Theor Appl Genet (1988) 75: 669–673

Review

Progress in grapevine breeding

G. Alleweldt [1] and J. V. Possingham [2]

[1] Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, D-6741 Siebeldingen, Federal Republic of Germany
[2] CSIRO Division of Horticultural Research, Adelaide, Australia

Received September 19, 1987; Accepted October 5, 1987
Communicated by H. F. Linskens

Table of contents

1 Summary. The European, or bunch grape, *Vitis vinifera,* is widely grown because of its high fruit quality and its capacity to grow in a wide range of climatic conditions. However, they are susceptible to fungal diseases and insect pests, especially when grown in cool, wet climates. The aim of a number of grapevine breeding programs throughout the world is to develop new varieties resistant to diseases using complex hybrids between European and American species of *Vitis.* Within these breeding programs it is essential to maintain heterozygosity and desirable hybrids are multiplied by asexual propagation. New approaches to grapevine improvement include the use of protoplast fusion to overcome sexual barriers, however the routine regeneration of plantlets from protoplasts and calluses is difficult. In vitro rescue of ovules from varieties with stenospermocarpic seeds shows considerable promise for breeding new seedless grapes. Eventually the use of plant transformation techniques to insert specific pieces of DNA coding for desirable genetic characteristics will provide opportunities for equipping well known grape cultivars with new characteristics.

Key words: *Vitis vinifera* – Fungal diseases – Asexual propagation – Stenospermocarpic

2 Introduction

Vineyards occupy more than 10 million hectares throughout the world. They are mainly located in regions with mild to hot summers and with cool, wet winters. Grapevines are able to grow in a wide range of soils and climates and have the capacity to produce high yields of fruit. They are productive in areas where many other crop plants fail.

During the last 30 years they have been successfully established in a number of tropical countries such as Thailand, India and Venezuela. It is probable that grapevines would be more extensively grown in the tropics if cultivars could be developed that were resistant to fungal diseases and adaptable to high temperatures.

The grapevine is a climbing perennial with coiled tendrils and a pronounced acrotonic pattern of shoot growth. Under cultivation it is normally given support and is pruned to a manageable form. Pruning is also used to regulate fruitfulness so that the crop is concentrated into a reduced number of moderate sized bunches. Although most cultivars possess hermaphrodite flowers, which are highly self-fertile, a few varieties have female flowers that require pollination by varieties with fertile pollen.

Grapevines were propagated from cuttings until the 1860's when a soilborn aphid, phylloxera, was introduced from North America to Europe. This insect attacks the roots of all *vinifera* vines and causes death within a year or two. Phylloxera-tolerant or resistant rootstocks are currently used in Europe as grapevine rootstocks, to which *vinifera* scions are grafted. The breeding of resistant rootstocks and of so-called "direct producers" (i.e., the grapes of resistant rootstocks are used for wine) represents the beginning of grape

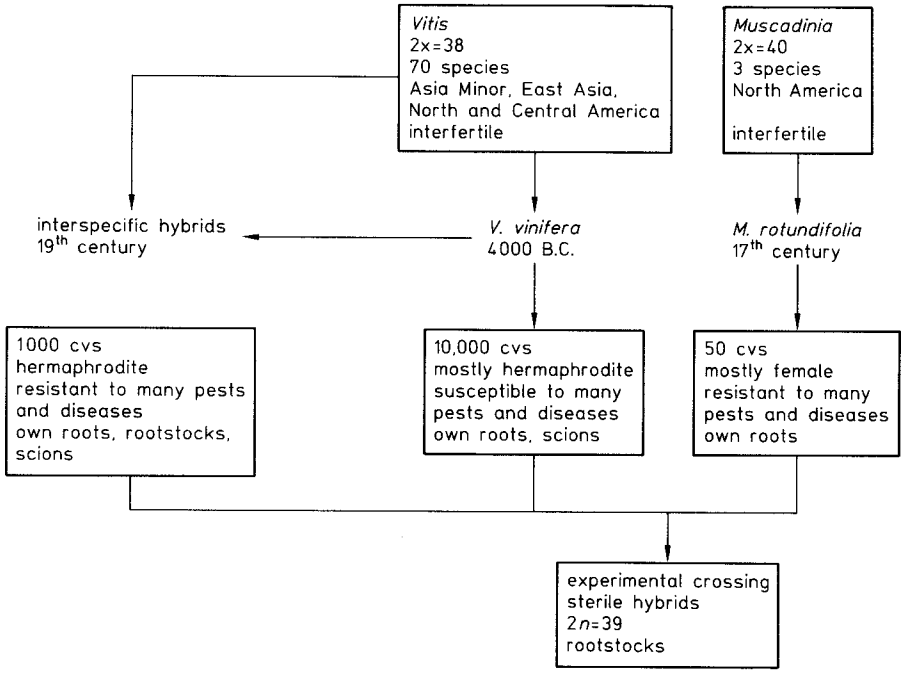

Fig. 1. The present stage of grape breeding

breeding in Europe, which occurred in the latter half of the 19th Century.

3 Grapevine germplasm

All commercial grapes are from the family *Vitaceae* and from the genus *Vitis.* The genus *Vitis* is now subdivided into two subgenera: *Euvitis* with 38 chromosomes, and *Muscadinia* with 40 chromosomes. Since the three known species of *Muscadinia* do not hybridize naturally with those of *Vitis,* they have been recognized by Olmo (1986) as a separate genus. The successful hybridization of *Muscadinia rotundifolia* with a range of *Vitis* species has made this genus a useful germplasm for improving the disease resistance of *Vitis* species and cultivars. Alternatively the fruit quality of domesticated *M. rotundifolia* vines has been improved by crossing them with *vinifera* cultivars. Thus, both *Vitis* and *Muscadinia* may be regarded as the natural genetic resources of grapevine breeding (Fig. 1).

The genus *Vitis* contains more than 70 species that can be divided into distinct geographical groups. In the terminology of *Vavilov* (1928) the various *Vitis* species belong to the three gene centers, which are located in South Europe and Asia Minor, in East Asia, and in North and Central America. Our botanical knowledge of the different species is still incomplete. The most renowned species is *Vitis vinifera,* domesticated some 5,000 years ago somewhere in Asia Minor or Armenia, where it spread to other countries throughout the world. The high morphological and genetic diversity of *vinifera* and the ease with which it is asexually propagated gave rise to an estimated number of more than 10,000 cultivars. Different cultivars are cultivated to produce either fresh fruit, fruit juice, dry raisins or wines. In Islamic countries, concentrated grape juice is a major sugar source of the people and is used to sweeten food, pastries and pies.

As *vinifera* grapes were introduced into regions beyond their natural habitat they were often hybridized with native *Vitis* species, for instance in North America, Central America and Japan. This resulted in hybrids better adapted to local environments, and enabled viticulture where *vinifera* vines could not survive due to their susceptibility to fungus diseases or severe winter frosts.

4 Vine improvement techniques

4.1 Clonal selection

The European, or bunch grape, *V. vinifera,* is characterized by a broad morphological and physiological variability and has attractive fruits of excellent quality. Negrul (1938) separated existing cultivars into three broad groups. First, the orientalis group with large berries, large bunches (clusters) and a high temperature requirement. These are utilized as table grapes. Second, the occidentalis group with small berries but with many per shoot. These are winterhardy and well adapted to

mild summers. The fruit of this group give the most famous wines. The third group, pontica, is intermediate between group 1 and 2 and are used either as table grapes or wine grapes.

The grapevine is highly heterozygous and propagation by cuttings or layers maintains their heterozygosity, and thus the intensity of heterosis. Clonal selection has lead to homogenous cultivars of well determined yield and fruit quality. The genetic stability of grapevines is high as some of the best known varieties have existed for many centuries. However, gene mutation does occur naturally over time and contributes to the clone yield of established cultivars.

Virtually all *vinifera* grapes are susceptable to the effects of virus diseases, which reduce both yield and fruit quality. The production of virus-free plant material has become one of the most important features of modern viticulture. The introduction of methods to eliminate the virus either by thermotherapy alone or in combination with meristem tip cultures has markedly increased yields (Barlass et al. 1982). Clonal selection, especially when carried out in combination with virus elimination, can bring about significant increases in the average yields of old cultivars of grape vines.

Breeders have attempted increased genetic variability of grapevines by the application of chemical mutagens or by irradiation, but so far these approaches have not produced improved clones or cultivars. This is partially because where mutations have been induced genetic changes occur only in specific cell layers or chimeras, and the separation of these from the rest of the tissue is difficult.

Many tetraploids have arisen spontaneously or have been induced by colchicine, but only a few are of commercial value (Gargiulo 1957, 1960). Most autotetraploids are periclinal chimeras and have large berries and loose clusters. They have attracted the attention of table grape breeders, but in general are less fruitful than the diploids from which they have been derived. Nevertheless, a more intense breeding program including the generation of both auto- and allotetraploid vines may be worthwhile.

4.2 Cross-breeding

Systematic grapevine breeding was first initiated by French scientists, nurserymen and winegrowers in the last half of the 19th century when phylloxera and fungal diseases began to decimate the vineyards of France and later those of all grape growing European countries. The fungal diseases were *Uncinula necator* (1845) and *Plasmopara viticola* (1878), which attack the leaves and fruits of *vinifera* grapes while the aphid, *Phylloxera vastatrix* (1860), attacks the root system and kills the vines.

Table 1. The main germplasm for resistance utilized in breeding programs in Europe

Abiotic stress factors	
Winter hardiness	*V. amurensis, V. riparia*
Chlorosis	*V. vinifera, V. berlandieri*
Fungus diseases	
Plasmopara viticola	*V. riparia, V. rupestris, V. lincecumii, V. aestivalis, V. cinerea, V. berlandieri, V. labrusca*
Uncinula necator	*V. aestivalis, V. cinerea, V. berlandieri, V. labrusca*
Botrytis cinerea	American species, *V. vinifera*
Pseudopeziza tracheiphila	*V. vinifera*
Bacteria	
Agrobacterium tumefaciens	*V. amurensis, V. labrusca*
Nematodes	
Meloidogyne sp.	*V. champini*
Xiphinema sp.	*V. rufotomentosa*
Insects	
Phylloxera vastatrix	*V. riparia, V. rupestris, V. berlandieri, V. cinerea, V. champini*

As it was not possible to control phylloxera with chemicals in the last century and new strategies had to be developed to protect the vines against American fungus diseases, governmental programs were initiated in France to import germplasm from America and to initiate breeding programs to combine fungus- and phylloxera resistance with the high wine quality of *V. vinifera.* Table 1 represents the main genetic resources used by European breeders.

These efforts proved to be partially successful as dying vineyards were gradually replanted with hybrids of French and American *Vitis* species and with *vinifera* cultivars grafted on to phylloxera-tolerant rootstocks. Today, the original own-rooted *vinifera* vineyards have largely been replaced by grafted vines with traditional varieties as scions and phylloxera-tolerant or -resistant vines as rootstocks. The old interspecific hybrids between American *Vitis* species and *vinifera* grape have mainly been removed and replaced by grafted vines. However, a few hybrids of American and French origin remain scattered throughout Europe.

The general condemnation of interspecific hybrids was due to undesirable flavor compounds introduced from American *Vitis* species. These compounds are polygenically inherited. Therefore, both F_1 hybrids or F_1 backcrosses are carriers of genes which give rise to unpalatable wines. Breeding programs to improve

Table 2. Yield and must quality of some fungus resistant cultivars of the Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof compared with Riesling ($\bar{x}$ 1977–1986); $LSD_{5\%}$ 42 kg/ar, 10°Oe, 1.8‰ acid

Variety	Yield kg/ar	Sugar content °Oe	Acid content g/l
Phoenix	178	71	10.2
Sirius	190	65	9.1
Silva	153	72	10.3
Orion	168	77	9.3
Riesling	111	67	15.7

interspecific hybrids were stopped or reduced in most countries soon after the turn of the century.

Subsequently the improvement of the *vinifera* grape by intraspecific hybridization became successful. The Müller-Thurgau variety in Europe, the table grape variety "Cardinal" from California and new wine varieties from Argentina and Australia are some examples. Currently the catalogue of new *vinifera* cultivars is progressively increasing in a number of countries including Hungary, Germany, France, Australia, Argentina and South Africa (Antcliff 1978, Csizmazia 1978).

As far as wine grapes are concerned, the controversy about the quality and usefulness of new *vinifera* cultivars tends to be emotional and not based on objective criteria. enthusiastic and often polemic discussions are explained by traditionalism versus progressiveness. The introduction and acceptance of new *vinifera* cultivars by grape growers is dependent on developing varieties with increased yield and with perceptable and acceptable flavor.

In spite of the discouraging results from developing disease resistant cultivars by utilizing the genetic resources of *Vitis* native to America, breeding has continued in some countries such as Germany, or has been recently initiated in other countries such as those in Eastern Europe. Economic considerations and ecological criteria make reductions in the use of toxic chemicals inevitable. Indeed, continuous breeding has resulted in new cultivars resistant to the most important fungus diseases in Middle Europe, i.e., powdery and downy mildew, and of a wine quality indistinguishable from the *vinifera* wines; acceptable new varieties are essentially free of the undesired flavor components of American *Vitis* species. Table 2 represents new cultivars from Germany that are being officially tested by German authorities within the regulations of the European Community. The genetic improvement of grapes is characterized by the following features:

1) Grapes are generally improved by outcrossing and the main cultivars are highly heterozygous.

2) Inbreeding leads to growth depressions as some old varieties appear to be carriers of deleterious recessive genes. The inbreeding effect varies between cultivars.

3) The maintenance of heterozygosity is achieved by crossing the best representatives of unrelated lines. In order to attain heterosis diallel crossings are carried out to determine the specific combining ability of different cultivars and breeding lines. New varieties are then stabilized by asexual propagation.

4) The efficiency of grape breeding depends on the development of suitable screening methods for fruit quality, yield, disease resistance, winter hardiness, tolerance to chlorosis or salinity and a range of other characteristics.

5 Reflections and future

Grape breeding is time consuming, laborious and expensive. The potential of producing grapes with increased flavor, yield, nutritional value and for the production of wines with distinctive flavors are good reasons for continuing grape breeding programs. As cultivar improvement is increasingly directed toward disease and insect resistance, the classical means of breeding has to be supported by new techniques.

A most promising technique at the moment is the in vitro-culture of grapevines (Barlass and Skene 1978). The meristematic tips of most grapevines regenerate readily, thus enhancing the rate of propagation, which in turn increases screening possibilities and shortens the duration of test procedures. To date, no evidence of somaclonal variation has been reported in grapevines. In vitro methods can be used for the production of own-rooted vines, but experiments are progressing toward micrografting of in vitro plantlets to test new scion/rootstock combinations.

A second use of in vitro techniques for grapes is the regeneration from isolated protoplasts and from undifferentiated calluses. The introduction of current genetic engineering strategies for the selection of stress-tolerant clones from cell populations depends on having a successful technique for the production of plants from single cells. Although some encouraging studies, resulting in somatic embryos from parenchyma cells or from callus have been presented (Krul and Mowbray 1984), the technology has not been consistently successful with *vinifera* grapes, and further research is necessary. The remaining barriers do not appear to be unsurmountable, and the prospect of combining the existing germplasm of *Vitis* and *Muscadinia* is exciting. It is possible that the problem of somaclonal variation may arise when reliable techniques for regenerating grapevines from single cells becomes available.

A third, already successful, field is the culture of pollinated embryos of cultivars with a stenospermocarpic seed. A number of cultivars used for raisin or table grape production undergo an incomplete seed development in as much as seed abortion occurs soon after fertilization. Their improvement by crossbreeding is thus limited. Spiegel-Roy et al. (1985) succeeded in culturing ovules from seedless varieties after hybridization opened a further prospective field of grapevine breeding.

Currently, plant transformation techniques are being applied to grapevines using *Agrobacterium tumefaciens* as the vector for DNA movement and fragmented meristems as tissue capable of being both transformed and regenerated into plantlets. Experiments to transform grapevines are in progress in Davis (Meredith), Sydney (Mullins) and Adelaide (Skene and Scott). These have as their aim the improvement of well established cultivars by the insertion of specific genes selected from grape vines, other plants and other biological systems such as bacteria.

With time it is probable that solutions of the above approaches will be found to the benefit of grape breeding. Tissue culture of grape material combined with intensifying efforts to identify in detail the genetics of resistance to winter hardiness, drought tolerance to fungal diseases and to insect infestations are of high priority.

6 References

Antcliff AJ (1978) Breeding grapes for hot climates. 2nd Int Symp Grapevine Breed, Bordeaux, pp 341–344

Barlass M, Skene KGM (1978) In vitro propagation of grapevine (*V. vinifera*) from fragmented shoot apices. Vitis 17:335–340

Barlass M, Skene KGM, Woodham RC, Krake LR (1982) Regeneration of virus-free grapevines using in vitro apical culture. Ann Appl Biol 101:291–295

Csizmazia J (1978) Sélection pour la résistance au mildiou: résultats obtenus en Hongrie. 2nd Int Symp Grapevine Breed, Bordeaux, pp 235–241

Gargiulo A (1957) Spontaneous tetraploid mutation Barbera d'Asti. Vitis 1:156–158

Gargiulo A (1960) Artificial induction of polyploidy in *V. vinifera* with colchicine. Vitis 2:181–189

Krul WR, Mowbray GH (1984) Grapes. In: Sharp WR, Evans DA, Ammirato PV, Yamady Y (eds) Handbook of plant cell culture, vol 2. Crop species. MacMillan, New York, pp 396–434

Negrul AM (1938) Evolution of cultivated forms of grapes. CR Acad USSR 18:585–588

Olmo HP (1986) The potential role of (vinifera × rotundifolia) hybrids in grape variety improvement. Experientia 42: 921–926

Spiegel-Roy P, Sahar N, Baron J, Lavi U (1985) In vitro culture and plant formation from grape cultivars with abortive ovules and seeds. J Am Soc Hortic Sci 110: 109–112

Vavilov NI (1926) Studies on the origin of cultivated plants. Bull Appl Bot Gen Plant Breed 16:1–248