Conservation of the genetic resources of *Vitis*

M. HARST-LANGENBUCHER and G. ALLEWELDT

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, F. R. Germany

Summary: As an alternative or supplement to field collections, a repository of 35 grapevine genotypes and 15 clones of cv. Optima was set up under minimal growth conditions. To prolong the storage period between subculturing to at least 12-18 months, some factors of long-term storage were optimized and the causes responsible for early senescence of sensitive genotypes were investigated.

Besides indirect effects due to excision time, origin of the starting material and preculture, there is a direct influence of the light conditions on the survival rate of cultures. CCC application showed, in some cases, positive effects only after long-term storage.

An increase of carbon dioxide concentration during storage is considered to be responsible for the early death of some cultures.

Key words: gene resources, tissue culture, long-term storage, preculture, light condition, growth regulator, carbon dioxide.

Introduction

For grapevines, as with most heterozygotic plants, maintainance of homologous material is normally carried out vegetatively in living collections. This form of preservation, however, is highly vulnerable by biotic and abiotic factors e. g. pests, pathogens, climatic stress or air pollution.

An ideal alternative or supplement to field collections is preservation *in vitro*. Under normal culture conditions (NC), maintainance *in vitro* is laborious, time and space consuming. For storage purposes plants are maintained under reduced culture conditions (RC) to prolong the period between subculturing.

In 1986 a repository of 35 grapevine genotypes and 15 clones of cv. Optima was set up under minimal growth conditions. A wide range of genotypical reactions was observed during a storage period of 12 months. To optimize the storage conditions and to determine the causes of early senescence, various examinations were carried out.

Material and methods

Plant material

Rooted plants deriving from nodal cuttings were used as storage material. The plantlets were cultivated under NC up to a shoot length of 5-7 cm and then transferred to RC.

Culture conditions

- NC: 25 °C, 16 h photoperiod, 50 $\mu E \cdot m^{-2} \cdot s^{-1}$
- RC: 8 °C, 10 h photoperiod, 10 $\mu E \cdot m^{-2} \cdot s^{-1}$

The medium of LINSMAIER and SKOOG (1965) supplemented with hormones (0.01 mg/l NAA and 0.03 mg/l BA), vitamins (0.40 mg/l thiamine), 30 g/l sucrose and 8 g/l agar, was used under NC and RC.

CCC was applied to the nutrient medium before cultivation of the cuttings according to ALLEWELDT and HARST-LANGENBUCHER (1987).

Carbon dioxide determination

The determination of CO_2 was carried out with a special CO_2 detector Ultramat 22p Siemens modified for use with *in vitro* cultures.

Abbreviations used

NAA, 1-naphtaleneacetic acid; BA, 6-benzyladenine; CCC, chlorocholinechloride (2-chlorethyl-trimethyl-ammonium-chloride); NC, normal culture conditions; RC, reduced culture conditions.

Results and discussion

Optimization of the long-term storage conditions

For a successful *in vitro* cultivation and long-term storage of nodal cuttings the starting material is important.

Best results were obtained when the explants were taken in the months of June and July. Cuttings from greenhouse-grown plants performed better than cuttings from the field (Table 1).

Prior to long-term storage the plants have to be subcultured twice after excision and establishment *in vitro*. Plants which were stored directly after sprouting of just excised cuttings were highly infected or died within a few weeks of storage (Table 2).

Table 1: Survival rate (%) of *in vitro* cultures after 12 months of long-term storage in dependence on excision time and origin of the plant material (n = 20)

excision time / origin	JUNE	JULY	AUGUST
FIELD	70	80	0
GREENHOUSE	90	100	50

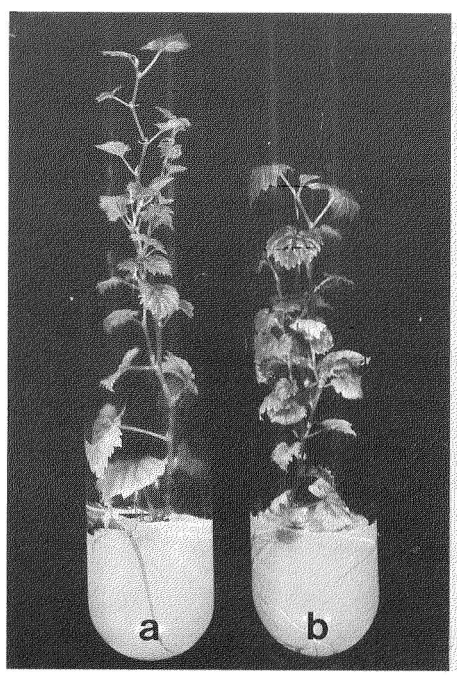
Table 2: Survival rate (%) of *in vitro* cultures after 3, 6 and 12 months of long-term storage in dependence on the number of subcultures (n = 20)

number of subcultures after excision	months of storage		
	3	6	10
0	100	100	55
1	100	95	37
2	100	90	80

Table 3: Survival rate (%) of *in vitro* cultures from cv. Riesling and the wild species *V. rupestris* after 9 months of long-term storage in dependence on light intensity (n = 16)

light intensity	10 $\mu E \cdot m^{-2} \cdot s^{-1}$	1 $\mu E \cdot m^{-2} \cdot s^{-1}$
genotype		
RIESLING	50	3
V.RUPESTRIS	88	81

Light conditions also seem to play an important role on survival rate. Within a few months of storage, the cultures stored under continuous light or in darkness died. Under short-day conditions and low light intensity ($10 \mu E \cdot m^{-2} \cdot s^{-1}$), most of the cultures survived a storage period of 15 months. A further reduction of the light intensity down to only $1 \mu E \cdot m^{-2} \cdot s^{-1}$ caused rapid losses of sensitive cultivars within a few weeks under RC (Table 3).



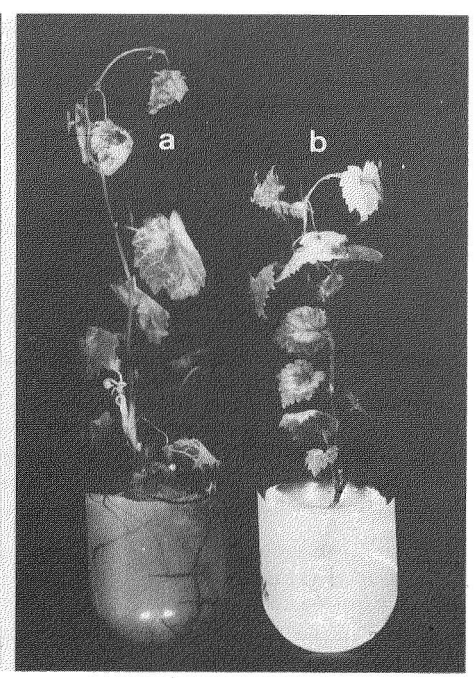


Fig. 1 (left): Improvement of sprouting and shoot development after subculturing onto fresh medium in dependence on CCC treatment during long-term storage. a) Subculture of a CCC-treated plant, b) subculture of an untreated plant.

Fig. 2 (right): Induction of senescence symptoms on *in vitro* plantlets by long-term storage under RC (a) and by increasing the CO_2 concentration under NC (b).

Comparable results had been achieved by BARLASS and SKENE (1983) and FAUSTINI (1984).

In a further attempt to improve the survival rate, CCC was applied to *in vitro* cultures. Under field conditions an increase in cold tolerance had been induced by treating grapevines with this growth inhibitor (BOURQUIN and ALLEWELDT 1970).

Table 4: Survival rate (%) of *in vitro* cultures of *V. riparia* in dependence on CCC treatment during different storage periods (n = 16)

treatment storage period (months)	untreated	CCC
3	100	100
6	71	100
12	36	64

Table 5: Comparison of the survival rates (%) from different genotypes of *Vitis* after a long-term storage of 12 months. Storage periods 1986/87 and 1987/88

genotype	storage period I 1986-1987	storage period II 1987-1988
V.RUPESTRIS	31	88
V.RIPARIA	0	63
SO 4	56	53
RIESLING	63	81
KERNER	81	100
FABERREBE	56	100
OPTIMA	56	100
ORION	6	100
VIDAL	50	81
mean	44	84

In vitro cultures of only a few cultivars showed a better survival rate in RC when treated with CCC (Table 4). Some cultivars could not be stored longer than when untreated. However, after long-term storage these CCC-treated plants exhibited earlier sprouting and faster shoot development after subculturing onto fresh medium (Fig. 1). This result has to be confirmed in subsequent examinations.

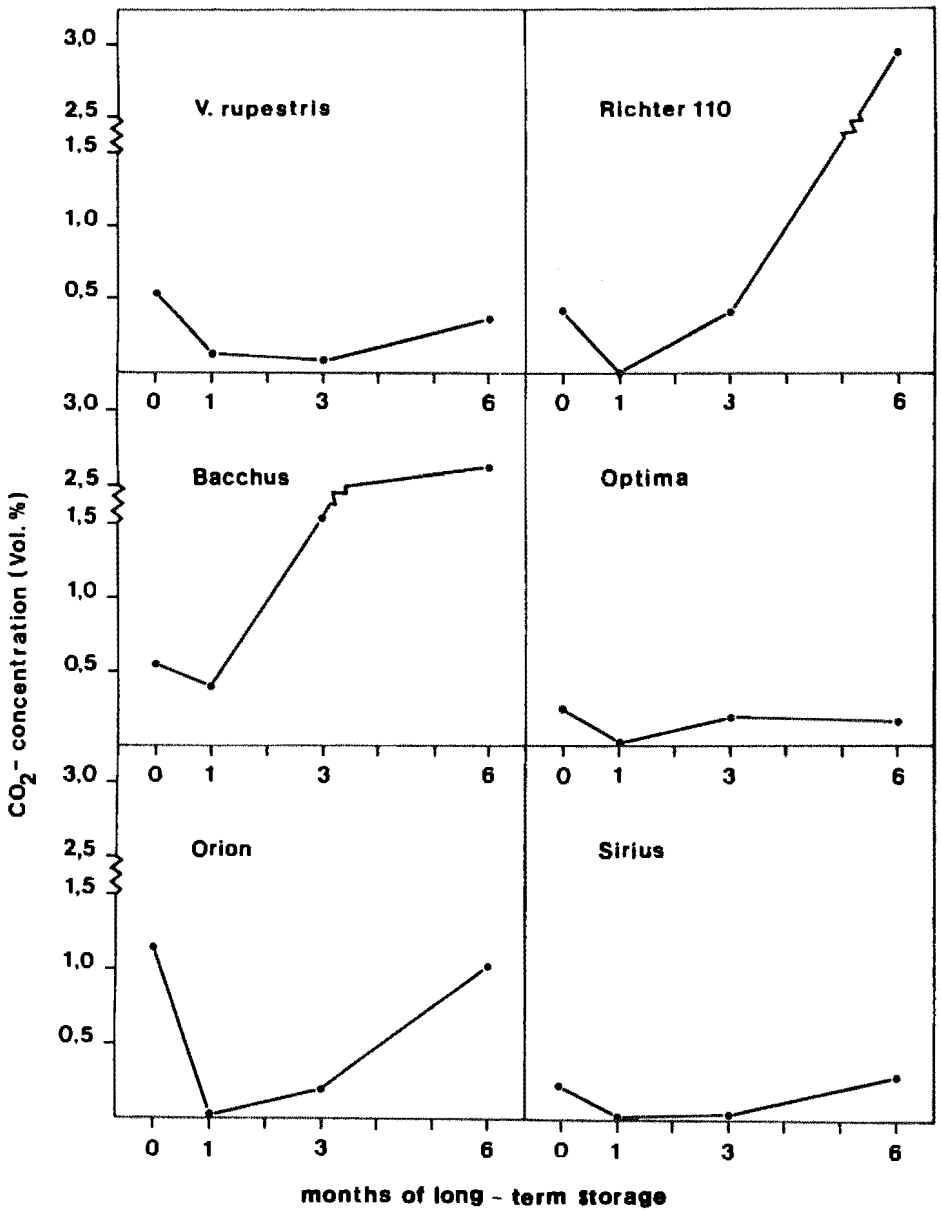


Fig. 3: CO_2 development during a storage period of 6 months in dependence on the genotype.

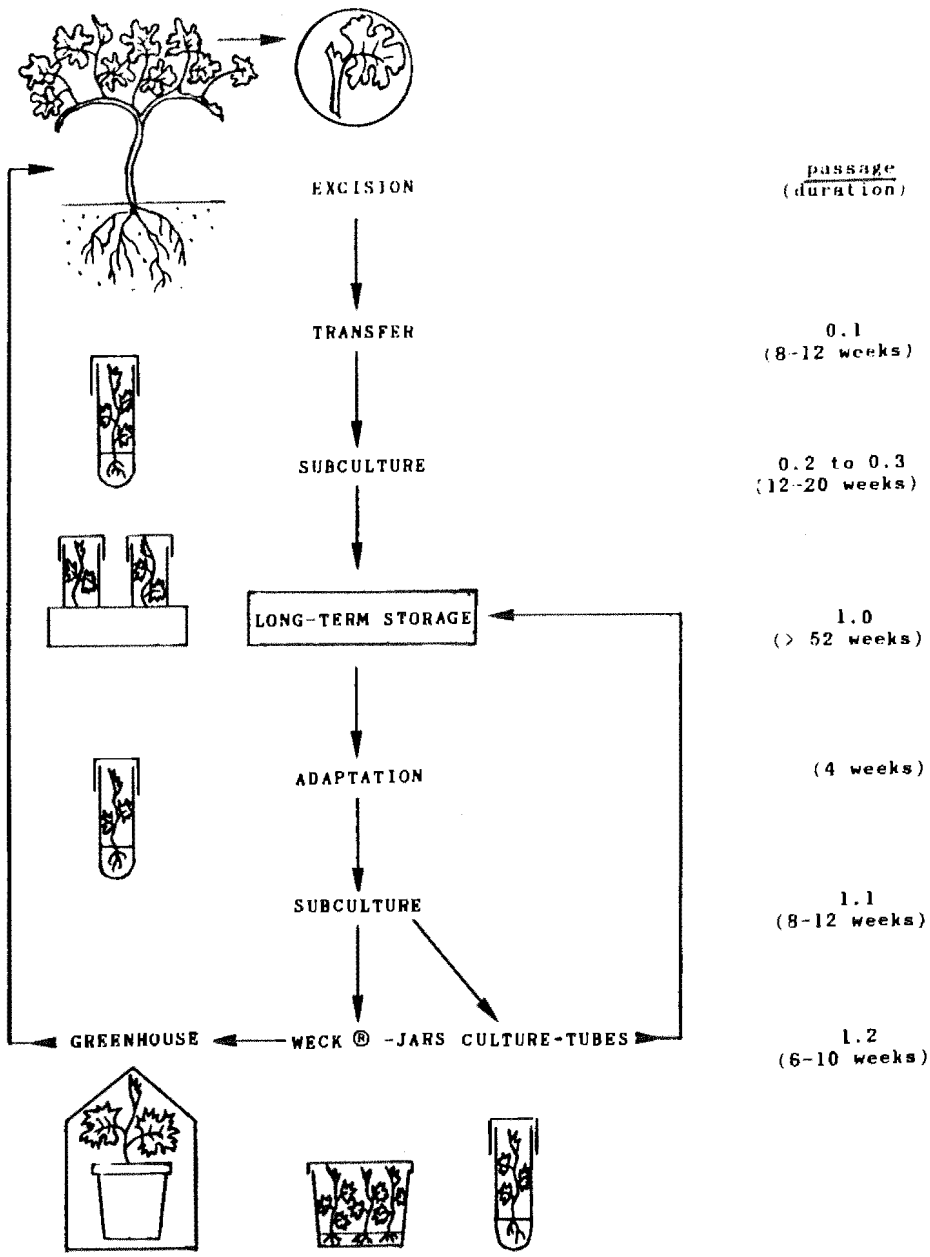


Fig. 4: Long-term storage under reduced culture conditions during maintainance and propagation of *Vitis* material by tissue culture techniques.

Causes of senescence

Besides these tests for optimizing the long-term storage conditions, the causes of early senescence exhibited by some genotypes were of great interest. Many authors have supposed that CO_2 becomes concentrated under *in vitro* conditions in the culture tubes (Donovan and Murashige 1979; De Proft *et al.* 1985; Fournioux and Bessis 1986; Zobel 1987).

In a special examination, senescence symptoms similar to those observed during long-term storage could be induced by artificial rising the CO_2 concentration up to 3 % (v/v) under NC (Fig. 2).

The development of CO_2 concentrations during a storage period of 6 months is demonstrated in Fig. 3. During the period under NC the CO_2 concentration increased to more than 0.5 % (v/v) of the environmental CO_2 content of the laboratory (start of storage). Within the first 4 weeks after transfer of the plantlets to RC, the CO_2 concentration declined rapidly (1 month of storage). During the following 5 months under RC the CO_2 concentration showed a characteristical rise dependent on genotype (6 months of storage).

Fig. 5: Resumption of growth vigour after 20 months of long-term storage when subcultured onto fresh medium. a) Plantlet after 20 months of long-term storage, b) subcultivation onto fresh medium, c) sprouting 7 d after subcultivation, d) shoot development 21 d after subcultivation.

In the culture tubes of cvs Richter 110, Bacchus and Orion, which showed a very marked response to RC, a high CO_2 concentration of almost 3 % (v/v) was determined.

To avoid high CO_2 concentration, the N_2 content in the atmosphere of the culture tubes could be risen to keep the CO_2 content on a non-toxic level. The long-term storage of ornamentals is carried out in this way (PREIL 1989, personal communication).

Due to these tests for optimizing the long-term storage conditions and the examination of the development of CO_2 concentration during the storage period, some general recommendations for carrying-out conservation of *in vitro* cultures of grapevine can be given (Fig. 4):

After excising the starting material from field- or greenhouse-grown grapes, the cultures have to be propagated twice under NC before they can be transferred to RC. If more than 50 % of each cultivar have died, the surviving cultures should be re-transferred into NC and adapted for about 4 weeks before subculturing onto fresh medium. After being subcultured twice, the plants can be stored again or, if necessary, adapted to soil.

By following this procedure the survival rate was almost doubled in a further storage period, as demonstrated on some cultivars (Table 5).

The stored material did not loose vitality even when the plants were subcultured onto fresh medium after a storage period of more than 20 months under RC (Fig. 5). After adaptation to soil and acclimatization to field, the long-term stored plant material is true to type (Fig. 6).

So far, some important factors influencing the survival rate under RC have been described. Other factors, like an adaptation to short-day conditions before and an adaptation to long photoperiod after the storage, have to be examined. A further perspective for optimization of the long-term storage can be the examination of the composition of the nutrient medium (full- or half-strength salt concentration, NH_4NO_3 concentration, reduction of sucrose concentration, activated charcoal, etc.).

For almost all of the stored genotypes a minimum storage period of 12 months was realized. There are no problems in subculturing and in the acclimatization to field conditions after long-term storage. By using differenciated explants like shoot-tips or nodal cultures, somaclonal variation can be excluded (D'AMATO 1978).

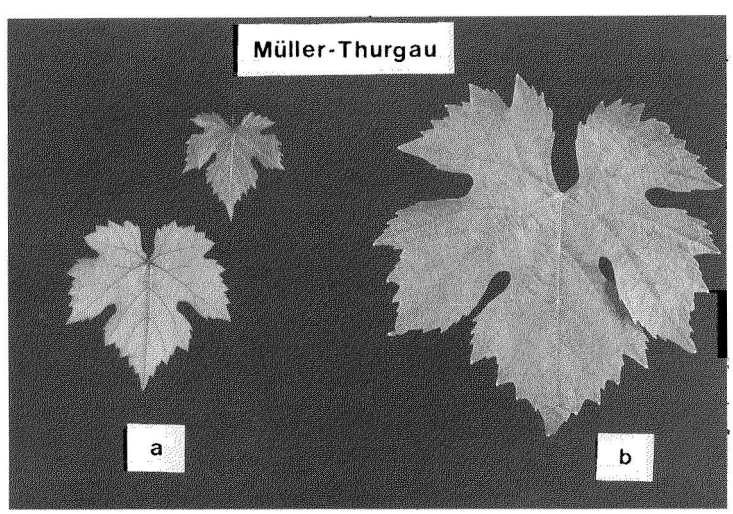


Fig. 6: Plants are true to type when adapted to field conditions. a) Leaves from an *in vitro* long-term stored plant after adaptation to field conditions, b) leaves from the same cultivar derived from the field collection.

At the present situation of plant cell and tissue culture research of the grapevine this conservation method of *in vitro* plantlets seems to be well-suited for a safe maintainance of healthy plant material. Thus, the establishment of a gene repository and the long-term storage of clonal material for breeding and/or propagation has been initiated.

Conclusion

A simple and safe maintainance procedure for rooted plantlets of *in vitro* cultures of grapevine has been established by improving the storage conditions (starting material, preculture, light conditions, CO_2 development). The period between two subcultivations could therefore be prolonged to 12-18 months. For those genotypes which show yet an unsatisfying survival rate, optimization of the long-term storage has to be continued.

References

ALLEWELDT, G.; HARST-LANGENBÜCHER, M.; 1987: Der Einfluß von Wachstumsinhibitoren auf die Langzeitlagerung von *in-vitro*-Kulturen der Rebe. Vitis **26**, 57-64.

BARLASS, M.; SKENE, K. G. M.; 1983: Long-term storage of grape *in vitro*. FAO/IBPGR Plant Genetic Resources Newsletter **53**, 19-21.

BOURQUIN, H. D.; ALLEWELDT, G.; 1970: Der Einfluß von CCC und B-995 auf das Triebwachstum der Reben. Vitis **9**, 105-120.

D'AMATO, F.; 1978: Chromosome number variation in cultured cells and regenerated plants. In: THORPE, T. A. (Ed.): Frontiers of Plant Tissue Culture, 287-295. International Association of Plant Tissue Culture, Calgary.

DONOVAN, S. T.; MURASHIGE, T.; 1979: Volatile emissions of plant tissue cultures. I. Identification of the major components. In Vitro **15**, 654-658.

FAUSTINI, R.; 1982: Metodi alternativi di conservazione del materiale genetico della vite: "colture *in vitro*". Vignevini **9** (9), 11-13.

FOURNIOUX, J. C.; BESSIS, R.; 1986: Influence de la teneur en gaz carbonique sur la morphogenèse de la vigne en culture *in vitro*. Can. J. Bot. **64**, 2608-2616.

LINSMAIER, E. M.; SKOOG, F.; 1965: Organic growth factor requirement of tobacco tissue culture. Physiol. Plant. **18**, 100-127.

PROFT, M. P. DE; MAENE, L. J.; DEBERGH, P. C.; 1985: Carbon dioxide and ethylene evolution in the culture atmosphere of *Magnolia* cultured *in vitro*. Physiol. Plant. **65**, 375-379.

ZOBEL, R. W.; 1987: Gaseous compounds of soybean tissue cultures: Carbon dioxide and ethylene evolution. Environ. Exp. Bot. **27**, 223-226.