron micrographs, possibly because the plasmalemma is not distinguishable from the electron-dense layer surrounding the