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Responses of grapevine genotypes to abiotic stress

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Background. Salt stress reduces water supply and causes ionic imbalance in the plant, eventually leading to a decrease in plant growth, functional activity, and productivity. Variable salinity levels in the field make it difficult to select salt-tolerant cultivars, so a need for other testing methods arises.
Materials and methods. The plant material consisted of two own-rooted *Vitis vinifera* (L.) genotypes: cv. 'Asma', and hybrid M. No. 8-08-8-4 ('Kok Pandas' × 'Zeibel 6357'). Salt stress was simulated by treating with NaCl at 0, 50, 80, 100, and 120 mM concentrations. Water status was measured by the leaf water potential (Ψ) using a pressure chamber. Changes in the leaf area and total root length were assessed *in vitro*.
Results. Salt stress affected growth characteristics and yield structure of both own-rooted vines, but hybrid M. No. 8-08-8-4 was more sensitive. The hybrid showed greater yield reduction (38.6%) than cv. 'Asma' (28.4%), while the mass concentration of sugars was higher in 'Asma'. The greatest differences in the predawn leaf water potential were observed for 'Asma' and M. No. 8-08-8-4 on the 45th day of irrigation with water containing different NaCl concentrations. The root length of the more salt-tolerant cultivar reduced *in vitro* to a greater extent.
Conclusion. The functional abilities of a cultivar depend on the level of salinization and the genotype. Cv. 'Asma' demonstrated higher salt tolerance compared to hybrid M. No. 8-08-8-4. Leaf water potentials characterizing the water status of plants were measured. The responses to salinization were the same in the vines grown *in vivo* and *in vitro*, so it is possible to perform testing for salt tolerance *in vitro*.

Keywords: NaCl, leaf water potential, growth indicators, yield, tolerance

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ИЗУЧЕНИЕ И ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ РАСТЕНИЙ

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Реакция генотипов винограда на абиотический стресс

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Актуальность. Солевой стресс вызывает уменьшение поступления воды и ионный дисбаланс; на этом фоне происходит снижение роста, функциональной активности и продуктивности растений. Различный уровень засоления в полевых условиях затрудняет выбор солеустойчивых сортов, в связи с чем возникает необходимость в других методах испытаний.
Материалы и методы. Исследованы два корнесобственных растения винограда вида *Vitis vinifera* (L.): сорт 'Асма' и гибрид М. № 8-08-8-4 ('Кок Пандас' × 'Зейбель 6357'). Солевой стресс моделировали введением NaCl в концентрациях 0, 50, 80, 100 и 120 мМ. Водный режим измеряли по методу водного потенциала листьев (Ψ) с использованием камеры давления. Изменения площади листьев и общей длины корня определяли *in vitro*.
Результаты. Солевой стресс повлиял на ростовые характеристики и урожай обоих корнесобственных генотипов винограда, но гибрид М. № 8-08-8-4 оказался более чувствительным. Снижение урожая было больше у гибрида М. № 8-08-8-4 (38,6%) по сравнению с сортом 'Асма' (28,4%), а массовая концентрация сахаров у сорта 'Асма' была выше. Наибольшие различия в предрассветном водном потенциале листьев у сорта 'Асма' и гибрида М. № 8-08-8-4 наблюдались на 45-й день полива водой с разной концентрацией NaCl. В условиях *in vitro* у более устойчивого к засолению сорта длина корня сократилась в большей степени.
Заключение. Функциональные способности сорта зависят от уровня засоления и генотипа. Сорт 'Асма' показал более высокую солеустойчивость по сравнению с гибридом М. № 8-08-8-4. Определены водные потенциалы листьев, характеризующие водный режим растений. У растений винограда, выращенных *in vivo* и *in vitro*, реакции на засоление совпадали, что свидетельствует о возможности тестирования на солеустойчивость *in vitro*.

Ключевые слова: NaCl, водный потенциал листьев, показатели роста, урожай, устойчивость

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Introduction

Salt-contaminated soils are spreading and have become a worldwide constraint for raising crop productivity. About 7% of the world's total land area is affected by salinity (Flowers, 2004). Adaptive properties and resistance to major environmental stressors are of greatest importance in obtaining high and stable yields (Safonova, Aniskov, 2023).

Two approaches could be used to minimize the impact of salt stress on plants: one is based on soil reclamation by leaching salts away from the soil profile, while the other relies on selecting genotypes with high genetic potential to tolerate salt stress.

Salinity causes serious declines in plant growth and development and crop productivity. Previous studies showed that saline osmotic stress led to plant growth retardation in grapes (Fozouni et al., 2012; Mamedova, Aliyeva, 2023). High salt concentrations reduce water potential of the soil solution, which prevents water entry through the plant root system. Ionic imbalance is another consequence of salinity, and sodium chloride is especially toxic (Walker et al., 2010). When affected by a stress, plants respond by reducing their functional activity (Deluc et al., 2011).

The choice of grapvine cultivars for planting should be based on a number of factors, such as pests and diseases, water availability, temperature regime, and soil salinity (Marín et al., 2021).

Salt-tolerant cultivars could be a solution to the problem. Salt tolerance of grapevine has been studied both in the field and in the glasshouse (Sinclair, Hoffman, 2003). Nevertheless, different levels of salinity in the field make it difficult to select salt-tolerant cultivars (Bayuelo-Jiménez et al., 2012), that is why there is an imperative need for alternative testing methods (Becker, 2019).

Research into salt tolerance of own-rooted grape cultivars and rootstocks with the aid of tissue culture has been limited (Sivritepe, Eriş, 1999; Troncoso et al., 1999; Volynkin et al., 2021). However, this approach has several advantages, as a large number of plants can easily be produced, experiments can be done in smaller locations, and results can be obtained within a relatively short period of time. The present study was conducted on own-rooted cultivated grapevine genotypes. According to Fisarakis et al. (2001), such cultivars have higher salt tolerance than grafted ones.

The objective of this study was to assess the tolerance of two own-rooted grapevine genotypes to sodium chloride both in an *in vitro* testing system and *in vivo*.

Materials and methods

Experiment site

The experiment was performed in the laboratory and at the experiment station of the All-Russian National Research Institute of Viticulture and Winemaking "Magarach" in Partenit, Crimea (76.88 m ASL; 44°34'39.684"N, 34°20'11.220"E).

Plant material and treatments in vivo

The plant material consisted of two own-rooted genotypes of *Vitis vinifera* (L.): cv. 'Asma', and hybrid M. No. 8-08-8-4 ('Kok Pandas' × 'Zeibel 6357').

Experiments with plants grown in vitro

Apical buds collected from the shoots of cv. 'Asma' and hybrid M. No. 8-08-8-4 were cultivated *in vitro* on the Murashige–Skoog (MS) medium, containing 6-benzyl aminopurine (BA) at 1 mg/L^{-1}. After 21–24 days, the shoots approximately 1.5 cm long were produced and transferred to the medium with α-naphtaleneacetic acid (NAA) at 0.1 mg/L^{-1} for rooting and further growth. The pH of the media was adjusted to 5.7 before autoclaving.

Fifty explants per genotype were obtained by microclonal propagation. The explants were transferred to the experiment media with 50 and 100 mM of NaCl and to salt-free control, and were grown in a climatic chamber at 33°C ± 1°C with a 16 h photoperiod. After 40 days, the leaf area and total root length were measured. Ten plants per each salinity level and the control were used.

Experiments with potted plants

In 2015, a total of 20 plants per each genotype (cv. 'Asma' and hybrid M. No. 8-08-8-4) were placed in 3,500 mL pots containing a mixture of diatomaceous breakstone and humus (1 : 1). Over the 2021–2023 growing seasons, the plants were treated for 75 days with salt-free water (control) or NaCl solutions (80, 100 and 120 mM). Ten plants per each salinity level and the control were used.

Salt tolerance of potted plants was assessed on the basis of a number of parameters. Leaf area, shoot length, and shoot number were measured, and shoot lignification (%) was calculated as a ratio of the green portion of a shoot to the lignified portion. Yield per vine was assessed by weighing the fruit on laboratory scales. Bunch number and the weight of one bunch were measured. For mechanical characteristics of bunches, their components (berries, stems, skin and pulp, and juice) were weighed, and the weight and volume of 100 berries were recorded. Total soluble solids in grape juice were assessed with a portable refractometer (Mettler Toledo). Titratable acids (g/L^{-1}) were determined by direct titration of a grape juice sample with alcaline solution until neutralization was observed. Grape juice pH was also measured.

Vine water status was measured according to the leaf water potential (Ψ) using a pressure chamber, and expressed in MPa (megapascals, the units of measurement for water potentials). The data were averaged for presentation in the article.

Results

Experiments with plants grown in vitro

The presence of NaCl at 50 mM reduced growth functions in both genotypes versus the salt-free controls, and those reductions were different (Table 1).

The root length of the more tolerant genotype decreased to a greater extent in order to reduce contact with the saline environment.

Leaf areas of cv. 'Asma' and hybrid M. No. 8-08-8-4 decreased by 45% and 60%, respectively, when compared to the salt-free control (Fig. 1). The reductions in total root lengths were by 55% in cv. 'Asma' and by 65% in hybrid M. No. 8-08-8-4 (Fig. 2). The data of statistical analysis are presented in Table 1. The latter genotype, with poor leaf and root growth, was more sensitive to NaCl.

Experiments with potted plants

Growth characteristics and yield structure were affected by salt stress in cv. 'Asma', but hybrid M. No. 8-08-8-4 was more sensitive (Table 2, 3).

The parameters of hybrid M. No. 8-08-8-4 decreased at any salt concentration, including shoot length, leaf area, shoot ripening, bunch weight, yield, sugars, and titratable acids, and significant differences were recorded between the control and the experiment.

Cv. 'Asma' at the NaCl concentration of 80 mM showed significant differences between the control and the experiment only in shoot ripening, sugars, and titratable acidity. As for the

Table 1. **Morphometric characteristics of the cultivar and hybrid *in vitro***

Таблица 1. **Морфометрические характеристики сорта и гибрида *in vitro***

Parameters	Salinity levels					
	'Asma'			M. No. 8-08-8-4		
	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM
Leaf area, mm^2	122 ±6.5	57 ± 2.1	0	79 ± 1.9	54 ± 0.9	0
Total root length, mm	325 ± 9.0	119 ± 5.0	0	232 ± 7.0	129 ± 4.0	0

Note: the differences are significant in comparison with the control ($p \leq 0.05$)

Примечание: различия достоверны по сравнению с контролем ($p \leq 0,05$)

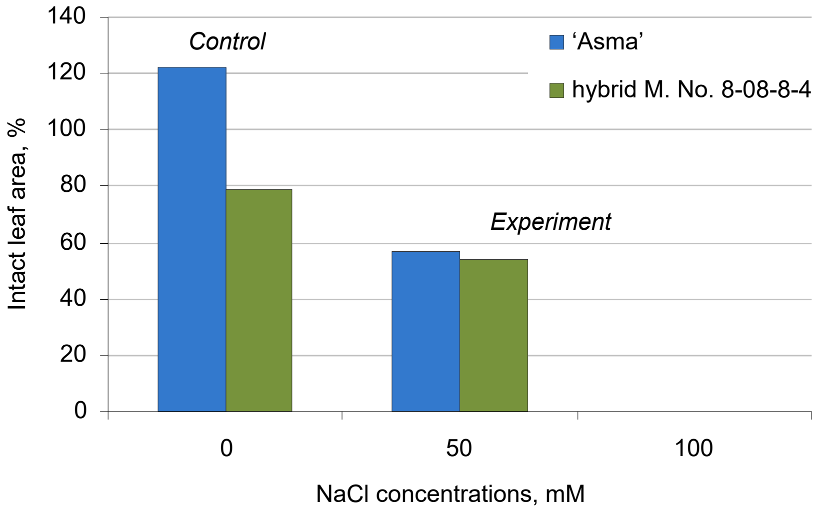

Fig. 1. **Percentage of intact leaf areas in the plants of cv. 'Asma' and hybrid M. No. 8-08-8-4 grown *in vitro* under different levels of salt stress (NaCl)**

Рис. 1. **Процент неповрежденной поверхности листьев при солевом стрессе *in vitro* у сорта 'Асма' и гибрида М. № 8-08-8-4**

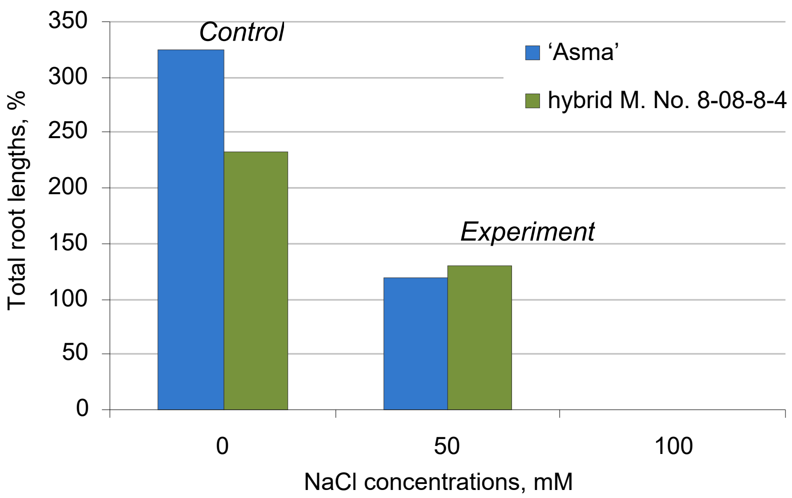

Fig. 2. **Percentage of intact total root lengths in the plants of cv. 'Asma' and hybrid M. No. 8-08-8-4 grown *in vitro* under different levels of salt stress (NaCl)**

Рис. 2. **Процент неповрежденной поверхности корня при действии соли (NaCl) *in vitro* у сорта 'Асма' и гибрида М. № 8-08-8-4**

Table 2. **Vine performance, yield components, and juice composition of hybrid M. No. 8-08-8-4 depending on NaCl concentrations in the irrigation water (2021–2023)**

Таблица 2. **Продуктивность винограда, компоненты урожайности и состав сока гибрида М. № 8-08-8-4 в зависимости от концентрации NaCl в поливной воде (2021–2023 гг.)**

Parameters	Salinity levels			
	0 mM	80 mM	100 mM	120 mM
Leaf area, cm^2	42568 ± 1108	35757 ± 578	0	0
Shoot number	14.4 ± 0.5	14.6 ± 0.5	14.2 ± 0.5	14.0 ± 0.6
Bunch number	5.0	5.0	5.0	5.0
Shoot length, cm	158.0 ± 7.0	129.0 ± 9.3	91.3 ± 7.2	86.5 ± 5.0
Shoot lignification, %	89.0 ± 1.4	62.4 ± 2.6	54.0 ± 2.2	42.0 ± 1.2
Yield per vine, kg	1.170 ± 0.127	1.018 ± 0.09	0.983 ± 0.179	0.719 ± 0.058
Bunch weight, g	234.0 ± 27.8	203.6 ± 24.4	197.0 ± 15.3	143.8 ± 10.9
Total soluble solids, °Brix	25.0	24.0	19.0	17.0
Titratable acidity, g/L^{-1}	7.4	8.0	8.5	9.2
pH (juice)	3.8	3.6	3.5	3.3

Note: the differences are significant in comparison with the control ($p \leq 0.05$)

Примечание: различия достоверны по сравнению с контролем ($p \leq 0,05$)

Table 3. **Vine performance, yield components, and juice composition of cv. 'Asma' depending on NaCl concentrations in the irrigation water (2021–2023)**

Таблица 3. **Продуктивность винограда, компоненты урожайности и состав сока сорта 'Асма' в зависимости от концентрации NaCl в поливной воде (2021–2023 гг.)**

Parameters	Salinity levels			
	0 mM	80 mM	100 mM	120 mM
Leaf area, cm^2	48650 ± 1190	48075 ± 1020	27974 ± 450	0
Shoot number	13.6 ± 0.7	13.0 ± 0.3	13.8 ± 0.2	14.2 ± 0.3
Bunch number	5.0	5.0	5.0	5.0
Shoot length, cm	162.0 ± 19.9	160.0 ± 18.0	140.0 ± 10.1	118.3 ± 9.2
Shoot lignification, %	91.3 ± 2.3	81.8 ± 1.0	69.0 ± 2.0	58.0 ± 2.8
Yield per vine, kg	1.990 ± 0.23	1.926 ± 0,186	1.690 ± 0.16	1.425 ± 0,156
Bunch weight, g	398.0 ± 20.0	385.2 ± 13.0	338.0 ± 9.8	285.0 ± 8.4
Total soluble solids, °Brix	19.0	18.5	18.5	17.5
Titratable acidity, g/L^{-1}	4.8	5.7	5.8	6.3
pH (juice)	3.8	3.6	3.6	3.5

Note: the differences are significant in comparison with the control ($p \leq 0.05$)

Примечание: различия достоверны по сравнению с контролем ($p \leq 0,05$)

other indicators (shoot length, leaf area, bunch weight, and yield), significant differences were manifested only at the concentrations of 100 mM and 120 mM. A slower decrease in the indicators under salinization points to a higher salt stress resistance of the cultivar.

Salt stress reduced shoot growth in both grapevine genotypes (Fig. 3): down to 74% of the control at 120 mM of NaCl in 'Asma'. The decrease in shoot growth was even more dramatic in hybrid M. No. 8-08-8-4: 57,8–54,7% of the control at 100 and 120 mM of NaCl. Shoot lignification in hybrid M. No. 8-08-8-4 and cv. 'Asma' was 54% and 69% at 100 mM, and 42% and 58% at 120 mM of NaCl, respectively (Fig. 4).

Leaf area was also affected by salt stress in both genotypes (Fig. 5). At 100 mM of NaCl, leaf area of cv. 'Asma' plants decreased by 42.5%, and that of hybrid M. No. 8-08-8-4 by 100%. The two genotypes lost their leaves at 120 mM of NaCl.

The effect of salt stress on yield was less pronounced, with reductions down to 28,4% and 38,6% in cv. 'Asma' and hybrid M. No. 8-08-8-4, respectively. However, the berries of both grapevine genotypes were not marketable, because they failed to ripen (Fig. 6).

Both tested grapevine genotypes showed a decrease in their bunch weight, berry indicators, and juice yield under salt stress, while their stem weight and skin weight increased (Table 4, 5).

There was a decrease in sugar accumulation in both grapevine genotypes under the impact of salt stress. However, the decrease in sugars was less in cv. 'Asma' than in hybrid M. No. 8-08-8-4. Sugar accumulation in hybrid M. No. 8-08-8-4 decreased by 0.5°Brix at 80.0 mM and 100 mM, and by 1.5°Brix at 120.0 mM of NaCl. Sugar accumulation in cv. 'Asma' became lower by 1.0°Brix at 80.0 mM, 6.0°Brix at 100.0 mM, and 8.0°Brix at 120.0 mM of NaCl (Fig. 7).

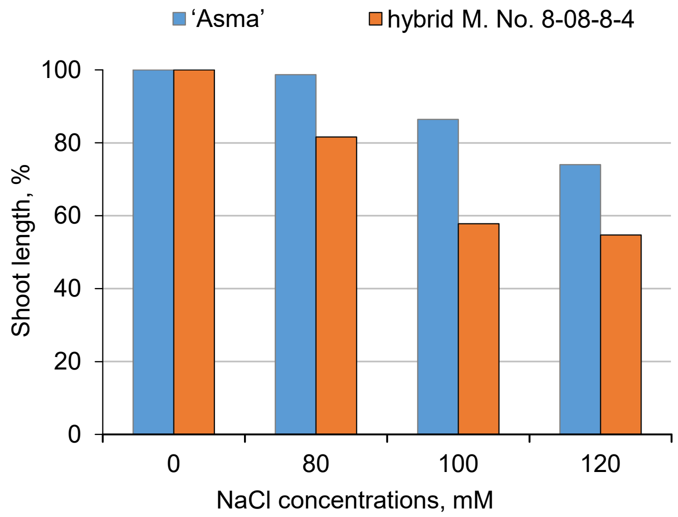

Fig. 3. Shoot length changes in cv. 'Asma' and hybrid M. No. 8-08-8-4 under the impact of NaCl concentrations in the irrigation water

Рис. 3. Изменение длины побегов сорта 'Асма' и гибрида М. № 8-08-8-4 под действием поливной воды с NaCl

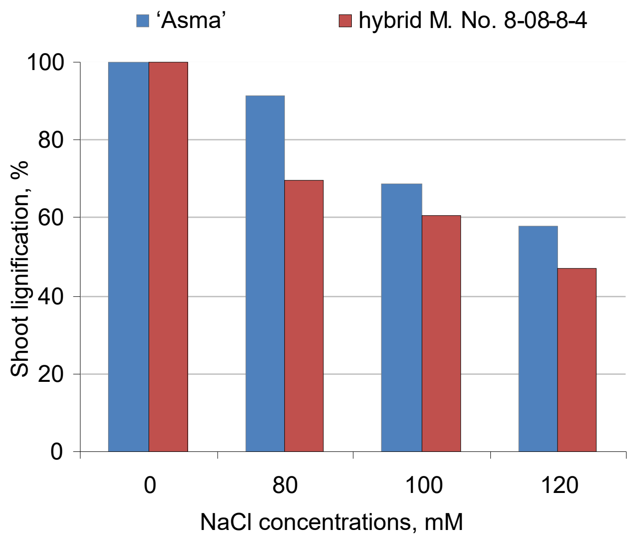

Fig. 4. Shoot lignification in cv. 'Asma' and hybrid M. No. 8-08-8-4 under the impact of NaCl concentrations in the irrigation water

Рис. 4. Вызревание побегов сорта 'Асма' и гибрида М. № 8-08-8-4 под действием поливной воды с NaCl

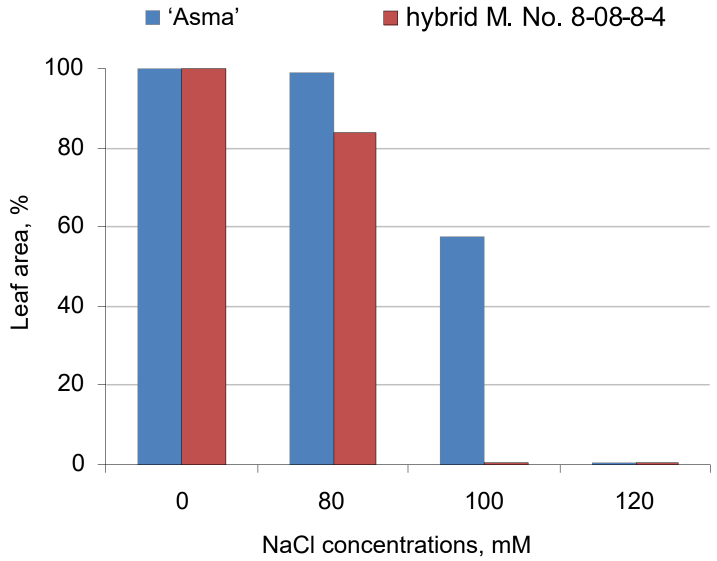

Fig. 5. Leaf area changes in cv. 'Asma' and hybrid M. No. 8-08-8-4 under the impact of NaCl concentrations in the irrigation water

Рис. 5. Изменение площади листьев сорта 'Асма' и гибрида М. № 8-08-8-4 под действием поливной воды с NaCl

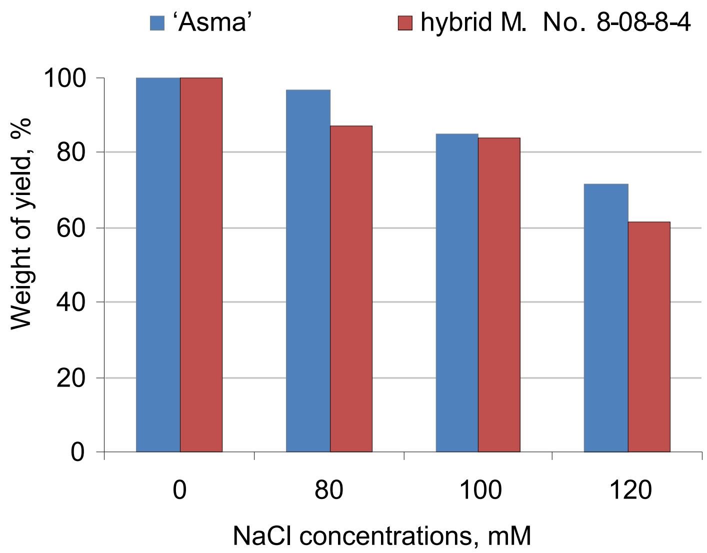

Fig. 6. Yields of cv. 'Asma' and hybrid M. No. 8-08-8-4 under the impact of NaCl concentrations in the irrigation water

Рис. 6. Урожаи сорта 'Асма' и гибрида М. № 8-08-8-4 при поливе водой с NaCl

Table 4. Bunch characteristics of hybrid M. No. 8-08-8-4 under the impact of NaCl concentrations in the irrigation water

Таблица 4. Характеристика гроздей гибрида М. № 8-08-8-4 под действием поливной воды с NaCl

Salinity level	Weight of						Weight of 100 berries, g	Volume of 100 berries, cm^3	Weight of			
	one bunch		stems		all berries				skin and pulp		juice	
	g	%	g	%	g	%			g	%	g	%
0 mM	234	100	3.7	1.6	230.3	98.4	205	200	35.1	15.0	195.2	83.4
80 mM	228	100	4.1	1.8	223.9	98.2	195	190	36.5	16.3	183.3	81.9
100 mM	210	100	4.6	2.2	205.4	97.8	158	150	46.8	29.6	111.2	70.4
120 mM	150	100	5.3	3.5	144.7	96.5	128	120	48.2	33.3	96.5	66.7

Table 5. **Bunch characteristics of cv. 'Asma' under the impact of NaCl concentrations in the irrigation water**

Таблица 5. **Характеристика гроздей сорта 'Асма' под действием поливной воды с NaCl**

Salinity level	Weight of						Weight of 100 berries, g	Volume of 100 berries, cm^3	Weight of			
	one bunch		stems		all berries				skin and pulp		juice	
	g	%	g	%	g	%			g	%	g	%
0 mM	382.0	100	7.4	1.9	374.6	98.1	307,5	300	103.0	27.0	271.6	71.1
80 mM	356.1	100	7.42	2.1	348.7	97.9	286.0	290	106.8	30.0	241.9	67.9
100 mM	335.0	100	7.8	2.3	324.9	97.0	264.6	270	106.2	31.7	221.1	66.0
120 mM	279.0	100	8.0	2.9	270.0	96.8	220	250	89.0	31.9	182.7	65.5

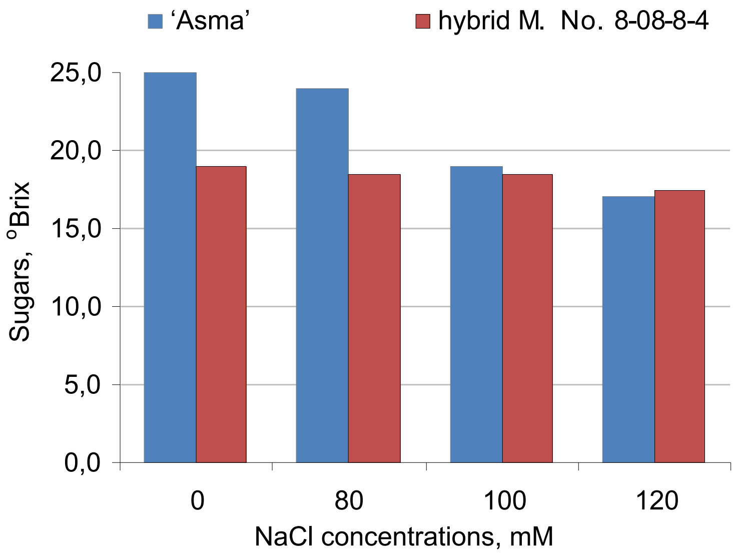

Fig. 7. **Sugar accumulation in berries of cv. 'Asma' and hybrid M. No. 8-08-8-4 under the impact of NaCl concentrations in the irrigation water**

Рис. 7. **Содержание сахаров в ягодах сорта 'Асма' и гибрида М. № 8-08-8-4 при поливе водой с NaCl**

Water stress was promoted by salt stress in both the cultivar and the hybrid. On the 30th day of irrigation with NaCl solutions in different concentrations, the plants failed to assimilate water normally, and water began to penetrate through the walls of the pots and leak out. This process continued to intensify (Fig. 8).

On the 45th day of irrigation, the filtrate amounts were about 5 mL at 0 mM, 25 mL at 80 mM, 100 mL at 100 mM, and 350 mL at 120 mM for cv. 'Asma', and 5 mL at 0 mM, 50 mL at 80 mM, 270 mL at 100 mM, and 500 mL at 120 mM for hybrid M. No. 8-08-8-4. Lower amounts of the filtrate recorded for cv. 'Asma' proves that the cultivar can absorb salt for a longer period of time.

By the end of the growing season, the filtrate amounts were 800–1000 mL for both genotypes at 120 mM of NaCl. Salt-induced scorching of the leaves was observed both in cv. 'Asma' and hybrid M. No. 8-08-8-4.

Electrical conductivity of the soil was increased by salt stress at the full field moisture capacity. The values were 8.6, 12.0, 15.0 and 16.3 for cv. 'Asma' versus 8.3, 11.3, 16.4 and 16.7 for hybrid M. No. 8-08-8-4 at 0, 80, 100 and 120 mM of NaCl, respectively.

Leaf water potentials of cv. 'Asma' and hybrid M. No. 8-08-8-4 decreased dramatically due to the salt stress simulation (Table 6, 7).

The most pronounced differences in predawn leaf water potentials of cv. 'Asma' and hybrid M. No. 8-08-8-4 were observed on the 45th day of irrigation with water containing different NaCl concentrations.

Discussion

Previously, T. Charbaji and Z. Ayyoubi (2004) studied a salt-stress response of *in vitro*-grown *Vitis vinifera* L. cultivars at 80 mM of NaCl in a non-agarized medium. They observed a greater effect of salt on the formation and development of roots compared to the ground part of rootstocks under *in vitro* conditions.

This study was performed on an agar nutrient medium containing a lower concentration of salt (50 mM); however, a negative effect of salt on the development of the root system was also observed.

Growth characteristics and yield structure were affected by salt stress in cv. 'Asma', but hybrid M. No. 8-08-8-4 ('Kok Pandas' × 'Zeibel 6357') appeared more sensitive. The same trend was observed by R. R. Walker et al. (2002): weights of one-year-old pruning wood and berry were reduced by high salinity for own roots and all rootstocks. Yields of cv. 'Sultana' on own roots were reduced by 30%.

Table 6. Leaf water potentials (MPa) of cv. 'Asma' and hybrid M. No. 8-08-8-4 during their growing seasons under the impact of NaCl concentrations in the soil (2021–2023)

Таблица 6. Водный потенциал листьев (МПа) сорта 'Асма' и гибрида М. № 8-08-8-4 в вегетационные периоды под влиянием концентрации NaCl в почве (2021–2023 гг.)

Salinity level	Growing seasons									
	Before bloom		Bloom		Berry growth		Berry ripening		Harvest	
'Asma'										
0 mM	−0.10 ± 0.01	−0.62 ± 0.02	−0.22 ± 0.02	−0.79 ± 0.02	−0.30 ± 0.02	−1.25 ± 0.06	−0.32 ± 0.02	−1.23 ± 0.03	−0.27 ± 0.03	−1.20 ± 0.02
80 mM	−0.10 ± 0.01	−0.62 ± 0.02	−0.22 ± 0.02	−0.80 ± 0.02	−0.38 ± 0.07	−1.35 ± 0.06	−0.40 ± 0.05	−1.37 ± 0.05	−0.30 ± 0.03	−1.30 ± 0.05
100 mM	−0.10 ± 0.01	−0.62 ± 0.02	−0.25 ± 0.03	−1.00 ± 0.02	−0.70 ± 0.05	−1.53 ± 0.05	−0.700 ± 0.03	−1.55 ± 0.05	−0.40 ± 0.02	−1.42 ± 0.03
120 mM	−0.12 ± 0.01	−0.65 ± 0.02	−0.30 ± 0.02	−1.20 ± 0.02	−0.80 ± 0.07	−1.60 ± 0.05	−0.82 ± 0.03	−1.62 ± 0.03	−0.50 ± 0.03	−1.53 ± 0.05
hybrid M. No. 8-08-8-4										
0 mM	−0.12 ± 0.01	−0.65 ± 0.02	−0.25 ± 0.01	−1.15 ± 0.01	−0.35 ± 0.02	−1.30 ± 0.01	−0.52 ± 0.01	−1.50 ± 0.01	−0.48 ± 0.02	−1.50 ± 0.02
80 mM	−0.12 ± 0.01	−0.65 ± 0.02	−0.25 ± 0.01	−1.18 ± 0.02	−0.42 ± 0.02	−1.52 ± 0.02	−0.65 ± 0.03	−1.65 ± 0.02	−0.58 ± 0.02	−1.60 ± 0.02
100 mM	−0.13 ± 0.02	−0.67 ± 0.02	−0.30 ± 0.01	−1.25 ± 0.02	−0.75 ± 0.02	−1.68 ± 0.03	−0.80 ± 0.03	−1.72 ± 0.02	−0.72 ± 0.04	−1.70 ± 0.02
120 mM	−0.13 ± 0.01	−0.65 ± 0.02	−0.35± 0.01	−1.35 ± 0.01	−0.85 ± 0.02	−1.85 ± 0.02	−0.98 ± 0.06	−1.90 ± 0.02	−−0.95 ± 0.03	−1.85 ± 0.02

Table 7. Average values of water potentials in the leaves of cv. 'Asma' and hybrid M. No. 8-08-8-4 during their growing seasons (2021–2023)

Таблица 7. Средние значения водных потенциалов листьев сорта 'Асма' и гибрида М. № 8-08-8-4 в вегетационных опытах (2021–2023 гг.)

Salinity level	Predawn values	Afternoon values
'Asma'		
0 mM	–0.20 ± 0.04	–1.00 ± 0.01
80 mM	–0.28 ± 0.03	–1.10 ± 0.01
100 mM	–0.43 ± 0.02	–1.20 ± 0.02
120 mM	–0.50 ± 0.02	–1.32 ± 0.02
hybrid M. No. 8-08-8-4		
0 mM	–0.34 ± 0.02	–1.20 ± 0.02
80 mM	–0.4 ± 0.02	–1.30 ± 0.02
100 mM	–0.54 ± 0.03	–1.40 ± 0.03
120 mM	–0.65 ± 0.02	–1.50 ± 0.03

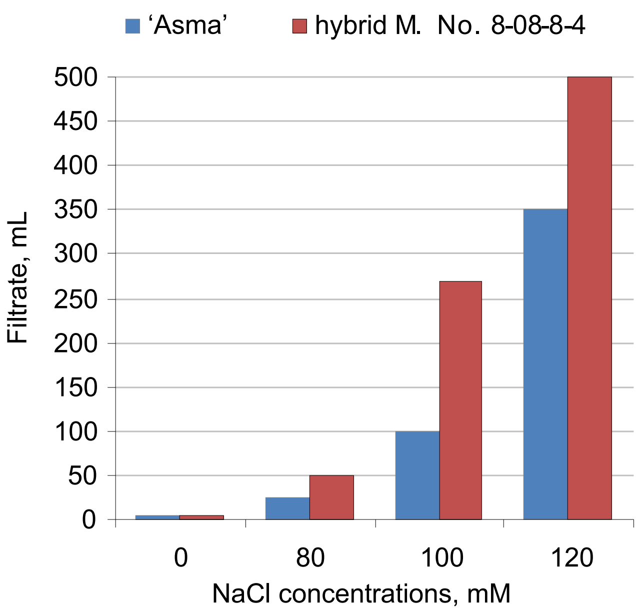

Fig. 8. Water filtration from the pots with plants on the 45th day of irrigation with water at different NaCl concentrations

Рис. 8. Фильтрация воды из сосудов на 45-й день полива растений водой с различной концентрацией NaCl

G. A. Gambetta et al. (2020) observed that grape plants usually functioned in the range from –0.3 to –2.0 MPa. Irrigated vineyards usually operate in a safe range of water potentials, not exceeding –1.5 MPa. A more severe water deficit exceeding –1.6 MPa can cause loss of turgor and xylem cavitation, which can lead to leaf drop in vine plants. In our experiments, the leaf water potential values of cv. 'Asma' did not fall below –1.62 MPa during its growing seasons. Water potentials reached such values only in the variant with the NaCl concentration of 120 mM. As for hybrid M. No. 8-08-8-4, its leaf water potential values dropped to –1.6 MPa even at 80 mM of NaCl. At 100 mM of NaCl those values decreased to –1.65 MPa, and at 120 mM to –1.75 MPa. Thus, a conclusion can be made that the plants of hybrid M. No. 8-08-8-4 experienced severe water stress in all variants with salization.

The differences in predawn leaf water potentials of hybrid M. No. 8-08-8-4, with its higher sensitivity to salt stress, and cv. 'Asma' were 0.2, 0.32, 0.15 and 0.12 MPa at 0, 80, 100 and 120 mM of NaCl, respectively. Predawn leaf water potentials (Ψpd) provide information on the root-zone soil water potential (Améglio et al., 1999). Under salinization, the value of leaf water potential reflects, along with biological productivity, the ability of the environment to oppose the stress (Voronin et al., 2021).

Water stress and increasing salt concentration in the soil exert a negative impact on vineyard performance. R. R. Walker et al. (2014) indicated different responses between the tested cultivars: cv. 'Shiraz' had been less affected by prolonged exposure to salinity when compared with cv. 'Chardonnay'.

Different resistance to salt stress was observed in grafted cultivars. The rootstocks differentially excluded Na and Cl from vines; '216/3' and 'Ruggeri' showed the best performance (Dag et al., 2015). Cessation of salt treatment in our experiments led to a promotion of shoot growth. This process occurs due to the progressive recovery in photosynthesis (Walker et al., 1981).

In the present study, a new indicator, total root length, which is easily measured *in vitro*, was used for evaluation of salt tolerance. Previously, a comparative analysis of grapevine root systems under drought pressure was carried out *in vitro* (Ryff et al., 2005). An assumption was made on the basis of such publications that under *in vitro* conditions it is possible to simulate and obtain data on the growth of roots under salinity. In this study, a decrease in the root length was observed. For plants grown *in vitro*, salt tolerance increased with root growth suppression. It may be supposed that a decreased root area admits lower amounts of toxic NaCl ions.

Roots remain in constant contact with saline soil and the cells of root hairs become damaged, which hampers water entry and supply of mineral nutrients. The effect of salt on root development in *in vitro*-grown rootstocks was observed by A. Troncoso et al. (1999) and T. Charbaji, Z. Ayyoubi (2004). Y. Lupo et al. (2021) also noticed a greater reduction in the root system in salt-tolerant cultivars.

In this investigation a decrease in leaf area was observed under salt stress. The leaf area of the salt-tolerant cultivar decreased less *in vivo* and *in vitro*. The same trend under salt stress was observed by other researchers (Sivritepe et al., 1999; Fozouni et al., 2012). Y. Netzer et al. (2014) examined vines of 'Superior Seedless' table grapes grafted on a salt-tolerant 'Paulsen' rootstock and irrigated with water containing NaCl concentrations. They reported trends of Na accumulation in vine tissue and the soil, stating that prolonged irrigation may pose a potential risk to the vines over time.

The results of the present study were similar to the findings by Y. Netzer et al. (2014) who reported that irrigation with NaCl-containing water was applied on table grapes for 8 years. As a consequence, an increase in visual salinity-like symptoms appeared on the leaves and, in some extreme cases, total collapse of yield-bearing vines occurred. The data obtained in this study on vegetative growth and yield parameters might be explained by the supposition that an increase in sodium concentration decreased the uptake of nitrogen and phosphorus, which had a direct effect on the plant growth.

With an increase in salt concentration, a decrease in water potentials was observed in cv. 'Asma' and hybrid M. No. 8-08-8-4. This decrease was less in the salt-tolerant cultivar 'Asma'. A smaller decrease in water potentials in a salt-tolerant cultivar was earlier observed by M. Fozouni et al. (2012). Apparently, a smaller decrease in water potentials can be considered as a sign of salt tolerance.

A conclusion can be made from this study that salinity has a negative effect on the parameters of vegetative growth. The same results were observed S. R. Bhagwat et al. (2021) who showed that salt-affected soils had an adverse effect on vegetative growth, yield, and quality parameters of vines.

Conclusions

The functional abilities of a grapevine depend on the salinity level and the genotype. Cv. 'Asma' demonstrated higher salt tolerance compared to hybrid M. No. 8-08-8-4 ('Kok Pandas' × 'Zeibel 6357'). It can be assumed on the basis of the conducted experiments that the best adaptation to increased salt content is associated with the ability of plants to reduce the areas of their leaves and roots and decrease their leaf water potentials under salt stress. Such characteristics of a genotype lead to a smaller change in the water-salt regime of the plant and indicate its ability to better adapt to an increase in salinity. Plant responses to salt stress were similar in the potted plants and those grown *in vitro*.

A reduction in root length is suggested as an indicator of salt tolerance for grapevines grown *in vitro*. Thus, tissue culture has good potential as an early diagnostic technology for salt tolerance evaluation in grapevine and for rapid screening of tolerant plants.

Further research is required to assess the long-term effects, particularly in view of important accumulations of chlorides and sodium in leaf tissues.

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