



Evaluating the Growth Response of *Ricinus communis* L. Cultivars to Salt Stress Using a Fast and Reliable Bioassay

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Abstract

Here is proposed an easy, fast, and economic technique to investigate plant physiological traits in response to salt stress by using castor bean (*Ricinus communis* L.). Four *Ricinus communis* L. cultivars (TUNI 1, TUNI 4, C1012, and C1028) were grown in a growth chamber for 15 days on a sand substrate and were watered regularly with deionised water or NaCl solution (water electrical conductivity (EC) 4, or 8 dS m⁻¹) to keep soil moisture close to field capacity. According to roots, stem, and germination traits and derived indices, TUNI 1 and TUN4 showed the best growth performance on sand irrigated with 4 dS m⁻¹ and 8 dS m⁻¹, respectively. Besides, comparing C1028 and C1012, the former showed a higher tolerance at the maximum electrical conductivity tested related to the latter, increasing salt tolerance index (STI) of roots (+25%) and stem (+20%). In conclusion, the present findings suggest that the TUNI 4 cultivar of *Ricinus communis* L. demonstrates superior overall performance, even under highly saline conditions, such as irrigation with 8 dS m⁻¹. Conversely, the TUNI 1 cultivar shows a good ability to perform in marginal soils with moderate salinity levels, such as those irrigated with 4 dS m⁻¹. Our results represent the first step towards optimization of a cost-effective method/bioassay for the selection of salt tolerant *Ricinus communis* L. cultivars and genotypes.

Keywords Abiotic stress · Cultivar screening · Salt tolerance · Sand · Salinity stress

1 Introduction

Sustainable agriculture is limited worldwide by the lack of fresh-water resources. As a consequence of climate change, freshwater reserves in many regions are running out, pushing to the use of lower quality water, such as saline water, for crop irrigation (Ahmed et al. 2007; Feng et al. 2019; Li et al. 2015). According to Cheng et al. (2021) meta-analysis, 17 world Countries are relying upon saline water to irrigate crops, which is defined as water with an electrical conductivity (EC) > 0.6 dS m⁻¹ (Paranychiakis and Chartzoulakis 2005). Saline irrigation lowers water potential, inducing drought stress in plants, and can result in specific ion

toxicity, nutrient imbalances, oxidative stress, and hormonal disruptions (Formisano et al. 2024).

In addition, the use of saline water for crop irrigation entails modifications of soil properties, which can be reflected in a loss of fertility due to physical characteristic alterations and waterlogging issues (Li et al. 2019; Mohanavelu et al. 2021; Munns and Tester 2008; Tedeschi and Dell'Aquila 2005). Saline stress particularly affects the early stages of plant development, such as seed germination and seedling growth (Wang et al. 2019, 2021b). It can also result in plant growth inhibition, leaf damage, and hinder root elongation (Arif et al. 2019; Munns 2002; Paranychiakis and Chartzoulakis 2005).

One of the possible strategies to ensure an adequate crop yield, while reducing pressure on fresh-water reserves (Sun et al. 2013), is the selection of plant species and genotypes tolerant to brackish water (Ahmed et al. 2007; Paranychiakis and Chartzoulakis 2005).

Phytotoxicity tests with plants and algae have been used since the 1980s for testing chemicals, substrates, and hazardous materials (Gopalan 1999; Lewis 1995; Meers et al. 2007; Wang 1985, 1991). These tests investigated mainly

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seeds germination to assess the toxicity of a substance. However, many researchers stress out the importance of root elongation studies, since roots are the first organs subjected to salinity stress (An et al. 2003; Julkowska et al. 2014). The root meristem acts as a salt-stress sensor, with high salt levels inhibiting root growth by suppressing cell elongation and division (Wu et al. 2015; Munns and Tester 2008). Wang (1985) found root elongation to be more sensitive than germination to low-concentration toxicity in millet, while Linder and McBee (1989) noted that germination reflects direct soil toxicity, and root elongation shows the effects of water-soluble constituents. Nakamura et al. (2021) further demonstrated that wheat roots are especially sensitive to salt stress in the early seedling stage. In any case, the study of the aboveground biomass must be kept into consideration (Campagna-Fernandes et al. 2016). Different methodologies, such as pot experiments, hydroponics, or agar plates, are used nowadays to evaluate the influence of salt stress on crop growth (Nakamura et al. 2021). However, many salinity tolerance bioassays fail to discern genotypic dissimilarities due to the short-term endpoint: the salt effects on the whole plant require time to be visible (Munns 2002; Munns and James 2003).

In light of these challenges, this study seeks to determine whether specific genotypes of castor bean (*Ricinus communis* L.) are capable of exhibiting higher germination rates and better physiological adaptation to saline conditions, thus making them suitable for cultivation in saline and arid lands. Castor bean is cultivated for numerous industrial and energy products and is known for its resilience in marginal lands (Carrino et al. 2020). However, its early developmental stages, such as germination (Severino and Auld 2013a), seedling establishment (Pinheiro et al. 2008), and root growth (Janmohammadi et al. 2012), are highly susceptible to salinity stress, with considerable intraspecific variation. The salinity tolerance threshold for castor bean is 7.1 dS

m^{-1} , though this is genotype-dependent (Babita et al. 2010; Lakshamma et al. 2017; Zhou et al. 2010). It is hypothesized that certain genotypes of *Ricinus communis* possess distinct physiological traits that confer greater tolerance to saline water, thereby improving germination, root growth, and overall plant development under saline conditions. To address this, we present a novel approach to evaluating plant physiological responses to salt stress, focusing on germination, root, and stem elongation inhibition, using castor bean (*Ricinus communis* L.). The study aims to identify salt-tolerant genotypes of *Ricinus communis* L. with high germination rates and physiological traits suited for saline tolerance. By identifying salt-tolerant and salt-susceptible cultivars, this study aims to contribute to the sustainable expansion of castor bean cultivation, providing a potential solution for utilizing saline and marginal lands that are otherwise prohibitive for agriculture.

2 Materials and Methods

The bioassay was made by using plastic boxes originally designed for dragée (Jordan almonds) ($30 \times 40 \times 240$ mm). The bottom of each box was closed with waterproof tape, while the longer side was closed with normal tape, leaving 2 cm between the two tapes (Fig. 1).

Ten seeds of each of the four tested cultivars were individually sown in containers filled with silica sand to simulate the extreme conditions of marginal lands such as the desert. Twenty-five containers were placed in a plastic tray ($150 \times 200 \times 60$ mm) that provided a standing position and acted as water reservoir. The trays were placed in a growth chamber (25 °C, day/night cycle: 12/12 h, 40% relative humidity) and moved every two days to avoid position bias. The plastic containers were watered regularly with deionised water or NaCl solution (water EC 4, or 8 dS m^{-1}) to

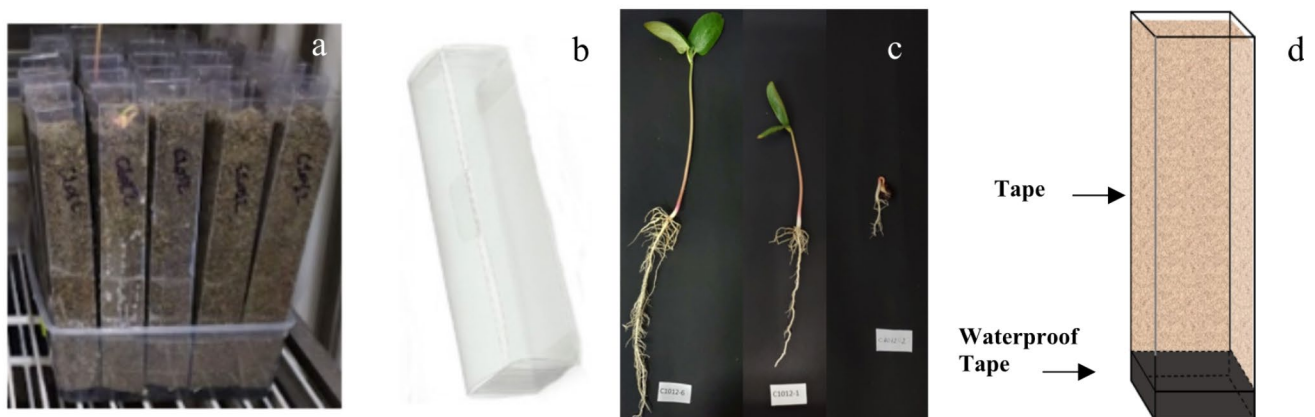


Fig. 1 (a) experimental set-up, (b) plastic box employed in the bioassay, (c) comparison at 0, 4 and 8 dS m^{-1} of C1012 cultivar and (d) schematic representation of the box closed with waterproof tape on the bottom and normal tape on the longer side

keep soil moisture close to field capacity. The EC of the water used in the experiment was monitored before each irrigation with a laboratory conductimeter (Conductimeter Basic 30, Crison). In addition, sand EC was evaluated at the begin and at the end of the experiment (1:5 soil: water solution ratio– Conductimeter basic 30, Crison). At emergence, germinated seeds were counted every day. The salt sensitivity of each studied cultivar was assessed 15 days after sowing. The length of plant main root and stem was measured, as their fresh weight. Subsequently, plants were dried at 50 °C until constant weight and then weighted to assess dry weight. Three replicates per each saline level were used, thus 30 seeds per each cultivar and each saline level were used (total: 360 seeds). The initial EC of the sand averaged 0.23 dS m⁻¹, while at the end of the experiment, the sand EC was 0.30 dS m⁻¹ for the sand treated with saline water at 4 dS m⁻¹, and 0.43 dS m⁻¹ for the sand treated with saline water at 8 dS m⁻¹.

2.1 Plant Material

Two tested cultivars (TUNI 1 and TUNI 4) were North African ecotypes, obtained from Tunisia, where castor bean is cultivated in pre-desert areas. The remaining two cultivars (C1012 and C1028) were provided by Kaiima Ltd. (Israel), a castor genetics and breeding company that develops castor hybrids. At first, an additional local ecotype, collected near the seaside in Pozzuoli (Naples's province, Italy), was tested but, being its germination rate lower than 65% at 0 dS m⁻¹, it was not included in the bioassay according to Wang et al. (2001). Average weight of 100 seeds was: 562 g for TUNI 1; 577 g for TUNI 4; 318 g for C1012; 278 g for C1028.

2.2 Data Elaborations

The saline tolerance of plants was assessed by different indices associated to the number of germinated seeds and to the measurements done at the end of the monitoring period on roots and stems. Seeds were considered germinated when the main root length was > 2 mm from the tip to the beginning of the hypocotyl. Seeds were considered emerged after the appearance of the seedling through the sand. Root length was measured from the base to the tip of the main root. Rotten seeds were considered dead. Stems were measured from the hypocotyl to the cotyledons.

Germination Percentage (GP) was calculated as follows:

$$GP = \frac{SG}{\text{total number of seeds}} \times 100$$

where SG is the number of germinated seeds.

RSG is Relative Seed Germination and calculated as:

$$RSG = \frac{SG_s}{SG_c}$$

where: *SG_s* is the relative seed germination in saline treatment and *SG_c* is the relative seed germination in the control (Luo et al. 2018).

Germination index (GI) was calculated according to Macagno et al. (2019) and Visioli et al. (2014) as:

$$GI = \frac{RSG}{RE} \times 100$$

In addition, Emergence Percentage (EP) and Relative Emergence percentage (RE) were assessed as:

$$EP = \frac{E}{\text{total number of seeds}} \times 100$$

$$RE = \frac{RE_s}{RE_c} \times 100$$

where E is the number of seedlings, *RE_s* is the relative emergence at the different salt levels and *RE_c* is the same parameter in the control.

The root parameter considered in our bioassay was Main Root Length (MRL).

Furthermore, Root to Mass Fraction (RMF) was calculated according to Ozaslan et al. (2016) as

$$RMF = \frac{\text{root d.w.}}{\text{plant d.w.}} \times 100$$

Stem Length (SL) was measured at harvest, and Stem-to-Mass Fraction (SMF) was assessed as RMF (Poorter et al. 2011).

2.3 Salt Tolerance and Susceptibility Indices

Stress Susceptibility Index (SSI) and Salt Tolerance Index (STI) were used to evaluate the potential of each cultivar to grow under saline stress (Dehnavi et al. 2020; Fernandez 1992).

$$SSI = \frac{1 - \frac{y_s}{y_p}}{1 - \frac{Y_s}{Y_p}}$$

$$STI = \frac{y_p \times y_s}{(Y_p)^2}$$

where $\bar{y}_s$ is the mean of the considered parameter in the control, and $\bar{y}_p$ is the one under saline stress. $\bar{Y}_s$ instead is the mean of the studied character of all the tested cultivars in the control and $\bar{Y}_p$ is that in salt stress condition. A higher value of SSI corresponds to lower stress tolerance; conversely, a higher value of STI suggests a higher stress tolerance. All the indices employed are reported in Table 1.

2.4 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) through GLM (General Linear Model) with Minitab 19 statistical software package (Minitab Inc., State College, PA, USA), and mean values were separated according to least significant difference test (LSD, $P \leq 0.05$).

3 Results

3.1 Germination and Emergence Parameters

The average emergence for all the cultivars was delayed by 0.4 days at medium EC treatment, and by 3.7 days at high EC compared to the control (data not shown), with average of 6.6 days to emerge at 0 dS m⁻¹, 7 days at 4 dS m⁻¹, and 10 days at 8 dS m⁻¹. The effect of the interaction of cultivars and saline levels on the different germination and emergence parameters (SG, RSG, GP, E, RE and EP) is shown in Table 2. In general, C1012 and C1028 showed a decrease in the number of germinated seeds (SG) and of the derived indices (RSG and GP) at 4 dS m⁻¹ compared to the control, while TUNI 1 and TUNI 4 showed no differences. Moreover, TUNI 1 registered a tendency of increased germination parameters (SG, RSG, and GP) at 4 dS m⁻¹ (Table 2). For C1012, RSG at 4 dS m⁻¹ was (-37.5%), as well as for C1028 (-40.9%), compared to the non-saline treatment.

Differently, all cultivars showed a decrease of SG, RSG and GP at 8 dS m⁻¹. Comparing the tested cultivars at the different saline treatments from the best to the worst according to SG, RSG, and GP, TUNI 1 and TUNI 4 performed better than C1028 and C1012 both at 4 dS m⁻¹ than 8 dS m⁻¹.

Regarding emergence parameters (E, RE, and EP) all the tested cultivars showed a decrease at 4 and at 8 dS m⁻¹ with the exception of TUNI 1 which at 4 dS m⁻¹ was similar to the control, as already reported for germination features. The highest reduction of E, RE, and EP was recorded for C1012 at 8 dS m⁻¹ (92%).

The effect of the cultivar by saline level interaction on the germination index (GI) is shown in Fig. 2.

For all the cultivars GI decreased with increased EC and C1012 resulted the most susceptible to the higher salinity levels (Fig. 2) while TUNI 1 GI was significantly higher than the other cultivars at both saline levels. On the other hand, TUNI 1 GI reduction from 4 to 8 dS m⁻¹ was one of the highest (59%), surpassed only by C1012 (78%). TUNI 4 and C1028 decreased their GI by 31.4% and 48.8% at 4 dS m⁻¹, and by 38.8% and 32.5% at 8 dS m⁻¹, respectively.

3.2 Growth Parameters

The effect of the interaction of cultivars and saline levels on MRL and SL is shown in Fig. 3.

For all the tested cultivars, MRL and SL decreased as saline EC of irrigation water increased (Fig. 3).

A significant reduction of MRL and SL was recorded for all the tested cultivars at 4 dS m⁻¹ compared to the control, while no significant reduction was recorded for TUNI 1. The longest main root at 4 dS m⁻¹ was found for TUNI 1 (18.7 cm), followed by TUNI 4 (12.3 cm), C1012 (8.9 cm) and C1028 (6.7 cm). On the contrary, all cultivars MRL and SL showed a significant reduction at 8 dS m⁻¹ compared to the control. The effect of the interaction cultivars x saline

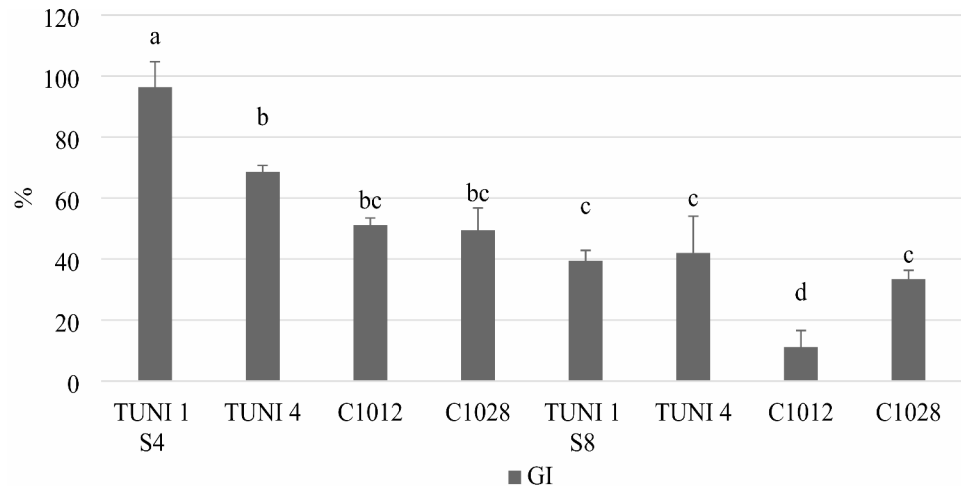
Table 1 List of the indices investigated, their abbreviation, formula and references

Abbreviation	Index	Formula	References
<i>SG</i>	Seed germination	Number of germinated seeds	
<i>RSG</i>	Relative Seed Germination	(SGs/SGc) x 100	Luo et al. 2018
<i>GP</i>	Germination Percentage	(SG/ total number of seeds) x 100	Rekik et al. 2017
<i>GI</i>	Germination index	(Gs*RLs/Gc*RLc) x 100	Macagno et al. 2019; Visioli et al. 2014
<i>E</i>	Emergence	Number of seedlings	
<i>EP</i>	Emergence Percentage	(E/ total number of seeds) x 100	Sun et al. 2013
<i>RE</i>	Relative EP	(EPs / EPc) x 100	Sun et al. 2013
<i>MRL</i>	Main Root Length	Main root length (cm)	
<i>RMF</i>	Root Mass Fraction	(Root d.w./ plant d.w) x 100	Ozaslan et al. 2016
<i>SL</i>	Stem Length	Stem length (cm)	
<i>SMF</i>	Stem Mass Fraction	(Stem d.w. / plant d.w) x 100	Poorter et al. 2011
<i>SSI</i>	Salt Susceptibility Index	(1-ys/yp)/(1-Ys-Yp))	Dehnavi et al. 2020; Fernandez 1992
<i>STI</i>	Salt Tolerance Index	(yp x ys)/(Yp) ²	Dehnavi et al. 2020; Fernandez 1992

Table 2 Number of germinated seeds (SG), relative seed germination (RSG), germination percentage (GP), number of seedlings (E), emergence percentage (EP) and relative emergence (RE) in the four cultivars (TUNI1, TUNI 4, C1012 and C1028) at 0, 4 and 8 dS m⁻¹. S0 is the saline treatment at 0 dS m⁻¹, S4 at 4 dS m⁻¹ and S8 at 8 dS m⁻¹. Values (mean of three replicates) followed by the same letter do not differ significantly according to the LSD test ($P < 0.05$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

		SG	RSG (%)	GP (%)	E	RE (%)	EP (%)
Cultivar (C)	TUNI 1	23 a	88 a	78 a	21 a	82 a	71 a
	TUNI 4	24 a	94 a	81 a	18 b	72 b	60 b
	C1012	15 b	64 b	51 b	13 c	56 c	44 c
	C1028	15 b	68 b	50 b	13 c	61 c	44 c
Saline level (S)	S0	25 a	100 a	82 a	24 a	100 a	81 a
	S4	19 b	77 b	64 b	17 b	68 b	56 b
	S8	14 c	60 c	48 c	8 c	35 c	28 c
C x S	TUNI 1 - S0	26 a	100 a	87 a	26 a	100 a	87 a
	TUNI 1 - S4	27 a	105 a	90 a	26 a	100 a	87 a
	TUNI 1 - S8	17 bc	65 cd	57 bc	12 c	46 bc	40 c
	TUNI 4 - S0	27 a	100 a	83 a	25 ab	100 a	83 ab
	TUNI 4 - S4	22 ab	88 ab	73 ab	16 c	64 b	53 c
	TUNI 4 - S8	18 bc	72 bc	60 bc	13 c	52 b	43 c
	C1012 - S0	24 a	100 a	80 a	24 ab	100 a	80 ab
	C1012 - S4	15 cd	62 cd	50 cd	14 c	58 b	47 c
	C1012 - S8	7 e	29 e	23 e	2 e	8 d	7 e
	C1028 - S0	22 ab	100 a	73 ab	22 b	100 a	73 b
	C1028 - S4	13 cd	60 cd	43 cd	11 c	50 b	37 c
	C1028 - S8	10 de	48 de	33 de	7 d	32 c	23 d
Significance	C	***	***	***	***	***	***
	S	***	***	***	***	***	***
	C x S	*	*	*	***	***	***

Fig. 2 Effect of cultivar (TUNI 1, TUNI 4, C1012 and C1028) by saline level interaction on germination index (GI). Data represent the mean of three replicates. S4 at 4 dS m⁻¹ and S8 at 8 dS m⁻¹. Columns sharing the same letter are not statistically different at $P < 0.01$ according to LSD test



levels on roots, stems and plant dry weight, and on RMF and SML are shown in Table 3.

All cultivars root dry weight showed a significant reduction both at 4 dS m⁻¹ and at 8 dS m⁻¹ compared to the control. In all the cultivars, root dry weight at 4 dS m⁻¹ decreased on average by 55% compared to the control, ranging from 35% in TUNI 1 to 81% in C1028, (Table 3). At 8 dS m⁻¹, an average root biomass decrease of 82% (compared to the control) was recorded, with a reduction of 70% and 91% for TUNI 4 and C1028, respectively. On average, roots were 1.9-fold lighter for all the cultivars at medium saline

level, besides for C1028 (5.2-fold). At the highest saline treatment, TUNI 4 roots were 3.3-fold lighter compared to the control, followed by TUNI 1 (5.7-fold), C1012 (6.9-fold), and C1028 (11.3-fold). The mean root biomass allocation (RMF) highlighted an increased root production at the highest saline level compared to the medium one (Table 3). However, cultivars had a different behaviour since the relative growth of roots in TUNI 1 and TUNI 4 was higher at 8 dS m⁻¹ than at 4 dS m⁻¹, while C1012 and C1028 showed the same RMF regardless of the saline levels (Table 3).

Fig. 3 Main root (MRL) and stem length (SL) mean value $\pm$ SE of the four cultivars according to water EC and cultivar (TUNI1, TUNI 4, C1012 and C1028). S0 is the saline treatment at 0 dS m^{-1} , S4 at 4 dS m^{-1} and S8 at 8 dS m^{-1} . Mean values with the same letter do not differ according to the LSD test $P < 0.001$. Capital letters are for MRL, lower case for SL

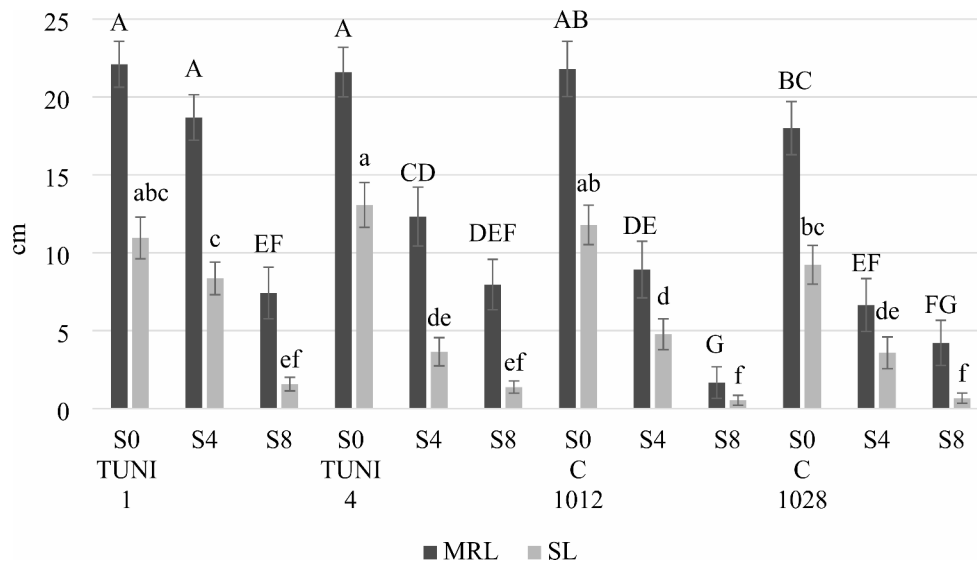


Table 3 Roots, stem and whole plant dry weight (d.w.), mean root-mass-fraction (RMF), and mean stem-mass-fraction (SMF) as affected by NaCl level in irrigation water supplied to the tested cultivars (TUNI1, TUNI 4, C1012 and C1028). S0 is the saline treatment at 0 dS m^{-1} , S4 at 4 dS m^{-1} and S8 at 8 dS m^{-1} . Values sharing the same latter are not statistically significant at $P < 0.05$ according to LSD test. $P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns, not significant

		d.w. roots (g)	d.w. stem (g)	d.w. plant (g)	RMF	SMF
Cultivar (C)	TUNI 1	0.083 a	0.072 a	0.269 a	29.0	21.9 a
	TUNI 4	0.060 b	0.058 b	0.178 b	28.1	19.3 a
	C1012	0.042 c	0.026 c	0.110 c	20.7	12.1 b
	C1028	0.048 bc	0.021 c	0.115 c	25.1	10.4 b
Saline level (S)	S0	0.107 a	0.067 a	0.278 a	33.2 a	20.5 a
	S4	0.049 b	0.050 b	0.204 b	18.0 c	15.6 b
	S8	0.019 c	0.017 c	0.053 c	26.0 b	11.6 b
C x S	TUNI 1 - S0	0.137 a	0.095 a	0.395 a	32.1 ab	27.1 abc
	TUNI 1 - S4	0.089 bc	0.093 a	0.356 ab	22.7 bcd	24.7 ab
	TUNI 1 - S8	0.024 ef	0.027 cde	0.057 fg	32.2 ab	19.9 abcd
	TUNI 4 - S0	0.101 bc	0.091 a	0.315 bc	29.8 abc	27.0 a
	TUNI 4 - S4	0.049 de	0.055 b	0.273 cd	15.8 d	13.0 cde
	TUNI 4 - S8	0.031 ef	0.027 cde	0.074 de	38.6 a	17.9 bcd
	C1012 - S0	0.076 cd	0.044 bc	0.191 e	30.9 abc	20.3 abc
	C1012 - S4	0.036 ef	0.029 cd	0.114 f	15.5 d	13.2 cde
	C1012 - S8	0.011 f	0.005 e	0.036 g	15.8 d	2.5 f
	C1028 - S0	0.113 ab	0.036 bc	0.212 de	40.0 a	613.7 cde
	C1028 - S4	0.022 ef	0.021 cde	0.073 fg	18.1 cd	11.5 de
	C1028 - S8	0.010 f	0.007 de	0.044 fg	17.3 cd	5.9 ef
Significance	C	***	***	***	ns	***
	S	***	***	***	***	***
	C x S	*	*	***	*	*

A significant reduction of stem dry weight was recorded at 8 dS m^{-1} for all cultivars, while at 4 dS m^{-1} only TUNI 4 and C1028 showed a significant stem dry weight reduction, compared to the control (Table 3). The magnitude of stem dry weight decrease was higher at 8 dS m^{-1} compared to the control, from 70% for TUNI 4, up to 81% for C1028. The mean stem mass fraction (SMF) was less impacted than the RMF. In TUNI 1, SMF did not differ between saline levels, while in TUNI 4 the reduction of SMF was the same at 8 dS

m^{-1} and at 4 dS m^{-1} . The greatest reduction of SMF was observed in C1012 at 8 dS m^{-1} .

3.3 Salt Tolerance and Susceptibility Indices

Salt tolerance index (STI) and salt susceptibility index (SSI) results are reported in Table 4.

The highest score of STI at 4 dS m^{-1} was obtained by TUNI 1 for all the studied characteristics, suggesting its

Table 4 Salt tolerance index (STI), salt susceptibility index (SSI) for the studied characteristics in saline treatment supplied to the tested cultivars (TUNI1, TUNI4, C1012 and C1028). Bold number represent the higher value for STI, and underlined one for SSI, i.e., the best and the worst cultivar, respectively. MRL = main root length; SL = stem length; SG = number of germinated seeds. S4 is the saline treatment at 4 dS m⁻¹ and S8 at 8 dS m⁻¹

EC	Cultivar	STI					SSI						
		d.w. plant (g)	d.w. roots (g)	d.w. stem (g)	MRL	SL	SG	d.w. plant (g)	d.w. roots (g)	d.w. stem	MRL	SL	SG
S4	TUNI 1	1.81	1.06	2.00	0.94	0.72	1.15	0.27	0.64	0.08	0.35	0.43	-0.17
	TUNI 4	0.58	0.43	1.13	0.61	0.38	0.97	1.44	0.95	1.55	0.67	1.32	0.83
	C1012	0.26	0.24	0.29	0.45	0.44	0.59	1.21	1.00	1.33	0.64	1.09	1.69
	C1028	0.25	0.21	0.17	0.27	0.26	0.47	<i>1.53</i>	<i>1.48</i>	<i>1.63</i>	<i>0.69</i>	1.12	<i>1.84</i>
S8	TUNI 1	0.29	0.30	0.58	0.38	0.14	0.72	<i>1.06</i>	0.99	0.95	0.89	0.94	0.73
	TUNI 4	0.30	0.27	0.56	0.39	0.14	0.79	0.94	0.85	0.94	0.85	0.99	0.70
	C1012	0.09	0.08	0.05	0.08	0.05	0.27	1.00	1.04	<i>1.18</i>	<i>1.24</i>	<i>1.05</i>	<i>1.49</i>
	C1028	0.12	0.10	0.06	0.17	0.05	0.36	0.98	<i>1.11</i>	1.07	1.03	1.02	1.15

good tolerance to medium saline stress. The lowest score was found for C1028. At 8 dS m⁻¹, TUNI 4 achieved the highest STI value for plant d.w., MRL, and SG, and the same STI as TUNI 1 for SL. The highest STI for roots and stem d.w. was achieved by TUNI 1. At the same saline level, the lowest STI for all the parameters was obtained by C1012. Regarding salt susceptibility, similar results were obtained for the two saline treatments, with TUNI 1 scoring the lower value at medium EC. It is interesting to point out that all the cultivars acted differently, according to SSI index. At medium saline level, the highest value of SSI for stem length (SL) was achieved by TUNI 1, consistent to the corresponding reduction in SL achieved by the same cultivar (-72%). Similarly, the greatest reduction in plant d.w. showed by TUNI 1 at the highest saline treatment (-86%), is followed by the highest SSI for the same characteristic on the same cultivar. Salinity tolerance and susceptibility indices are related to plant biomass, therefore, to crop yield. The SSI index is useful in predicting the most tolerant cultivar with a higher yield stability by comparing their biomass yield in the absence and with saline or other abiotic stresses (Fernandez 1992). Furthermore, the combined use of SSI and STI can be used to highlight the higher biomass yield in different conditions for different cultivars, genotypes, or subspecies (Fernandez 1992). Therefore, according to SSI and STI, TUNI 1 showed the highest biomass stability at 4 dS m⁻¹, and TUNI 4 at 8 dS m⁻¹.

4 Discussion

The emergence was delayed at 4 dS m⁻¹ and 8 dS m⁻¹ suggesting that salinity can lengthen the establishment time for castor seedlings, preventing root development (Severino et al. 2012; Silva et al. 2005; Wang et al. 2021; Zhou et al. 2010).

In addition, at the medium saline level of irrigation water (4 dS m⁻¹), all cultivars reported a decrease of germination indices (SG, RSG and GP) and emergence parameters (E, RE and EP) at 4 dS m⁻¹, compared to the control with a significant reduction in all cultivars besides of TUNI 1, suggesting a high adaptation of this cultivars to the medium EC level. In addition, TUNI 1 showed an increasing tendency of the above-mentioned indices coherently with literature reporting an enhanced germination of some plant species under saline irrigation (Mbarki et al. 2020; Sun et al. 2013). The high GI of TUNI 1 at the medium saline level can be explained by the stimulation in germination reached in this treatment by the cultivar. Increased germination features and improved seedling establishment is in accordance with various authors, who found the germination ability of castor bean seeds positively correlated with their weight, meaning

that heavier seeds perform better than lighter ones (Carvalho et al. 2010; Severino and Auld 2013b).

However, at 8 dS m⁻¹ all tested cultivars showed an evident stress response resulting in a significant reduction of germination (SG, RSG and GP) and emergence (E, RE and EP) indices whose values were lower for C1028 identified as the cultivar with the lowest tolerance to high water salinity.

Stem length proved to be less sensitive to salt stress in accordance with Nakamura et al. (2021), who had the same results on wheat. This can be seen also from SMF values, that were less impacted than RMF. Being the roots the first organs to encounter salt stress, it is reasonable to imagine that, at least at the seedlings stage, the roots are more impacted than stems. This is in accordance with the results obtained by Jiao et al. (2019), where the outcome caused by saline stress on the height of castor bean plants was not significant between 0, 200 and 250 mM of NaCl treatment after 10 days, while it was significant after 30 days of salt stress.

All tested cultivars reported a significant root biomass reduction at both 4 dS m⁻¹ and 8 dS m⁻¹. It is well recognised that plants can modulate the architecture of their root system, revealing great root plasticity in response to hostile abiotic conditions (Karlova et al. 2021). Root plasticity, enabling roots to avoid highly saline soils while maximizing water and nutrient uptake, is part of the salt-tolerant root ideotype (Shelden and Munns 2023). Poorter et al. (2011), state that when plant biomass is reduced in response to abiotic stress by more than 50% (compared to the control), a strong increase in RMF is found at the expense of stems. This can explain why the greatest values of RMF in this study were found at the highest saline level. Salinity reduction of all roots-related traits is in accordance with different studies conducted on *R. communis* (Janmohammadi et al. 2012; Jeschke and Wolf 1988; Wang et al. 2021; Zheng et al. 2021).

The dry weight of stems showed an analogous trend as the one of roots, with the only exception at 8 dS m⁻¹ with the two North-African cultivars (TUNI 1 and TUNI 4) that presented dry weight of the stem higher, than the two Kaiimas (C1012 and C1028). However, the reduction of stem biomass was not so marked as for roots even if a more subtle response was also recorded for SMF values. In a study by Temme et al. (2020), the response to salt stress of sunflower plants changed the biomass allocation with a 50% increase in RMF, and a reduction of 35% in SMF. The dry weight difference at 4 dS m⁻¹ was much more intense for roots than for stems. Similar results were also obtained by Jiao et al. (2019) and Zhou et al. (2010).

All the cultivars subjected to saline treatment showed a greater decrease in root dry weight than in stem dry weight. A higher reduction of root traits in response to salinity was

observed also by other authors. For instance, Janmohammadi and coworkers (2012) reported a 78% reduction of root dry weight, and a stem decrease only by 56%. Zhou et al. (2010) described a stem length decrease of 37% and a main root length reduction of 49%. As reported by several authors, roots are more susceptible to saline water irrigation (Nakamura et al. 2021), perhaps for their proximity to Na⁺ and Cl⁻ ions (Janmohammadi et al. 2012) that can lead to a decline of cell division and elongation.

The trends of STI and SSI were consistent with germination and plant growth at both 4 dS m⁻¹ and 8 dS m⁻¹, confirming that TUNI 1 and TUNI 4 are the cultivars best adapted to salt stress. These findings suggest that these two cultivars are well-suited for cultivation in soils irrigated with highly saline water, highlighting their potential feasibility in such challenging conditions.

5 Conclusions

In conclusion, the present findings suggest that the TUNI 4 cultivar of *Ricinus communis* L. demonstrates superior overall performance, even under highly saline conditions, such as irrigation with 8 dS m⁻¹. Conversely, the TUNI 1 cultivar shows a good ability to perform in marginal soils with moderate salinity levels, such as those irrigated with 4 dS m⁻¹. The two genotypes exhibit higher germination rates and better physiological adaptation to saline conditions, enabling their cultivation in saline and arid lands. The current manuscript represents the first step towards optimization of a cost-effective method/bioassay for the selection of salt tolerant *Ricinus communis* L. cultivars and genotypes. Further studies will be undertaken with a larger number of cultivars, coupled with genetic and molecular mechanisms analysis, to implement the obtained findings.

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Data Availability Data will be made available on request.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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