Evaluation of interspecific populations of grapevine in breeding for complex resistance to fungal diseases and phylloxera

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S u m m a r y : Roentgenoscopy was used as a method to determine the quality of hybrid seeds and to predict the development of viable plants from interspecific hybridization. The seeds were grouped into five classes of quality (embryo classes) depending on embryo size and degree of endosperm development. As the index number of a class increased, the proportion of plantlets and vigorous plants produced also increased.

In order to evaluate genotypic peculiarities of the original forms and seedlings, the seedlings were studied at the juvenile stage of ontogeny.

Analysis of development of the hybrids studied during 5-6 years under conditions of complex infection pressure at a special planting site made it possible to evaluate the degree of their resistance to phylloxera, pathogenic soil microflora and fungal diseases and to eliminate susceptible genotypes.

The heritability of resistance to fungal diseases (mildew, oidium, grey rot) and phylloxera was studied, conclusions were made concerning the combining ability of the original forms, and these forms were evaluated as donors of the desirable characters.

Using transgressive resistant hybrids as donors in backcrossing provided improved quality with a broad range of resistance variability, which made it possible to select promising genotypes.

K e y w o r d s : resistance, plasmopara, oidium, botrytis, phylloxera, hybrid, Muscadinia, seed, roentgenoscopy, screening, genetics, breeding, USSR.

Introduction

Among all worldwide cultivated fruit-bearing plants, apple trees are the most widely spread (24 mill. ha); they are followed in the decreasing order of acreages by species such as pear (7 mill. ha), peach (6 mill. ha), and plum (5 mill. ha). The spread of grapes is rather high, it amounts to about 10 mill. ha. This distribution is not occasional. Grapes have universal therapeutic properties; fresh grape as well as juice, wine, vine leaves, seeds and grape marc are of great importance (Kiskin 1984).

Generally, the demands of USSR in fresh grape are ill-satisfied. During the 10th and 11th five-year plan periods, the annual consumption of this valuable product was only 0.5 kg per capita, while according to the data of the Institute of Nutrition of the USSR Academy of Sciences the average annual physiological rate of fresh grape consumption should be 10 kg per capita. In perspective, about 13.5 kg of fresh grape per capita will be required annually to organize the rational nutrition of the country population (Railianu 1985).

As a result of the extensive selection researches, a wide range of high-quality grape varieties which satisfy consumers' demands has been obtained; but the fact that the overwhelming majority of these varieties is susceptible to frosts, phylloxera, mildew, oidium and grey rot results in significant complication of cultivation techniques due to the use of grafted cultures and numerous protective treatments with toxic chemicals and the restriction of cultivation to the southern regions of the country.

The prospects of vinegrowing should correspond to the ecological demands. This is conditioned by the present and predicted pollution of environment with chemicals used for plant protection, as well as to the increasing economic demands under conditions of increasing primary production costs and the transition to self-repayment and self-financing.

The breeding of high-quality productive grape varieties with complex resistance to pests and diseases and their wide industrial introduction will play the major role in the future intensification

of this branch of economy, and the accelerated breeding of these varieties will evidently be carried out by selecting promising combinations of crosses (specific combining ability) as well as by decreasing the duration of the selection process.

Analysis of data presented in the literature and our own experimental results show that the resistance to phylloxera, mildew and grey rot and good quality of yield are independently inherited and this fact makes it possible to choose promising genotypes using selection to resistance (GOLODRIGA *et al.* 1979).

A considerable progress in the field of breeding for resistance was due to the use of the donors of resistance in the hybridization process obtained as a result of over 100 years of breeding researches which were conducted by French originators (SEYVE VILLARD, SEIBEL, RAVAT, OBERLIN, JOANNES SEYVE etc.).

Several species of plants in Vitaceae family have been distinguished as having resistance to pests and diseases. *Vitis rotundifolia* MICHX., a resistant species, and its DRX hybrids (DUNSTAN 1962) are of particular interest in this respect. Some difficulties arising in hybridization were due to differences in the chromosome number in *V. vinifera* L. belonging to *Euvitis* with 2n = 38 and in *V. rotundifolia* belonging to *Muscadinia* with 2n = 40. For a long time, the breeders could not obtain fertile plants using such crosses. The first fertile hybrid between *Euvitis* and *V. rotundifolia* was obtained in the USA (OLMO 1954, 1971; PATEL and OLMO 1955). Later DRX hybrids were obtained from such crosses (DUNSTAN 1962) and introduced into France (BOUQUET 1980). In 1974, some hybrid seeds were kindly supplied to us by the French breeders.

Materials and methods

The objects under investigation in determining the quality of grape seeds, the new original material in breeding for resistance, were 18 grape seed samples obtained by interspecific hybridization of *V. vinifera* (diploids and polyploids) with pure *V. rotundifolia* and its DRX hybrids (these hybrids were encoded as 'Magarach N 100'), as well as seeds obtained by the random pollination of these hybrids. Generally, 3257 seeds were investigated with 1434 of them having full weight (those which sunk). All seeds obtained both as a result of crossings and random pollinations were included into study. At first, the seeds were dipped into water. Some of them sank. Examination of the floating seeds showed them to be hollow, i. e. without embryo and endosperm. The number of the full weight seeds was determined by counting (in % of the total number). Subsequently only sunk seeds were studied. Their quality was determined by roentgenoscopic method (NEKRASOV and SMIRNOVA 1961; SMIRNOVA 1978) using a Svetlana X-ray emitter [1]). Seeds under investigation were placed on a frame having a sticky film bottom; this frame was put under the X-ray tube of the emitter. The roentgenogram was obtained under defined conditions. The quality of seeds on roentgenograms was determined by the degree of endosperm and embryo development taking into account the intensity of their fixation on the film and classifying them into categories of seeds and classes of embryo development. The content of seeds of all classes of development (in %) was determined for each sample studied.

At the stage of plantlets, 2308 seedlings and 15 combinations with the single pollinator (cv. Podarok Magaracha) were investigated. The variability was estimated according to the following characters: germination capacity, size of cotyledons, presence of deformed cotyledons, differences in the number of cotyledons, teratologic changes, and seedling survival rate.

The juvenile gene pool screening, i. e. the mass screening of hybrids at the juvenile stages of ontogeny using simple and rapid methods for determining the morphological-phenological variability was used for the early selection of the objects studied (KLIMENKO 1986).

[1]) Investigations were carried out in cooperation with T. MUKHORTOVA and V. NOVIKOVA in Nikitsky Botanical Garden.

Resistance to phylloxera and pathogenic microflora was estimated using the method developed by P. N. NEDOV and A. P. GULER (1971); resistance to mildew was rated using the 5-point scale according to the method approved by the Section of Viticulture of the National Academy of Agricultural Sciences (Methodical Instructions Concerning the Grape Breeding, Erevan, 'Aistan', 1974).

Susceptibility to oidium was estimated using the method proposed by I. N. NAIDIONOVA (1985) at an infected planting site established in the greenhouse. Hybrid seeds and two-bud cuttings were placed in hydroponic channels. Controls were positioned along the axis of the channels. Samples were inoculated with conidia suspension (35-30 conidia/0.001 ml of suspension). Suspension rate was 25 ml/m^2 of the channel area. Inoculations were repeated every 2 weeks.

The following grape varieties investigated by a number of authors were used as controls: highly resistant varieties: SV 12-375 (BOUBALS 1961), Kishmysh Vatkana (Methodical Instructions Concerning the Grape Breeding, 1974; NAIDIONOVA 1985), Gangal kara (SOHI and SRIDHAR 1972; SHTIN *et al.* 1986); resistant varieties: Seibel 15062 (BOUBALS 1961), Kishmysh rozovy (NAIDIONOVA 1985); susceptible varieties: Ravat 6 (BOUBALS 1961), Riesling Italico (NAIDIONOVA 1985).

In order to reveal the genetically dependent grey rot resistance, a laboratory method of estimation was used (GOLODRIGA *et al.* 1979).

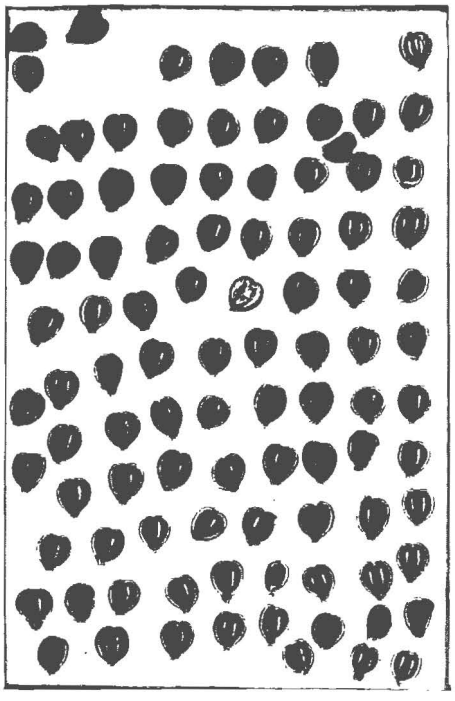

Fig. 1: Roentgenogram of the hybrid grape seeds.

Results and discussion

Potential advantages of the vast hybrid collections of the Breeding and Ampelography Department of the National Institute for Grape and Products of Grape Processing 'Magarach' in ontogeny and pathogenesis were investigated in order to develop scientific bases and concepts for complex resistance.

Promising hybrids destined for the use as original forms in the process of breeding for resistance were selected among the seedlings of heterogenous populations (Golodriga and Kireyeva 1981; Golodriga *et al.* 1986). Seedlings were selected among the seed progeny of DRX x *V. vinifera* hybrids; they were characterized by high fertility and productivity and combined the great vigor and the disease resistance of muscadine varieties with berry characteristics of *V. vinifera*. So Magarach N 100-74-1-5 seedling (DRX N 60-24 x *V. vinifera*) meets the requirements of the standard European table grape varieties – it has berries and clusters of large size and gives a high yield.

The aims of the research work conducted by the breeders of the 'Magarach' Institute include the mobilization of different forms of *V. rotundifolia* and its hybrids in order to carry out hybridization with *V. vinifera* varieties and hybrids, and the investigation of the probability of introgression (when the genetic material of some species gradually penetrates into another one through incomplete interspecific isolation barrier) of *V. rotundifolia* resistance in order to form new sources of selection material. Seedlings of heterogenous populations obtained using DRX N 58-5 and DRX N 60-24 were included into the program of breeding for resistance. The degree of resistance of the most promising seedlings among those mentioned above was estimated in the complex infection planting site and these seedlings were included into the breeding program as original forms. However, as a result of genetic abnormalities in the process of interspecific hybridization non-viable seeds which absolutely do not differ in their appearance from the viable ones are frequently formed. The roentgenoscopic method allows preliminary estimation and selection of viable seeds. This method was used for the first time to determinate the quality of grape seeds. To make comparisons between the combinations, the average class of the seed development (K_{av}) was calculated by determination of the arithmetical mean of the weighted values using the expression:

$$K_{av} = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{100}$$

where n_1, n_2, n_3, n_4 and n_5 are the numbers of the seeds of the corresponding class as % of the total number of seeds in the sample ($n_1 + n_2 + n_3 + n_4 + n_5 = 100$).

Thereafter, seeds were germinated in the laboratory at 26-30 °C. Germination in the laboratory was determined by the number of germinated seeds (in %) compared to the total number of the seeds sown. Germinated and ungerminated seeds were sown in the hydroponic greenhouses with the aim of obtaining seedlings and investigating their biological characteristics.

The roentgenograms (Fig. 1) clearly show endosperms of different degrees of development and comparatively small embryos also of different sizes and degrees of development. Hybrid seeds obtained by crosses and the random pollination were classified into 3 categories: 1. Filled seeds which have a well developed endosperm, 2. empty seeds which have no endosperm and embryo, and 3. deficient seeds which are partly filled with an endosperm and have an embryo which is less than $^1/_8$ in volume compared to the developed endosperm of the seed; and into 5 classes of embryo development: I. Empty seeds which have no endosperm and embryo; II. seeds with embryos which are less than $^1/_8$ in length compared to the developed endosperm of the seed; III. seeds with embryos which are $^1/_8$ in length of the seed endosperm, clearly seen and not closely adjacent to the endosperm; IV. seeds with embryos which are more than $^1/_8$ in length compared to the seed

Table 1: Distribution of hybrid grape seeds from different cross combinations to embryo classes

Material studied	Total number of full weight seeds	Embryo classes, %					
		I	II	III	IV	V	K_{av}
Cross combinations							
Muscat VIRa x Mag. N 100-74-1-5	266	0	19	39	33	9	3.3
Polyvitis x Mag. N 100-79-34	32	0	36	48	8	8	3.3
Mag. N 100-74-1-5 x Podarok Magaracha	59	0	0	7	20	73	4.5
Mag. N 100-74-3-1 x Tsvetochny	28	0	14	18	18	50	4.0
Mag. N 100-74-2-1 x Tsvetochny	146	0	19	38	41	7	3.4
Mag. N 37- 77-44 x Mag. N 100-79-52	38	0	8	40	26	26	3.7
Mag. N 44-77-18 x Mag. N 100-74-1-5	109	1	8	35	41	15	3.6
Mag. N 100-74-1-5 x Tsvetochny	43	0	0	18	50	32	4.1
SV N 52-76 x Mag. N 100-79-58	23	0	0	17	35	48	4.3
Mag. N 40-69-11 x Mag. N 100-74-3-1	63	0	0	0	0	100	5.0
Mag. N 38-77-5 x Mag. N 100-79-52	101	0	11	39	27	23	3.6
Mag. N 10-69-17 x Mag. N 100-79-15	36	0	14	14	14	58	4.2
Random pollination							
Mag. N 100-74-1-5 (DRX 60-24 x Vv)	101	0	5	26	47	22	3.4
Mag. N 100-79-17 (Polyvitis x Mag. N 100-74-1-5)	81	0	4	5	54	37	4.3
Mag. N 100-74-3-3 (DRX x 58-5 x Vv)	100	0	3	12	50	35	4.2
Mag. N 100-74-3-1 (DRX 60-24 x Vv)	49	0	0	8	16	76	4.7
Mag. N 100-74-2-4 (DRX 60-24 x Vv)	37	0	3	22	35	40	4.6
Mag. N 100-79-52 (Polyvitis x Mag. N 100-74-1-5)	15	0	0	27	47	26	4.0

endosperm, they are positioned much closer to the endosperm and comparatively clearly seen; V. seeds with embryos which are 1/4 or more in length compared to the seed endosperm, white in colour, positioned close to the seed endosperm in the most cases, not clearly seen on roentgenograms and often leading to confusion when seeds are classified into classes; the section of such seeds shows a developed embryo close to the endosperm.

Among the full-weight seeds studied, the empty seeds are almost absent, generally there are developed and incompletely developed seeds. The number of developed and incompletely developed seeds varies depending on the cross combination from 35 % to 100 % and from 0 % to 65 %, respectively; in the random pollination, the number of developed and incompletely developed seeds varies from 69 % to 92 % and from 8 % to 31 %, respectively.

When hybrid grape seeds obtained from various combinations of crosses and from the random pollinations were classified according to the embryo classes, 5 embryo classes were established (Table 1). Embryo class I seeds were practically absent in all cross combinations and in the random pollination. However, the number of seeds of embryo classes II and III varies greatly depending on cross combinations in the limits of 8-36 % and 7-48 %, respectively; in the random pollination, this variation is in the limits of 3-5 % and 5-27 %, respectively. The number of seeds of embryo classes IV and V also varies: in crosses from 8 % to 50 % and from 7 % to 100 %, and in the random pollination from 16 % to 54 % and from 22 % to 76 %, respectively. All seeds belonging to embryo class V were obtained only in one combination of Magarach N 40-69-11 x Magarach N 100-74-3-1. The value of the average embryo class is also different and varies in the random pollination and in crosses from 3.4 to 4.7 and from 3.3 to 5.0, respectively.

A wide-range variation of the embryo classes indices suggests the presence of genetic abnormalities in the distant incompatible crosses. Thus, using new sources for interspecific crosses and for obtaining resistant varieties, we should carry out a careful cytogenetic investigation and determine the biological mechanism of the formation of the fully developed seeds.

Estimation of the germinated hybrid grape seeds classified according to embryo classes in the laboratory showed that the number of germinated seeds varied according to embryo classes and combinations of crosses.

In the random pollination, the number of germinated seeds of embryo classes II, III, IV and V was 0-3, 7-25, 14-33 and 44-86 %, respectively. In crosses, the number of germinated seeds of embryo classes II, III, IV and V was 6-18, 12-25, 20-34 and 32-100 %, respectively. In the random pollination and in different cross combinations, the total number of plantlets obtained was 59-89 % and 16-78 %, respectively. In the first case, the variability range was 30, and 62 in the second case. A single combination, Magarach N 40-69-11 x Magarach N 100-74-3-1, showed 100 % of germinated seeds. However, occasional germinations of embryo class I seeds were noted in Magarach N 44-77-18 x Magarach N 100-74-1-5 combination. In general, the germinated seeds belonged to embryo classes III, IV and V. The total number of germinated seeds obtained from the random pollination and from cross combinations was 20 % compared to the initial number of seeds, and 45 % compared to the number of seeds used for germination in classes I-V. The number of germinated seeds of embryo classes I-V was 0.3, 0.5, 15, 28 and 52 %, respectively.

After estimating the number of germinated seeds in the laboratory, the number of germinating seeds and their ability to produce seedlings were determined according to cross combinations and embryo classes. Embryo class I seeds failed to produce plantlets. The percentages of plantlets obtained from the seeds of the random pollination belonging to embryo classes II, III, IV and V were 0.8, 8-38, 8-66 and 0-84 %, respectively. The variability range according to embryo classes was 8, 28, 59 and 84 units, respectively. Embryo classes II-V seeds obtained from different cross combinations gave 0-36, 0-42, 0-46 and 4-100 % of plantlets, respectively, the variability range being 36, 42, 46 and 96, respectively.

Seedlings were investigated during the period from the appearance of plantlets up to the development of mature plants. The number of survived seedlings was determined according to the

embryo classes. The highest number of embryo class V viable seedlings was obtained in Magarach N 40-68-11 x Magarach N 100-74-3-1 combination. 58 viable plants were obtained from 62 plantlets. Survival rate of seedlings belonging to different embryo classes varied exceedingly.

Depending on cross combinations, the percentage of survived seedlings obtained from embryo classes III-V seeds were 0-18, 0-42, 0-48 and 4-100 %, respectively, and the variability range was 18, 42, 48, 96, respectively. The number of seedlings surviving from embryo classes II-V seeds in the random pollination was 0-8, 8-33, 15-67 and 0-77 %, the variability range was 8, 25, 52, 77, respectively. The total number of seedlings obtained was 395. The survival rate for all combinations and for the random pollination averaged 77 %.

The number of vigorous plants varies depending on cross combinations. In Magarach N 40-69-11 x Magarach N 100-74-3-1 combination, for example, 23 vigorous plants were obtained from 58 seedlings, and in Magarach N 37-77-44 x Magarach N 100-79-52 combination only 3 vigorous plants were obtained from 11 seedlings generally produced by embryo class V seeds. Most vigorous hybrid plants proved to be obtained from the seeds belonging to embryo classes III, IV and V. As a whole, 118 vigorous hybrid plants were obtained. Fig. 2 shows the hybridization data according to the classes of seed quality in breeding for resistance.

The greatest number of plantlets, viable seedlings and vigorous hybrid plants was shown to be obtained generally in all populations from classes IV and V seeds and partially from class III seeds.

Breeding for resistance in grapes as well as in other perennial plants is a labor-consuming and long-term process, therefore, rapid and simple methods of estimation of parents and their hybrid progeny must find great application in practical selection. To evaluate the grape hybridization effectiveness, it is desirable and practicable to consider the objective information as soon as possible, particularly at the stage of plantlets.

The results show that the seed germination, the number of plants with defective cotyledons, the number of plants with deviations from the normal size of cotyledons, the number of plants with teratologic changes, seedlings survival rate towards the end of the first vegetation season vary in the range of 3.8-90.9, 0-50.0, 0-9.6, 0-13.2 and 1.0-100 %, respectively (Table 2).

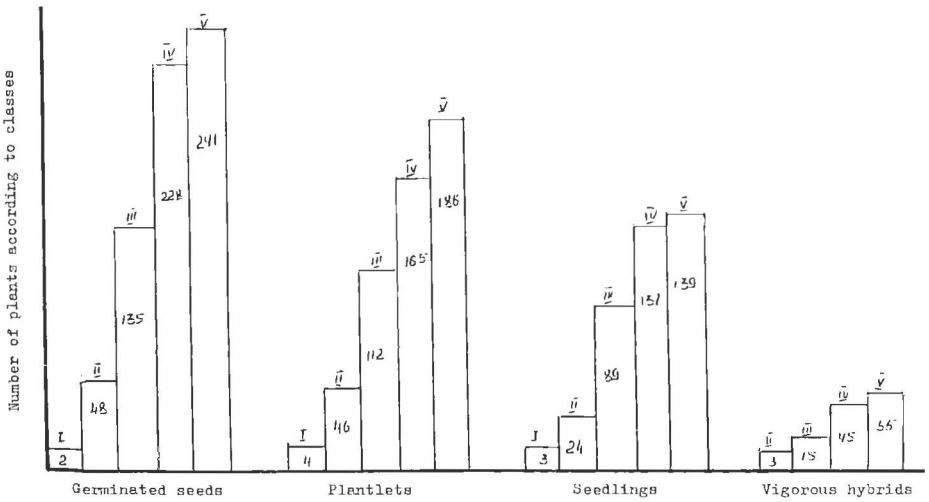

Fig. 2: Dependence of the development of viable seedlings on the class of seed quality.

Table 2: Variability of hybrid grape populations at the stage of plantlets

NN	Cross combination	Plant-lets number	Seed germi-nation %	Cotyledon size: length mm	Cotyledon size: width mm	Abnormalities: defor-med coty-ledons %	Abnormalities: deviati-ons in number of seeds %	Abnormalities: terato-logic changes %	Seed-ling survi-ving capa-city %
1.	Tavrida x Podarok Magaracha	300	22.7	24	17	6.7	0	2.0	63.3
2.	Madeleine Angevine x - - " -	68	34.3	24	19	1.5	0	1.5	97.1
3.	Muscat blanc x - - " -	40	19.6	22	15	50.0	0	0	57.5
4.	Muscat VIRa x - - " -	32	3.8	22	15	50.0	0	3.1	84.4
5.	Sauvignon vert x - - " -	238	51.2	24	16	4.2	0	2.1	16.0
6.	M.47-68-2 x - - " -	355	45.0	25	18	11.3	0.05	2.3	46.8
7.	M. 7-69-12 x - - " -	87	73.1	24	17	0	1.2	0	100
8.	M.56-75-1 x - - " -	400	35.7	22	16	0	0	0	1.0
9.	M.37-77-41 x - - " -	69	49.3	20	10	30.4	0	0	36.2
10.	M.44-77-11 x - - " -	56	69.0	22	16	8.6	0	0	55.2
11.	M.44-77-13 x - - " -	100	50.8	24	16	15.0	0	1.0	58.0
12.	M.44-77-19 x - - " -	114	52.5	20	14	33.3	9.6	13.2	39.5
13.	M.44-77-22 x - - " -	300	45.4	14	10	26.0	1.3	1.7	61.7
14.	M.34-78-7 x - - " -	110	90.9	22	14	6.4	0	0	43.6
15.	M.34-78-4 x - x -	37	40.2	22	14	1.1	0.5	0	73.0
Variation coefficient			47.9	12.2	16.9	107.0	272.7	185.1	56.2

While analysis at juvenile stage is based on the investigation of phenological variability, the genotypic specificity of recombinants is to a considerable degree expressed during the first year of plant development (KLIMENKO 1987). The high value of variation coefficients suggests that seed (and possibly seedling) variability is generally determined by the female genotype (OLMO 1942). The highest values of variation coefficients of the cotyledon number and teratologic changes indicate the great asymmetry in the distribution due to the genotypic determination. The highest number of abnormal plants in Magarach 44-77-19 x Podarok Magaracha hybrid population is supposed to be due to aberrations in the genome of Magarach 44-77-19 hybrid which has a mutant in its genealogy.

When planning subsequent crosses in order to get an increase in the yield of hybrid seedlings, it is necessary to exclude those parents that have low seed germination and survival rate (for example, Muscat VIRa and Magarach 56-75-1) or to use unconventional methods of obtaining seedlings from them.

Correlations between morphological characters of juvenile seedlings and economically attractive features are known to exist and to be used for the early selection of the promising hybrids (POGOSIAN and KHACHATRIAN 1983). In particular, the study of cross combinations was aimed at

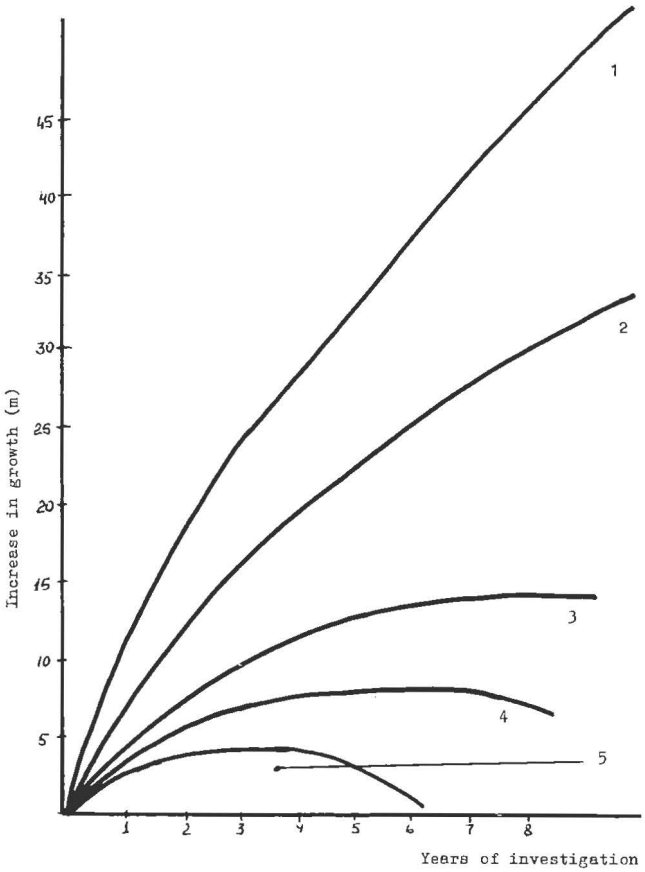

Fig. 3: Dynamics of the development of varieties and hybrids with different degrees of phylloxera resistance related to years. Resistance rating: 1-5.

Table 3: Estimation of donors of resistance as producers of the resistant progenies in breeding for mildew and phylloxera resistance

Cross combination	Hybrid number	Phylloxera resistance % according to scores						Mildew resistance % according to scores						Breeding value of the F_1 population
		population av.	1	2	3	4	5	population av.	1	2	3	4	5	
V.vinifera x S.V. 12309	58	3.2	0	25	42	22	11	2.4	12	50	29	5	4	0.16
- " - x S.V. 20366	115	3.1	0	23	48	23	6	3.1	9	32	17	26	16	0.20
- " - x Seibel 7053	51	4.0	0	11	5	58	26	3.9	4	4	26	31	25	0
- " - x S.V. 18315	109	3.7	0	7	37	32	24	3.5	0	20	34	24	22	0.03
- " - x(S.V. 18315 x V.vinifera)*	136	3.4	0	18	30	27	25	3.1	13	23	21	29	14	0.04
- " - x Seibel 13666	127	3.8	0	16	22	28	34	3.3	9	20	23	25	23	0.06
- " - x(Seib.13666 x V.vinifera)*	12	3.2	0	10	70	10	10	4.0	0	8	25	25	42	0
- " - x(Ravat 6 x V.vinifera)*	549	3.8	0	7	28	46	19	2.8	22	29	12	17	20	0.06
- " - x(S.V. 20365 x V.vinifera)*	79	3.5	0	9	45	32	14	3.3	0	3	72	16	9	0
- " - x(S.V. 20347 x V.vinifera)*	56	3.4	0	9	18	37	36	3.9	4	21	13	5	57	0.02
													Total for all populations	0.06

* Pollen mixture of the best European-Asiatic varieties

producing wine varieties but judging from the size of cotyledons, it is quite possible to obtain large-berry seedlings in Magarach 47-68-2 x Podarok Magaracha hybrid population.

The successful use of breeding for resistance is to a great extent determined by the corresponding selection of parents and by the precise evaluation of their resistance. The estimate of the resistance of parents and of a vast hybrid fund, consisting of many thousands of hybrids, to phylloxera and pathogenic microflora under conditions of the planting site complex infection during a 15-year period indicates the dependence of the dynamics of the hybrid development on the degree of resistance expressed in scores (Fig. 3). As the increase in growth is the integrated index of the phylloxera resistance which correlates well with the root system state and development, the analysis of the dynamics of the development of the hybrids studied (with the rare exceptions of genotypically weakly growing hybrids) conducted during a 5 to 6-year period provides a rather accurate estimate of their resistance degree. Hence, analysis was carried out concerning the regularities of the inheritance of the hybrid populations resistance to phylloxera and mildew (Table 3).

The main aim of this analysis was to elucidate the role played by the male parent, the donor of resistance, in transfering this character to the progeny. As the data presented concerning crosses between European-Asiatic varieties and Seyve Villard (S. V.) and Seibel hybrids show, characters of phylloxera and mildew resistance have a wide range of variability. A considerable proportion of seedlings has a high resistance to mildew (scored 1) and to phylloxera (scored 2). These seedlings are good parents for the use in selection as indicated by high indices of the value of populations (Table 3).

To improve the interspecific hybrid quality, backcrosses to European-Asiatic varieties, donors of high fruit quality, are known to be used. From this point of view, it is considerably interesting in which way the resistance will be inherited, if selected transgressive resistant hybrids obtained as a result of the first backcross of Seyve Villard and Seibel to European-Asiatic varieties are used as parents. As the data of Table 3 show, the number of resistant seedlings generally decreases in such populations; however, if the selection of parental pairs is successful, a certain proportion of hybrids will be of sufficiently high resistance.

Estimation of the varieties and the hybrid populations indicated that S. V. 13-303, S. V. 12-283 interspecific hybrids and Magarach N 100-75-1-5 hybrid showed a resistance to oidium. A highly resistant group (scored 1) consisted of Podarok Magaracha variety, a number of Seyve Villard interspecific hybrids and their hybrid progeny (Moldova, Magarach N 97-75-1, etc.) as well as Magarach N 13-88-2 and Magarach N 33-78-11 elite forms (Nimrang x Magarach N 124-66-26). Varieties such as Antei Magarachsky, Liana, Lanka, Mogis, Muscat Bessarabsky, Margaritar, Pamiaty Verderevskogo, Frumoasa Albe, Pukhliakovsky Magaracha, Nimrang Magaracha, Magarach N 17-82-1 hybrid (Magarach N 57-75-1 x Antei Magarachsky) showed an increased resistance to oidium (scored 2) (Oleinikov 1988).

Control varieties resistance was in close agreement with the literature data.

The evaluation of hybrid populations indicated that some donors of oidium resistance were of value for breeding and that Podarok Magaracha variety used as a male parent gave rise to seedlings of different degrees of resistance, but in many cross combinations plants which were scored 1 prevailed. The highest percentage of seedlings scored 1 was obtained in Madeleine Angevine x Podarok Magaracha and Magarach N 44-77-13 (Italia x Podarok Magaracha) x Podarok Magaracha combinations (12 and 11 %, respectively) (Table 4).

When crossing Magarach N 31-77-8 (Nimrang x Seibel 13666) with Ranny Magaracha variety, 16 % of seedlings were scored 1. The use of Ranny Magaracha variety as a female in crosses with Podarok Magaracha, JS 26205 and S. V. 20-366 did not result in highly resistant hybrids.

The donor of complex resistance, S. V. 12-397, in crosses with variety Madeleine Angevine allowed to obtain 4 % of hybrids scored 1. In Madeleine Angevine x Magarach N 124-66-26 and

Table 4: Heritability of oidium resistance in interspecific hybridization (seedlings from crosses made in 1986)

Cross combinations	Hybrid number	parental pairs ♀	parental pairs ♂	Oidium resistance: populations (according to scores, %) 1	2	3	4	5	av. score for a population
Madeleine Ang. x Podarok M.	41	4	1	12	32	52	4	0	2.5
N 44-77-13 x Podarok M.	53	3	1	11	15	43	25	6	3.0
N 31-77-44 x Podarok M.	13	4	1	8	38	46	8	0	2.6
N 44-72-11 x Podarok M.	15	3	1	7	27	53	13	0	2.8
N 44-77-22 x Podarok M.	144	4	1	6	29	39	21	5	2.9
N 47-68-2 x Podarok M.	156	4	1	4	10	59	18	9	3.2
Muscat VIRa x Podarok M.	22	5	1	0	9	77	14	0	3.1
Ranny M. x Podarok M.	13	2	1	0	23	64	13	0	2.9
Ranny M. x J.S. 26-205	117	2	2	0	11	63	17	9	3.2
Ranny M. x S.V.20-366	72	2	0	0	14	72	14	0	3.0
N 31-77-8 x Ranny M.	110	2	2	16	43	39	2	0	2.3
Muscat VIRa x N100-74-1-5	11	5	1	2	16	19	36	27	3.7
N 44-77-18 x N100-74-2-2	75	4	1	1	20	40	34	5	3.2
Antei Mag. x Tavria	23	2	3	0	17	44	22	17	3.4
Muscat Yant. x Antei Mag.	9	2	2	0	11	53	33	0	3.2
N 10-51-1 x Antei Mag.	28	4	2	4	11	39	32	14	3.4
N 4-57-66 x Antei Mag.	14	3	2	0	14	79	7	0	2.9
Madeleine Ang. x Antei Mag.	28	4	2	0	4	42	36	18	3.6
Madeleine Ang. x S.V.12-397	154	4	1	4	22	49	20	5	3.0
Madeleine Ang. x N124-66-26	9	4	1	0	11	78	11	0	3.0
Nimrang x N124-66-26	37	5	1	21	49	21	9	0	2.2
Bican x N124-66-26	10	4	1	0	20	60	20	0	3.0
Chaush x N 124-66-26	11	5	1	0	18	73	9	0	2.0
Tashly x S.V.20-366	55	4	2	0	20	62	16	2	3.0
N 4-68-25 x Crymskaia zhemchuzhina	22	1	4	12	23	60	5	0	2.6

Madeleine Angevine x Antei Magarachsky combinations, highly resistant plants were not produced.

Nimrang x Magarach N 124-66-26 population where the yield of resistant seedlings was 21 % proved to be the most profitable. In the progeny of crosses between Madeleine Angevine, Bican, Chaush varieties and Magarach N 124-66-26, hybrid seedlings were of low resistance.

The high yield of resistant hybrids was provided by Magarach N 4-68-25 (S. V. 20-366 x *V. vinifera*) x Crymskaia Zhemchuzhina and Magarach N 10-51-1 x Antei Magarachsky combinations. The latter was the only combination of those studied in which Antei Magarachsky variety provided the production of 4 % of transgressive hybrids concerning the resistance to oidium.

In breeding for grey rot resistance, the use of various sources of disease resistance genes may be recommended. In this respect it is possible to use species such as *V. armata* (Staudt 1980)

Table 5: Heritability of grey rot resistance in F_1 grapevines

Cross combinations		Parents resistance ♀	Parents resistance ♂	Hybrid number	In % according to scores: resistant 1	resistant 2	resistant 3	susceptible 4	susceptible 5
Moldavsky Chiorny	x Mtsvane	1	3	33	30.3	12.1	21.2	21.2	15.2
Moldavsky Chiorny	x Rkatsiteli	1	3	26	19.2	30.8	19.2	15.4	15.4
Moldavsky Chiorny	x Seyve Villard 18315	1	2	29	24.1	13.8	13.8	27.6	20.7
Moldavsky Chiorny	x Seyve Villard 20347	1	4	34	5.9	8.8	17.6	29.4	38.3
Moldavsky Chiorny	x Seibel 7053	1	5	11	0	27.2	18.2	18.2	36.4
Rkatsiteli	x Magarach 376	3	5	14	0	0	14.3	35.7	50.0
Rkatsiteli	x Magarach 2-57-72	3	2	23	0	13.0	13.0	21.8	52.2
Rubinovy Magaracha	x Magarach 6-68-27	1	1	32	28.1	28.1	21.9	12.5	9.4
Tavkveri	x Magarach 6-68-27	4	1	33	27.3	33.3	18.2	12.1	9.1
Tagobi	x Amursky Oboepoly	4	1	14	7.2	14.3	35.7	21.4	21.4
Nimrang	x Amursky Oboepoly	3	1	12	8.3	16.7	41.7	8.3	25.0
Bastardo Magarachsky	x Khindogny	4	3	14	0	7.2	42.8	35.7	14.3
Bastardo Magarachsky	x Portugieser	4	4	13	0	0	7.7	38.5	53.8

Magarach N 376 - Riesling x Muscat blanc
Magarach N 2-57-72 - Mtsvane x Sochinsky Chiorny
Magarach N 6-68-27 - Ravat 6 x pollen mixture

having practical resistance to botrytis, *V. riparia* (ALLEWELDT 1985), *V. labrusca* (VASILIEVA 1975), *V. amurensis* (TAMASHI 1964), complex interspecific hybrids of Seyve Villard, Seibel and other breeders as well as highly resistant *V. vinifera* varieties and hybrids (GOLODRIGA *et al.* 1979; SUPOSTAT 1986).

These findings are supported by the results obtained which are presented in Table 5. Crossing of variety Moldavsky Chiorny with varieties Mtsvane and Rkatsiteli is an example showing the possibility of obtaining grey rot resistant hybrids in F_1 by breeding within *V. vinifera* L. A high percentage of resistant hybrids is obtained in both cases, but in the first case the maximum number of highly resistant hybrids is observed (30.3 %), and the maximum number of resistant hybrids is detected in the second case (30.8 %). It should be noted in this context that Moldavsky Chiorny belongs to highly resistant varieties and Mtsvane and Rkatsiteli are considered to be varieties of intermediate resistance.

The use of complex hybrids (for example, Seyve Villard 18315, Seyve Villard 20347, Seibel 7053) in the hybridization with the highly resistant variety Moldavsky Chiorny resulted in F_1 hybrids which have high grey rot resistance, but the high percentage of susceptible hybrids obtained in crosses with susceptible and highly susceptible parents indicates the insufficient degree of genetic dominance of Moldavsky Chiorny in the first generation, concerning disease resistance.

Crossing between Rkatsiteli intermediately resistant females and highly susceptible Magarach 376 hybrid results in progeny where percentages of intermediately resistant and susceptible forms are 14.3 and 85.7 %, respectively; better results are obtained using Magarach 2-57-72 interspecific resistant hybrid, but in this case also practically no highly resistant forms are produced.

The use of *V. amurensis* in crosses when breeding for grey rot resistance can be considered promising, and this is supported by the example of crosses with Tagobi and Nimrang varieties.

The use of some varieties in crosses results in F_1 positive transgressions. When crossing Bastardo Magarachsky and Portugieser varieties (both are susceptible), 7.7 % of positive transgressive intermediately resistant forms were obtained in the first generation, and crossing between Bastardo Magarachsky and Hindogny (susceptible and intermediately resistant varieties) gave practically the same quantity of intermediately resistant forms (7.2 %).

So, in breeding for grey rot resistance, the resistance of parents should be taken into account and in spite of the possible production of resistant transgressive recombinants resistant forms should preferably be used.

On the basis of the data presented, analysis of the specific combining ability was possible. The highest combining ability in breeding for grey rot resistance was shown by Magarach 6-68-27 interspecific hybrid whose crosses with varieties having different resistance (Rubinovy Magaracha – scored 1 and Tavkveri – scored 4) resulted in equally large groups of resistant progenies, 78.1 and 78.8 %, respectively.

At the same time, the use of highly resistant Amursky oboepoly males results in a considerably smaller quantity of disease resistant forms compared to Magarach 6-68-27 hybrid.

Thus, a high combining ability of Magarach 6-68-27 provides a considerably higher number of resistant forms compared to Amursky oboepoly which has a lower combining ability.

Comparative analysis of Moldavsky Chiorny x S. V. 20347, Tavkveri x Magarach 6-68-27, Tagobi x Amursky oboepoly populations which can be considered as reciprocal crosses according to grey rot resistance (scored 1 x 4 x 4 x 1) suggests the lack of female effects in breeding for this character.

Conclusions

The results obtained indicate the possible use of the roentgenoscopic method in the preliminary estimation of parental forms. Using the roentgenoscopic method, data on the quality of

seeds were obtained concerning the filling with endosperm and the embryo development. Seeds were classified according to quality classes and preserved for sowing and subsequent investigation of seedlings. The development of plants obtained from different classes of seeds was monitored, the number of plantlets, developed seedlings and vigorous hybrid plants was determined.

In all cross combinations and in the random pollination, an increase in the class (I-V) resulted in an increase in the proportion of plantlets, viable and vigorous hybrid plants produced. Despite the difficulties in obtaining hybrid plants using interspecific hybridization, 3-8 % of viable plants were produced depending on cross combinations and embryo classes.

Thus, the roentgenoscopic method makes it possible to determine the seed quality and to predict the development of viable plants under conditions of conventional cultivation as well as to select seeds for the use in embryo and tissue culture.

The results of investigation of grapevine seedlings at the stage of plantlets can serve as an estimate of the parent and seedling genotypic characteristics, they should be taken into account when planning crossings and selection of hybrids at the subsequent stages in the breeding process.

Analysis of the hybrid development dynamics under conditions of the high infection pressure (phylloxera and pathogenic microflora) during a 5 to 6-year period at a special planting site provides a sufficiently accurate estimate of the degree of hybrid resistance to phylloxera and allows the rejection of susceptible genotypes. Detailed analysis of the root system should be carried out only on the isolated resistant forms.

The use of the resistant hybrids as donors of resistance in backcrosses to *V. vinifera* L. varieties allows the improvement of fruit quality while retaining a sufficiently high variability range of hybrid resistance characters, which makes it possible to select promising genotypes.

S.V. 12309, S.V. 20366 and Seibel 13666 are considered to be the most promising donors of resistance to phylloxera, pathogenic microflora and mildew.

At the oidium infected planting site established in the greenhouse, a considerable number of promising oidium resistant genotypes were for the first time isolated from the hybrid populations according to the intensity of the conidiophore development on 1-year seedling leaves in Madeleine Angevine x Podarok Magaracha, Magarach N 44-77-13 x Podarok Magaracha, Magarach N 31-77-8 x Ranny Magaracha, Nimrang x Magarach N 124-66-26, Magarach N 4-68-25 x Crymskaia Zhemchuzhina populations.

The highest yield of transgressive hybrids was provided by Nimrang x Magarach N 124-66-26 combination.

The results of our investigations made it possible to elucidate some of the problems connected with directed breeding of grey rot resistant varieties and to come to the conclusion that the increased production of the first-generation disease resistant grapevine hybrids was possible by both interspecific and intraspecific crosses and that the heritability of this character in both cases depended on the genotype structure of parental forms and on their combining ability; this provides the possibility of obtaining positive transgressions concerning the disease resistance, but the female parent effect connected with this character was not detected.

The development of the bases of the particular grapevine genetics, the evaluation of parents according to their ability to produce resistant progeny, the use of the complex infection planting site in the practical selection and the selection of promising forms at the juvenile stage will make it possible to create a wide range of complex resistant grape varieties of different uses. The wide use of resistant grape varieties in agriculture will result in a decrease in labor and energy requirements in grapevine cultivation, and a nearly complete elimination of treatments with toxic chemical substances used as means of plant protection against pests and diseases will decrease the pesticide pollution of environment and will allow the production of dietary grapes without residual quantities of toxic substances.

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Resistance to phylloxera and pathogenic microflora was estimated using the method developed by P. N. NEDOV and A. P. GULER (1971); resistance to mildew was rated using the 5-point scale according to the method approved by the Section of Viticulture of the National Academy of Agricultural Sciences (Methodical Instructions Concerning the Grape Breeding, Erevan, 'Aistan', 1974).

Susceptibility to oidium was estimated using the method proposed by I. N. NAIDIONOVA (1985) at an infected planting site established in the greenhouse. Hybrid seeds and two-bud cuttings were placed in hydroponic channels. Controls were positioned along the axis of the channels. Samples were inoculated with conidia suspension (35-30 conidia/0.001 ml of suspension). Suspension rate was 25 ml/m^2 of the channel area. Inoculations were repeated every 2 weeks.

The following grape varieties investigated by a number of authors were used as controls: highly resistant varieties: SV 12-375 (BOUBALS 1961), Kishmysh Vatkana (Methodical Instructions Concerning the Grape Breeding, 1974; NAIDIONOVA 1985), Gangal kara (SOHI and SRIDHAR 1972; SHTIN *et al.* 1986); resistant varieties: Seibel 15062 (BOUBALS 1961), Kishmysh rozovy (NAIDIONOVA 1985); susceptible varieties: Ravat 6 (BOUBALS 1961), Riesling Italico (NAIDIONOVA 1985).

In order to reveal the genetically dependent grey rot resistance, a laboratory method of estimation was used (GOLODRIGA *et al.* 1979).

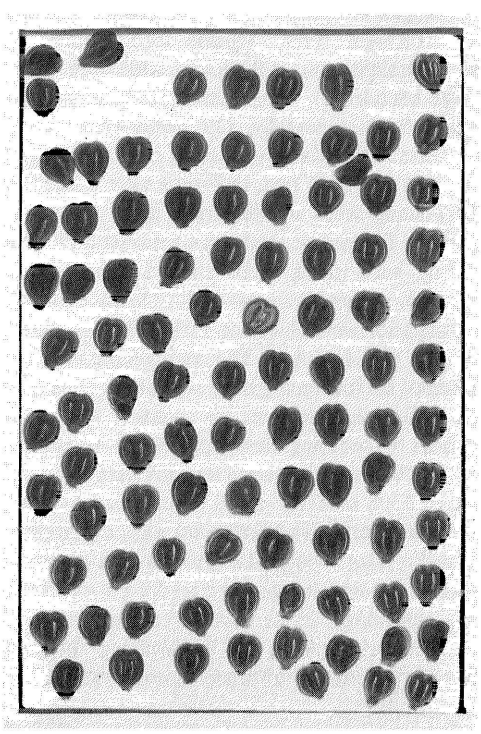

Fig. 1: Roentgenogram of the hybrid grape seeds.