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SALT TOLERANCE BASED ON MORPHOLOGICAL VARIABILITY IN SPRING WHEAT (*TRITICUM AESTIVUM* L.)

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SUMMARY

Soil salinity appears as one of the most relevant abiotic factors negatively affecting wheat yield and quality, and creating varieties that can adequately respond to this factor is an urgent task today. The presented study assessed the response of wheat (*Triticum aestivum* L.) genotypes to salinity at the ontogenetic stage based on variations in morphometric parameters. Eight spring wheat cultivars' evaluation identified salt-tolerant genotypes beneficial in future breeding programs. The study of salt stress with varied influence on the shoot and root length of wheat seedlings ensued. The results revealed substrate salinity leads to a significant decrease in seed germination, length and weight of shoots and roots, and leaf area (by 12.8%–97.6%). Wheat cultivars displayed varied responses to different salinity concentrations based on morphometric indicators. According to the degree of negative impact on wheat plants, the salinity types can be as NaCl < Na_2SO_4. The highest sensitivity of wheat cultivars to salinity was evident at the seed germination and the growth of seven-day-old seedlings. Based on the presented study, some spring soft wheat (Kayraktash, Pakhlavon, Es-4, and Es-61) cultivars showed distinction with different levels of salt tolerance that can benefit future breeding programs.

Keywords: Spring wheat (*T. aestivum* L.), cultivars, salt tolerance, chloride salinity, sulfate salinity, response to salinity, germination and growth traits

Key findings: Four wheat (*T. aestivum* L.) cultivars were tolerant to chloride and sulfate salinity at the juvenile stage of development from the eight studied samples. These are Kayraktash, Pakhlavon, Es-4, and Es-61, providing the highest germination at different salinity levels. These genotypes can be a valuable source in future breeding programs.

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INTRODUCTION

Soil salinity is one of the major limiting factors negatively affecting wheat (*Triticum aestivum* L.) growth and development, ultimately leading to decreased growth and productivity, and even with other crops' yield worldwide (Song and Wang, 2015; Shrivastava and Kumar, 2015; Abd-Elgawad *et al*., 2016; Ding *et al*., 2018). Globally, about a quarter of arable soils are saline with varying degrees, and by 2050, the soil salinity share may be more than 50% in cultivated areas (Liu *et al*., 2020; Chaurasia *et al*., 2022).

In Uzbekistan, the total irrigated area is 4.2 million hectares, of which 1.956million hectares of land (46.6%) is saline with varying degrees. About 1.316 million hectares is slightly saline (31.3%), 541,500 hectares is moderately saline (12.9%), and 987,000 hectares (2.4%) is highly saline, preventing further expansion of crop areas (Khamidov and Khamrayev, 2019).

Wheat is the chief crop, ranking first among grain crops consumed by humans worldwide (FAO, 2019; Ramzan *et al*., 2020; Saddiq *et al*., 2021). However, despite its importance as a staple food crop, the considerable decrease in grain yield and quality is largely due to soil salinity. Development of salt-tolerant cultivars is one of the most effective ways to solve this problem, which involves the search for donors of such traits as effective sources (Oyiga *et al*., 2016). Therefore, for the effective assessment of different genotypes, early diagnostic methods are vital in significantly reducing the time wastage of breeders (Balandrán-Quintana *et al*., 2015).

For determining salt tolerance, using several methods are used that differed in seed germination techniques, salt solution concentrations, and accounting diverse elements (rate of seed swelling in salt solutions, germination energy, seed germination, and length and weight of seedlings) (Cuin *et al*., 2010; Yakovets, 2011). Different ideas exist about the generality of plant responses to stressors and the nonspecific nature of stress resistance (Bome *et al*., 2006).

Laboratory diagnostics allow evaluating genotypes' resistance to stress conditions in a shorter time, especially in the early stages of ontogenesis. Studies revealed that crop plants in the seedling phase were most sensitive to stress, and differences appeared among the cultivars during this period can remain as a genetic trait in adult plants (Ivanov *et al*., 2001; Lisitsyn, 2003). Therefore, in practice, the laboratory methods for diagnosing salt tolerance are widely applicable in different crop plants (Korobko and Volkov, 2013; Sandukhadze *et al*., 2015; Wahyuning *et al*., 2015; Dashtoian *et al.*, 2023).

Past studies suggested the study of characteristics of early ontogenesis with a detailed analysis of the primary root system and aboveground organs of seedlings. This considers a set of quantitative traits that could be valuable for the selection of salinity-resistant types of cultivated plants. Therefore, based on the above discussion, the presented study sought to assess the salt tolerance in spring wheat cultivars at the ontogenetic stage based on variations in morphometric parameters and predict the response of genotypes to salinity.

MATERIALS AND METHODS

Plant material

In the presented study, using eight spring soft wheat (*Triticum aestivum* L.) cultivars included Kayraktash, E'zoz, Ok Marvarid, and Pakhlavon from the Uzbek selection. The other four cultivars, Es-1, Es-4, Es-7, and Es-61, came from the International Maize and Wheat Improvement Center (CIMMYT). These underwent screening for salt tolerance.

Salt tolerance at seed germination phase

The wheat experiments commenced at the Samarkand State University, Sh.R. Rashidov, Samarkand, Uzbekistan. The evaluation of spring soft wheat cultivars for salt tolerance continued under laboratory conditions using two methodological approaches: a) germination of seeds in saline solution in Petri dishes and b) germination of seeds on BioGro soil mixture in growing vessels (Vadez *et al*., 2007). Before the experiment, wheat seeds' sterilization with 90% alcohol for two minutes disinfected the surface from harmful microflora and washed twice with distilled water. Developing chloride salinity employed 1.40% NaCl solution, and for the sulfate salinity, 2.78% Na_2SO_4 solution, which corresponds to an osmotic pressure of solutions of 1 MPa. In the first approach, each sample's placement for germination, consisting of 30 grains, proceeded in sterile Petri dishes on filter paper with two-layers, moistened with a 1.4% solution of sodium chloride (NaCl), a 2.8% solution of sodium sulfate (Na_2SO_4), and distilled water (control). Each variant was in triplicate. The seed dishes placed in a MEMMEPT UH 30 thermostat had a constant temperature of 24 °C ± 1 °C for seven days. On the seventh day of the experiment, the seed germination, the length of shoots and roots, the number of roots, and the wet and dry weight of shoots and roots incurred accounting.

In the second methodological approach, BioGro soil mixture served as a substrate, filling the growing vessels 2.6 kg of this mixture, measuring a diameter of 18 cm. Salinization materialized by spreading the seeds with salt solutions. In the control, the BioGro soil mixture moistening used distilled water only. The sample size was 100 seeds in triplicate for each option. Seed germination progressed in a climate chamber (growth chamber GC-1000) with a constant temperature of 24 °C ± 1 °C for seven days.

On the 10th day of the experiment, the data recording occurred for the seed germination ratio, length of shoots and roots, the number of roots, length and width of the first leaf, wet and dry mass of shoots and embryonic roots, with the first leaf area also calculated. All the data based on various parameters reached analysis and processing as per the standard methods (Lakin, 1988). The analyses ran through the data processing program Statistica 6.0 and STATAN.

RESULTS

Seed germination at early stages

For the first time, the local and foreign spring wheat (*Triticum aestivum* L.) cultivars' screening transpired in the early phase of the growing season. The results revealed the negative impact of saline solutions on the seeds' germinating viability. Salinity induced by NaCl and Na_2SO_4 causes several specific reactions to germinating spring wheat seeds. Violations can also affect the inhibition of swelling, impaired seed pecking, and at the later stages, causing variations in growth of the primary roots, lateral roots, and coleoptile.

It is a well-known fact that the highest sensitivity of spring wheat cultivars to stress factors manifested at stages of seed germination and seedling development (up to seven days), later (on the 10th day); however, the differences between chloride and sulfate salinity were less prominent (Belozerova and Bome, 2014). This was also consistent with the obtained data, and for this reason, we studied seven-day-old seedlings.

Wheat growth at initial stages

The wheat seeds' swelling (Figure 1a), pecking (Figure 1b), and seedling formation (Figure 1c-d) revealed all three different stages. At the initial stage of seedlings, plants' division can be into four groups, according to biometric indicators (Figure 2). These are two wheat cultivars with root growth inhibition and a slight decrease in shoot growth, two cultivars with shoot growth inhibition and a slight reduction in root growth, three cultivars with shoot and root growth inhibition, and one wheat genotype observed with a slight decrease in shoot and root growth.

The reaction of each spring wheat cultivar to salinity has individual varietal characteristics, demonstrating four different types of reactions. The inhibition of the growth of individual vegetative organs of wheat, corresponding to the scheme presented as

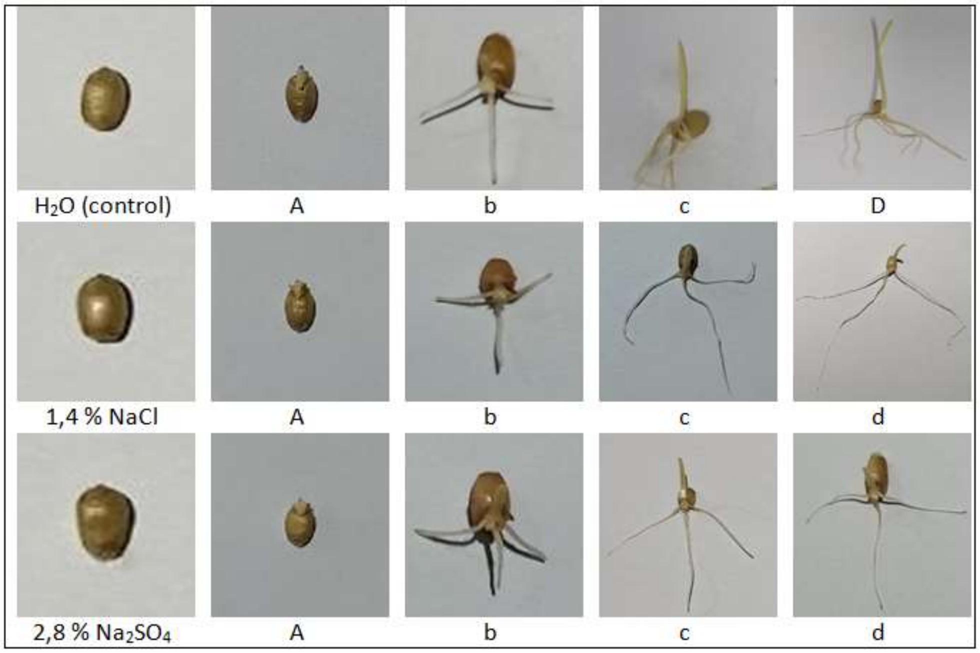

Figure 1. Initial stages of seed germination in spring wheat cultivars: a) swelling, b) pecking, and c-d) seedling formation.

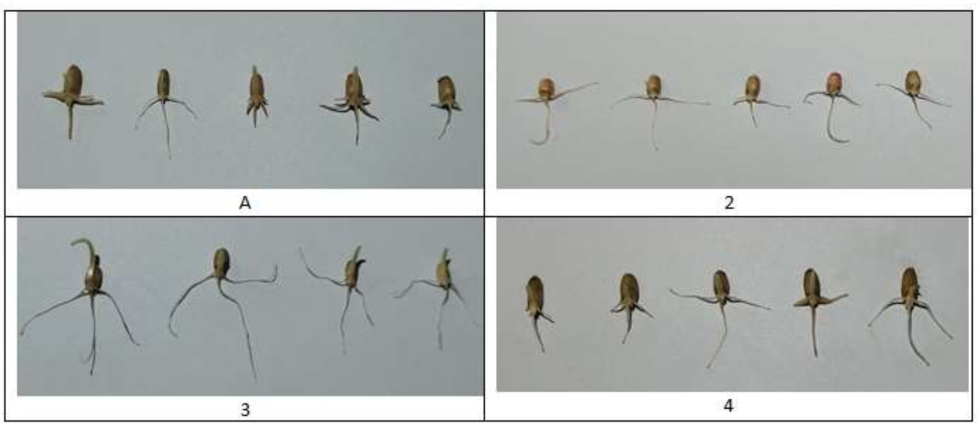

Figure 2. Salt-tolerant types in the seedlings of spring wheat cultivars (1 - 4).

above. The wheat cultivars Es-7 and Es-4 classified under the first type, cultivars Pakhlavon and Kayraktash as the second type, wheat cultivars Ok marvarid, E'zoz, and Es-61 as the third type, and genotype Es-1 as fourth (Figure 2). Based on the results, it was valid that under salinity conditions in Petri dishes and growing vessels, a significant decrease in seed germination appeared: against the NaCl (24.1%–36.8%) and against the background of Na_2SO_4 (45.4%–54.6%), compared with the genotypes used in the control (Figure 3).

The wheat cultivars Pakhlavon (90.2%) and E'zoz (87.4%) emerged with the highest seed germination in Petri dishes in the control variant. At the NaCl salinity, the cultivars Pakhlavon and Kayraktash (76.3%) were leading with 77.8% – 76.3% of viable seeds. At the Na_2SO_4 salinity, the cultivars Kayraktash and Pakhlavon stood out (61.6% and 51.4%,

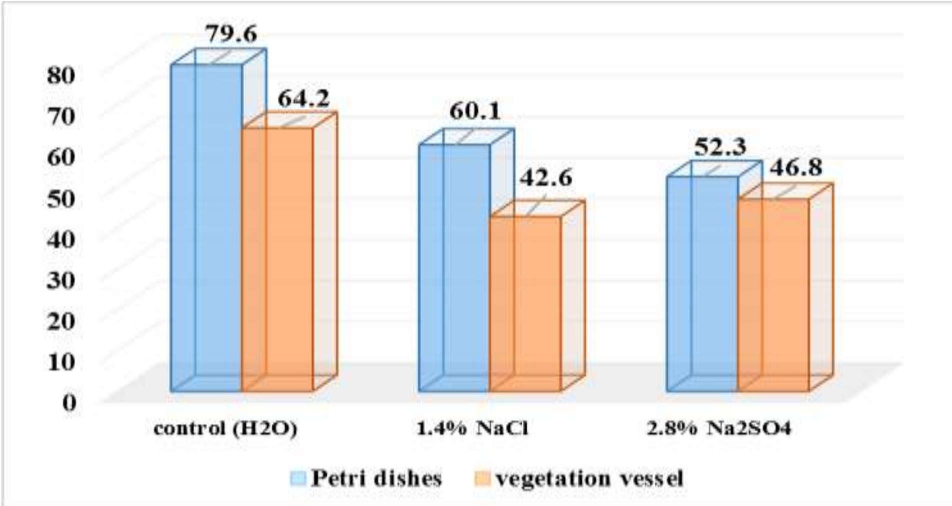

Figure 3. Seed germination (%) of 7-day-old spring wheat seedlings under different salinity conditions (average for 8 cultivars), % of control.

Table 1. The influence of various types of salinity on the manifestation of morphometric characteristics of spring wheat seedlings.

Characteristics	Control (H_2O)	NaCl		Na_2SO_4	
	X±m_x	X±m_x	±, %	X±m_x	±, %
Petri dish (average of 8 cultivars)					
Number of roots (#)	3.9±0.08	3.4±0.06*	-12.8	3.2±0.07*·	-17.9
Spine length (mm)	114.3±3.11	24.2±0.81*	-80.7	6.4±0.35*·	-94.4
Shoot length (mm)	82.5±1.96	4.1±0.32*	-95.0	2.0±0.07*·	-97.6
Vegetation vessels (on average for 8 cultivars)					
Number of roots (#)	4.3±0.05	4.9±0.06*	+22.3	4.8±0.06*	+11.6
Root length (mm)	118.2±2.48	42.6±0.71*	-63.9	44.3±0.67*·	-62.5
Shoot length (mm)	171.2±2.82	71.2±1.79*	-58.4	68.4±1.79*	-60.0
Sheet length (mm)	136.2±2.48	38.4±1.67*	-71.8	34.2±1.71*	-74.9
Sheet width (mm)	2.6±0.05	2.8±0.04*	+7.0	2.1±0.05*	-19.2
Sheet area (mm^2)	342.2±6.34	91.2±3.78*	-73.4	84.2±4.82*	-75.4

Note: Statistically significant differences * - with control, NaCl at $P < 0.05$, % - deviation from control.

respectively). The lowest percentage of seed germination was evident in the cultivar Es-61, i.e., control (49.3%), with NaCl (36.4%), and with the background of Na_2SO_4 (24.6%).

In vegetation vessels, the highest rates of seed germination against the chloride and sulfate salinity backgrounds resulted in the local cultivars Kayraktash (NaCl = 57.8%, Na_2SO_4 = 43.2%) and E'zoz (NaCl = 54.6%, Na_2SO_4 = 29.7%). Foreign cultivar Es-61 showed the smallest differences in germination with the control under sulfate salinity conditions, amounting to 15.4%. The exotic cultivar Es-61 also showed distinction by the greatest sensitivity to both types of salinity, and the decrease in the percentage of viable seeds relative to the control was 24.6% to 15.4%.

The salt tolerance assessment in the seven-day-old wheat seedlings used morphometric parameters to predict the response of various cultivars to salinity and identify the tolerant genotypes. Salinity led to suppressing most morphometric parameters, especially in seedlings in Petri dishes (Table 1). For the average number of roots compared with the control, a considerable decrease occurred for the said trait in experimental variants in Petri dishes (12.8% to 17.9%), while in vegetation vessels, an excess of 11.6% to 22.3% manifested. Cultivar Ok marvarid, in all variants of both experiments, formed the most number of roots. In the presented experiment, by comparing two types of salinity, it was evident that sulfate salinity in Petri dishes led to a significant decrease in the number of roots; however, no difference appeared in vegetation vessels.

By assessing wheat cultivars in Petri dishes in the control variant, the length of the roots varied from 82.4 ± 3.23 mm (Kayraktash) to 148.4 ± 8.16 mm (Ok marvarid), with an average value for cultivars of 114.3 ± 3.11 mm (Table 1). By germinating seeds in saline solutions, the embryonic roots length decreased to 6.4 ± 0.35 mm (with Na_2SO_4) to 24.2 ± 0.81 mm (with NaCl). For the said trait, the smallest deviation from the control (by 37.4% to 78.3%) was apparent in variants with salinity and cultivars Ok Marvarid and Kayraktash. Significant inhibition of this trait against the Na_2SO_4 background was visible in spring wheat cultivars Es-4 and E'zoz.

Growth features at early stages of ontogenesis

In the vegetation experiment, in the control treatment, the primary root length ranged from 140.3 ± 2.43 mm (Es-1) to 162.1 ± 2.68 mm (Ok marvarid), with an average of 118.2 ± 2.48 mm. In the experimental variants, the differences among the effects of different types of salinity were less prominent, compared with the experiment in Petri dishes for root length. In seedlings under NaCl, the primary root length decreased by 62.5% under stress conditions, while with Na_2SO_4 by 63.9%. By germinating seeds in Petri dishes, seedlings formed shorter shoots (82.5 ± 1.96 mm) than shoots in vegetation vessels (171.2 ± 2.82 mm). Sulfate salinity in Petri dishes significantly reduced shoots compared with chloride, and on average, the shoot length for various cultivars ranged from 2.0 ± 0.07 and 4.1 ± 0.32 mm, respectively. In vegetation vessels, this feature varied from 68.4 ± 1.79 mm (with Na_2SO_4) to 71.2 ± 1.79 mm (with NaCl) (Table 1).

The vegetation experiment further allowed comparing the effect of different types of salinity on the morphometric parameters of the first true leaf in spring wheat cultivars. It was impossible by conducting the said experiment in Petri dishes. The study also confirmed in vegetation vessels with sulfate salinity, a significant decrease in the leaf length, width, and leaf area surfaced, with average values for wheat cultivars (34.2 ± 1.71, 2.1 ± 0.05, and 84.2 ± 4.82, respectively) (Table 1).

By studying the morphometric characteristics of seedlings under sulfate salinity stress conditions, it was remarkable that cultivar Ok Marvarid gave more developed shoots with a shoot length of 105.0 mm, leaf length (62.1 mm), and leaf area (148.36 mm^2). Significant suppression of the aerial parts was the notable characteristic of the wheat cultivar Es-4 with the shoot length (34.5 mm), leaf length (11.6 mm), leaf width (1.3 mm), and leaf area (34.2 mm^2). Regarding the phenotypic manifestation of all morphological characteristics, the experimental seedlings were considerably inferior compared with the control variants.

Under salinity stress conditions, an increase in the variability of most studied characteristics of seedlings was distinctive, especially when assessing the salt tolerance of wheat cultivars in Petri dishes (Table 2). However, under salinity conditions, the number of roots varied to a lesser extent, regardless of the experimental variant, and the leaf width in vegetation vessels. With NaCl background in Petri dishes, the greatest variability emerged in the shoot length (98.46%), while in vegetation vessels, in the leaf length and leaf area (72.35 and 74.63 mm^2, respectively). For Na_2SO_4 during seed germination in Petri dishes, the root length significantly varied (71.24 mm). Meanwhile, in vegetation vessels, variations were in the leaf length and leaf area (74.65 and 76.28 mm^2, respectively). In the vegetation experiment with chloride salinity, the coefficient of variation varied from 13.64% (number of roots) to 74.28% (leaf area), while with sulfate salinity, the said variation was from 14.84% (number of roots) to 74.65% (leaf length).

Table 2. Variability of morphometric characteristics of spring wheat seedlings under the influence of various types of salinity (V, %).

Characteristics	H_2O (Control)	NaCl	Na_2SO_4
Petri dishes (average of 8 cultivars)			
Number of roots (#)	19.68	26.68	28.87
Root length (mm)	39.86	45.34	71.24
Shoot length (mm)	36.86	98.46	38.47
Vegetation vessels (average of 8 cultivars)			
Number of roots (#)	14.56	13.64	14.84
Root length (mm)	37.23	23.18	24.12
Shoot length (mm)	29.84	46.10	44.72
Sheet length (mm)	38.41	72.35	74.65
Sheet width (mm)	17.87	16.89	23.69
Sheet area (mm^2)	38.87	74.63	76.28

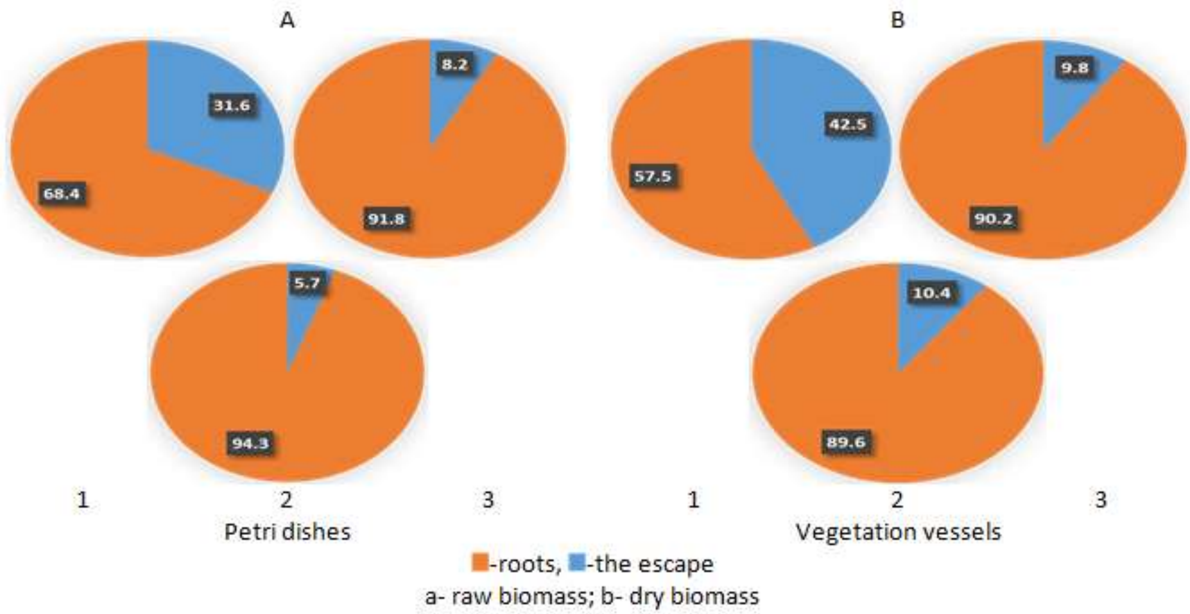

Figure 4. The ratio of the root system and shoots in the wet dry biomass of spring wheat seedlings (average for 8 cultivars), %. Note: 1-H_2O (control); 2-1.4% NaCl; 3-2.8% Na_2SO_4.

The analysis of wet and dry biomass showed a decrease in the dry mass of shoots under stress conditions, regardless of the salinity types and the assessment method used. On average, for wheat cultivars under salinity stress conditions, the biomass structure of raw and dry seedlings had dominance from roots in both Petri dishes and growing vessels (Figure 4). By growing in the BioGro soil mixture, the seedlings had more developed shoots, and accordingly, the highest wet and dry weight, compared with plants obtained from the seeds germinated in saline solutions.

In identifying salt-tolerant wheat cultivars, the scoring relied on the percentage reduction of the studied traits in relation to the control. According to the latest results, two local spring wheat cultivars (Kayraktash and E'zoz) were distinct for sulfate salinity, with exotic selection (Es-4) identified with the background of chloride salinity. Spring wheat cultivars Ok marvarid, Es-7, and Es-61's suppression was to a lesser extent than the control variant for most of the studied traits. The foreign cultivar Es-1 turned out to be the most sensitive to chloride and sulfate salinity. In vegetation vessels, the cultivars Kayraktash and Pakhlavon with sulfate salinity and cultivar Ok marvarid with chloride salinity were among the most resistant ones. The cultivar Es-1 showed the greatest sensitivity to the salinity effects of chloride and sulfate.

DISCUSSION

Soil salinity is one of the factors with a negative effect on wheat, manifesting the deterioration of its various properties and functions, which leads to a decrease in productivity (Houshmand *et al*., 2014; Balandrán-Quintana *et al*., 2015; Bai *et al*., 2013; Marchenkova *et al*., 2017). According to the literature, due to reduced level of metabolism, the more resistant types often have lower productivity, although production requires stable, environmentally flexible, and highly productive cultivars. In this regard, modern wheat breeding strives to develop new cultivars that combine high salt tolerance and productivity (El-Hendawy *et al*., 2007).

Overcoming the negative effects of environmental stress factors has a way to use the tolerant cultivars, especially the local selection (Vlasenko, 2016; Al-Ashkar *et al*., 2019). Several studies admit the possibility of combining such properties in one plant by breeding highly flexible cultivars. Model experiments serve to evaluate the breeding material at the seedling stage to isolate resistant types. Studies revealed relatively little variation among the cultivars in salt tolerance during germination (Malcolm *et al*., 2003). Although, according to the data provided by Munns *et al*. (2006), screening for salinity resistance during germination was moderately important. Oyiga *et al*. (2016) successfully screened the tolerant genotypes at the germination stage using different salinity concentrations (100, 150, and 210 mmol/L). Zhu *et al*. (2016) reported despite the significant genetic diversity in salt tolerance of wheat, modern elite cultivars could not grow with high soil salinity conditions.

Most of the genotypes produce low grain yield by exposing to salinity above 150 mmol/L NaCl, and this discrepancy may be due to differences in plant species and salt concentrations (Munns *et al*., 2006). The presented results based on the identification of bread wheat cultivars tolerant to elevated salt concentrations were consistent with the findings of Oyiga *et al*. (2016). They have established that, based on increased level of chloride and sulfate salinity concentrations, the maximum sensitivity of wheat in response to stress factor manifests in the early stages of ontogenesis. Thus, according to the results, it was obvious that salinity induced by different concentrations of NaCl and Na_2SO_4 causes several specific reactions in seed germination, seedling formation, and embryo growth.

The latest results also aligned with similar results obtained in wheat (Kononenko *et al*., 2019; Belozerova and Bome, 2014; Mubushar *et al*., 2022) and millet (Zhirnova *et al*., 2022). These studies also reported the effects of the stress factor manifest themselves at the stages of seed germination and seedling growth. The selected NaCl and Na_2SO_4 concentrations allowed us to evaluate the spring wheat cultivars for tolerance, with the genotypes ranked based on the percent reduction in the studied traits relative to the control. The presented result suggested the concentrations of NaCl and Na_2SO_4 proved effective in detecting the genotypic variation for the resistance during germination.

Salt tolerance measurement can also indicate less reduction in biomass and yield of a crop under saline conditions (Manaa *et al*., 2011; Majeed *et al*., 2022). It is knowledge that the power of biomass development is also one of the dominant characteristics while assessing resistance to salinity. The said approach was operational in the latest study, which facilitated rapid agroecological comparison of spring wheat cultivars under laboratory conditions. Based on the results, the study established substrate salinity (NaCl and Na_2SO_4) leads to a significant decrease in seed germination, shoot and root length and weight, and leaf area (by 12.8% to 97.6%).

Moreover, this effect was more distinct under conditions of sulfate salinity (Table 1).

The presented results were analogous to past findings in winter wheat under stressful conditions, and a decrease in morphometric parameters occurs, regardless of the type of salinity and the assessment methodology used (Taymazova and Muslimov, 2021; Cherkasova *et al*., 2024). The unequal response of wheat seedlings on growth parameters like roots, aerial parts, and the accumulation of biomass could refer with different mechanisms of salt tolerance in the studied genotypes.

CONCLUSIONS

Based on the laboratory and vegetation assessment of eight spring wheat (*Triticum aestivum* L.) cultivars, the four genotypes (Kayraktash, Pakhlavon, Es-4, and Es-61) were notable at the juvenile stage of development. They showed resistance to chloride and sulfate salinity in all morphometric traits and are of practical interest in future wheat-breeding programs.

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