biology and life sciences forum

MDPI

Type of the Paper (Proceedings, Abstract, Extended Abstract, Editorial, etc.)

Evaluation of durum wheat genotypes at germination stage under salinity stress †

Mauro Vaccarella *[1], Benedetto Frangipane [1], Sergio Saia [2], Luciano Raimondo [1], Antonino Rigoglioso [1], Rosa Petralia [1], Marco Genduso [1] and Claudia Miceli [1]

[1] Council for Research in Agriculture and Economics - Research Center for Plant Protection and Certification Viale Regione Siciliana sud/est, 8669 – 90121 Palermo – Italy; benedetto.frangipane@crea.gov.it; (B.F.); luciano.raimondo@crea.gov.it (L.R.); rosa.petralia@crea.gov.it;(R.P); marco.genduso@crea.gov.it; (M.G.); antonino.rigoglioso@crea.gov.it (A.R.); claudia.miceli@crea.gov.it (C.M.)

[2] University of Pisa Dept. Veterinary Sciences, sergio.saia.unipi.it (S.S.)

* Correspondence: mauro.vaccarella@crea.gov.it (M.V.)

† Presented at the 3rdt International Electronic Conference on Agronomy, 15-30 October 2023; Available online: https://iecag2023.sciforum.net/

Abstract: The aim of this work was to investigate several durum wheat genotypes (11 landraces, 2 old varieties and 7 modern cultivars) for salt tolerance at germination and early growth stages. Seeds were tested under 2 different concentrations of NaCl solution (50 mM and 100 mM) and the control (T) with distiller water. Experiments were laid out in a two-factorial with four replications. Two-way ANOVA was performed and means were compared with Duncan's Multiple Range test. Seven parameters were measured under laboratory conditions: germination, mean germination time, shoot length, root length, roots number, shoot dry matter and root dry matter. Rusticano, with the highest value of root number (5.44), was statistically different from all other genotypes Timilia reste bianche, Timilia reste nere, Ciciredda and Cappelli are highlighted for the best root length performance at 100 mM.

Keywords: durum wheat; Sicilian landraces; germination traits; salt stress

Citation: To be added by editorial staff during production.

Academic Editor: Maria Martínez Mena

Published: date

1. Introduction

Salinity is one of the most severe abiotic stress factors affecting plant growth and agricultural production worldwide. It affects almost 1 billion ha worldwide, globally representing about 7% of continental extent of the earth [1]. Agricultural crops exhibit a wide spectrum of responses under salt stress. Salinity affects almost all aspects of plant development including germination, vegetative growth and reproductive development. It strongly inhibits seed germination through osmotic stress, ion-specific effects, and oxidative stress. Sicily, with its variable pedoclimatic conditions, is an important source of agrobiodiversity. In this context, over the past centuries, farmers have made a continuous selection that has led to the creation of numerous landraces.

Landraces are named and maintained by traditional farmers to meet their social, economic, cultural, and environmental needs. Durum wheat [(*Triticum turgidum* L. subsp. *durum* (desf.) Husn.] is a typical Sicilian crop with a cultivated area of about 270,000 ha; although on small areas (about 5,000 ha), in the last decade the cultivation of landraces has been reintroduced [2]. Landraces, which have arisen through a combination of natural selection and the selection performed by farmers usually have a broader genetic base and can therefore provide valuable characteristics important for breeding. To date 24 Sicilian durum wheat landraces are listed in the national register of varieties. (https://www.sian.it/portale-sian/home.jsp consultation in 2023/07/22). Information on the genotypic tolerance to salt stress during the germination process are lacking in Sicilian

durum wheat landraces. Limited literature work is documented so far on these genotypes so knowing their behavior can help breeding programs in the selection of salt tolerant varieties to achieve optimum wheat growth under saline condition. The aim of this work was to investigate several durum wheat genotypes (11 landraces, 2 old varieties and 7 modern cultivars) for salt tolerance at germination and early growth stages.

2. Materials and methods

Laboratory experiments were conducted to evaluate the germination traits of 20 durum wheat genotypes at Council for Research in Agriculture and Economics – Research Centre for Plant protection and Certification Palermo, Italy in 2023. The seeds of all the genotypes tested were obtained in our experimental station during the growing season 2021/2022 and stored at 5 °C with 30 % relative humidity.

Seven modern varieties of durum wheat (Ciclope, Duilio, K-26, Mongibello, Quadrato, Rusticano and Simeto), two old varieties (Capeiti 8 and Trinakria) and eleven landraces (Cappelli, Castiglione glabro, Ciciredda, Francesa, Perciasacchi, Russello, Russello Ibleo, Scorsonera, Timilia reste bianche, Timilia reste nere and Urria) were assessed. Seeds were tested under 2 different concentrations of NaCl solution (50 mM and 100 mM) and the control (T) with distiller water. Seeds were surface sterilized in 5% sodium hypochlorite solution for 3 minutes, washed thoroughly under tap water for 10 minutes and at the end with distilled water. To avoid water losses, edges of Petri dishes were tightly sealed with an impermeable colorless parafilm. Seeds were allowed to germinate at 20 °C in the dark.

To each Petri dishes, sterilized in oven at 120 °C for 2 hours, 12 ml of solution was added to keep the filter paper uniformly soaked-wet without floading. Fifty randomly selected seeds for each variety were placed in Petri dishes having 13.5 cm diameter (one replication is made up of 2 petri dishes containing 25 seeds each) on double layer of Whatman filter paper No. 1 with the crease facing down and kept in a thermostatic cabinet (KW Scientific Equipment model WRS 85). Shoot and root length, fresh and dry weight were recorded after 7 days. Data for shoot and root length were obtained from 50 seedling in each replication.

The seedling's fresh and dry weight was taken with the help of digital balance (Mettler Toledo PR503 Delta Range); dry weight was measured by placing at 80 °C in a hot air oven for 24 hours until constant weight was observed.

Experiments were laid out in a two-factorial design with three replications using a complete randomized design (CRD). Two-way ANOVA was performed and means were compared with Duncan's Multiple Range test at 5% level of probability. The assumption of normality and homoscedasticity has been verified with Shapiro-Wilk and Levene's test, respectively.

Seven parameters were measured under laboratory conditions: germination, mean germination time (MGT), shoot length, root length, roots number, shoot dry matter and root dry matter.

Germination percentages were recorded daily up to 7th day using radicle extrusion (≥ 2 mm long) as a criterion. A seed was considered to show abnormal germination if shoot growth occurred in the absence of radicle extension. It expresses as the ratio of germinated seeds at the 7th day to the total number of seeds using the following formula:

$$G = (c / a) * 100$$

where a = total number of seeds, c = number of germinated seeds at 7th day.

Mean germination time (MGT, days): This parameter is determined according to the following formula [3]:

$$MGT = \sum (ni \times di) / \Sigma b$$

where n is the number of seeds germinated on day i, d is the incubation period in days, and b the total number of seeds germinated upon treatment.

To compute shoot and root dry matter, the following formula has been used:

Dry matter (%) = (Dry weight / fresh weight) * 100

3. Results

Genotype, concentration and their interaction showed significant differences in many of the parameters studied (Table 1).

Table 1. Factorial analysis of variance.

Source of variation	df	Germination (%)	MGT	Shoot length (cm)	Root length (cm)	Root number (n°)	Shoot dry matter (%)	Root dry matter (%)
Genotype	19	***	***	***	***	***	***	**
Concentration	2	**	**	***	***	***	***	n.s.
Genotype*Concentration	38	***	n.s.	n.s.	*	n.s.	n.s.	n.s.

*** significantly different at $p < 0.001$; ** significantly different at $p < 0.01$; * significantly different at $p < 0.5$; n.s. not significantly different.

As showed in table 2, Rusticano, Ciclope, Simeto and Mongibello are notable for the significantly shorter mean germination time (range from 2.15 to 2.20 days) compared to Cappelli, Francesa, Perciasacchi and Russello Ibleo (range from 2.43 to 2.68 days). In general, all the landraces have been reported for the better shoot length than modern varieties; the top values are for Ciciredda, Scorsonera, Timilia reste nere and Timilia reste bianche with 7.95, 7.85, 7.76 and 7.69 cm respectively, values significantly higher than all modern varieties.

Rusticano showed the highest value of root number (5.44), statistically different from all other genotypes, however, Timilia reste nere, Timilia reste bianche, Cappelli, Ciciredda were characterized by the lowest values.

In general, modern varieties show significantly higher shoot dry matter values than landraces except for Francesa, Scorsonera and Russello Ibleo.

Table 2. Influence of genotype on some parameters studied.

Genotypes	MGT		Shoot lenght (cm)		Root number (n°)		Shoot dry matter (%)		Root dry matter (%)	
Rusticano	**2.15**	**f**	6.03	efg	**5.44**	**a**	9.99	bc	13.17	abc
K-26	2.26	def	6.68	cde	4.83	cde	10.19	b	12.86	abcd
Capeiti 8	2.55	bc	7.61	abc	4.62	ef	9.28	cdef	13.29	ab
Ciclope	**2.19**	**ef**	5.66	fg	4.80	cde	10.52	ab	11.65	bcde
Cappelli	**2.43**	**cd**	7.09	abcd	**4.12**	**h**	8.57	fg	10.46	e
Scorsonera	2.29	def	**7.85**	**a**	4.46	fg	**9.37**	**cde**	12.89	abcd
Mongibello	**2.20**	**ef**	6.65	cde	5.13	b	9.89	bcd	10.92	cde
Quadrato	2.31	def	6.46	def	5.07	bc	10.42	ab	13.41	ab
Francesa	**2.43**	**cd**	7.13	abcd	4.66	def	**9.15**	**defg**	12.90	abcd
Ciciredda	2.24	def	**7.95**	**a**	**4.22**	**gh**	8.52	fg	11.63	bcde
Castiglione glabro	2.39	cd	7.21	abcd	4.62	ef	8.66	efg	12.85	abcd
Russello	2.33	def	7.32	abcd	4.44	fg	8.48	g	12.43	abcde
Russello Ibleo	**2.68**	**ab**	7.60	abc	4.48	fg	**9.77**	**bcd**	11.34	bcde
Duilio	2.35	de	6.04	efg	4.94	bcd	9.83	bcd	12.33	abcde

Timila reste nere	2.39	cd	**7.76**	**ab**	**3.96**	**h**	8.64	efg	10.47	e
Timilia reste bianche	2.39	cd	**7.69**	**ab**	**4.07**	**h**	8.49	g	11.41	bcde
Perciasacchi	**2.65**	**ab**	6.82	bcde	4.95	bc	8.98	efg	13.14	abcd
Simeto	**2.19**	**ef**	5.34	g	4.42	fg	11.02	a	14.00	a
Urria	2.29	def	7.05	abcd	4.42	fg	9.00	efg	11.44	bcde
Trinakria	2.74	a	6.49	def	4.57	ef	8.91	efg	10.85	de

In each column, mean followed by the same letter are not statistically different with Duncan's Multiple Range test at. 5% of probability level.

Raise of NaCl concentration determined an increase of the mean germination time and shoot dry matter; mean germination time passed from 2.31 days (control) to 2.38 days (50mM) and 2.43 days (100 mM). while shoot dry matter varied from 8.92 (control) to 10.04 (100 mM). Increasing the concentration of NaCl in the solution determined a significant reduction of shoot length from 7.73 cm (control) to 5.67 cm (100 mM). The root number was higher at 50 mM (figure 1).

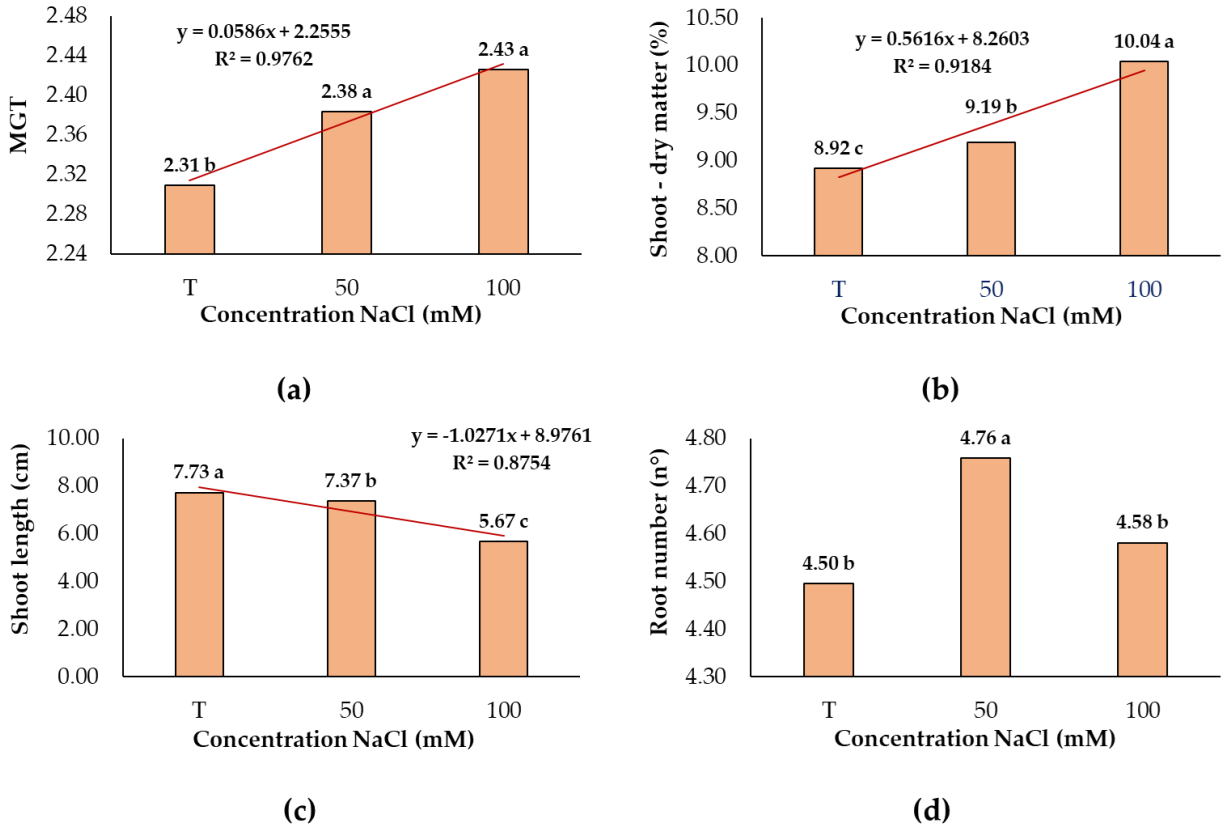

Figure 1. Influence of concentration on: (**a**) Mean Germination Time (MGT); (**b**) Shoot dry matter (%); (**c**) Shoot length (cm); (**d**) Root number. Mean followed by the same letter are not statistically different with Duncan's Multiple Range test at 5% of probability level.

Remarkable germination values, at 100 mM, were obtained by Timilia reste nere (98.67%), Ciclope (98.00 %), Mongibello (96.67%) and Timilia reste bianche (96%): in particular, Timilia reste nere showed the highest value statistically different from the two modern varieties Duilio and Quadrato; Trinakria, Scorzonera, Russello and Russello Ibleo reported the lowest ones statistically different from all other genotypes.

At 50 mM, the landraces Cappelli, Urria, Timilia reste bianche and Timilia Reste nere, showed good performance of germination while Trinakria, with the lowest value, was statistically different from the other varieties tested.

Root length best performances at 100 mM are for Timilia reste bianche (7.88 cm), Timilia reste nere (6.84 cm), Ciciredda (6.75 cm) and Cappelli (6.54 cm); Perciasacchi, Russello Ibleo and Duilio resulted statistically different to Ciciredda, Timilia reste bianche and Timilia reste nere (table 3).

Table 3. Influence of the interaction concentration*genotype on some parameters studied.

Concentration*Genotype		Germination %		Root lenght (cm)	
0 mM	Capeiti 8	97.33	abc	6.89	fghijklmnopqrstuv
	Castiglione glabro	97.33	abc	7.83	cdefghijk
	Ciciredda	95.33	abcdef	7.90	cdefghij
	Ciclope	92.67	abcdefghi	9.22	abc
	Duilio	94.00	abcdefghij	7.72	defghijkl
	Francesa	88.67	fghijkl	8.12	cdefgh
	K-26	90.00	defghijk	6.99	defghijklmnopqrst
	Mongibello	93.33	abcdefghi	7.91	cdefghij
	Perciasacchi	89.33	efghijk	7.05	defghijklmnopqrs
	Quadrato	94.33	abcdefgh	6.94	efghijklmnopqrstu
	Russello	88.00	ghijkl	8.45	bcd
	Russello Ibleo	83.33	klm	7.56	defghijklm
	Rusticano	96.67	abcd	7.26	defghijklmnopqr
	Scorsonera	86.67	ijkl	7.81	cdefghijk
	Cappelli	97.33	abc	8.01	cdefghi
	Simeto	95.33	abcdef	6.37	klmnopqrstuvwxy
	Timila reste nere	95.33	abcdef	9.53	ab
	Timilia reste bianche	91.67	abcdefghij	10.06	a
	Trinakria	82.00	lmn	6.69	hijklmnopqrstuvw
	Urria	93.33	abcdefghi	7.55	defghijklmn
50 mM	Capeiti 8	96.67	abcd	6.92	efghijklmnopqrstu
	Castiglione glabro	88.00	ghijkl	6.69	hijklmnopqrstuvw
	Ciciredda	88.67	fghijkl	6.99	defghijklmnopqrst
	Ciclope	95.33	abcdef	7.38	defghijklmnopq
	Duilio	92.00	abcdefghij	5.98	pqrstuvwxyz
	Francesa	91.33	bcdefghij	7.74	defghijkl
	K-26	92.67	abcdefghi	8.17	cdefg
	Mongibello	95.33	abcdef	6.55	ijklmnopqrstuvwx
	Perciasacchi	89.00	efghijk	6.04	opqrstuvwxyz
	Quadrato	92.00	abcdefghij	6.49	jklmnopqrstuvwx
	Russello	85.33	jkl	7.31	defghijklmnopq
	Russello Ibleo	92.67	abcdefghi	6.48	jklmnopqrstuvwx
	Rusticano	98.33	ab	7.42	defghijklmnop
	Scorsonera	87.33	hijkl	7.47	defghijklmno
	Cappelli	98.00	ab	8.40	bcde
	Simeto	94.00	abcdefgh	6.99	defghijklmnopqrst
	Timila reste nere	94.67	abcdefg	8.28	bcdef
	Timilia reste bianche	94.67	abcdefg	8.22	bcdefg
	Trinakria	74.00	o	6.55	ijklmnopqrstuvwx
	Urria	95.33	abcdef	6.99	defghijklmnopqrst
100 mM	Capeiti 8	94.00	abcdefgh	5.83	rstuvwxyz
	Castiglione glabro	88.67	fghijkl	6.18	mnopqrstuvwxy
	Ciciredda	92.67	abcdefghi	**6.75**	**ghijklmnopqrstuvw**
	Ciclope	**98.00**	**ab**	5.32	wxyz
	Duilio	90.67	cdefghij	5.14	xyz
	Francesa	90.00	defghijk	6.07	nopqrstuvwxyz
	K-26	95.00	abcdefg	6.00	opqrstuvwxyz
	Mongibello	**96.67**	**abcd**	5.77	stuvwxyz
	Perciasacchi	85.33	jkl	4.68	z
	Quadrato	87.33	hijkl	5.78	rstuvwxyz
	Russello	78.00	mno	5.56	tuvwxyz
	Russello Ibleo	78.33	mno	4.93	yz
	Rusticano	95.33	abcdef	6.27	lmnopqrstuvwxy
	Scorsonera	77.33	mno	6.02	opqrstuvwxyz
	Cappelli	95.00	abcdefg	**6.54**	**ijklmnopqrstuvwx**
	Simeto	94.67	abcdefg	5.50	uvwxyz
	Timila reste nere	**98.67**	**a**	**6.84**	**fghijklmnopqrstuv**
	Timilia reste bianche	**96.00**	**abcde**	**7.88**	**cdefghij**
	Trinakria	76.00	no	5.90	qrstuvwxyz
	Urria	94.00	abcdefgh	5.44	vwxyz

In each column, mean followed by the same letter are not statistically different with Duncan's Multiple Range test at 5% of probability level.

4. Discussion

Seed germination is a major factor limiting the establishment of plants under saline condition. As reported by the available literature (4 - 7), our results confirm that durum wheat seeds tend to germinate at a lower rate and consumed longer time when exposed to salt stress. Increasing NaCl concentrations determined a raise in mean germination time and shoot dry matter and a reduction of shoot length of the seedlings.

Interaction of wheat genotypes with salinity level was found to be significant for germination and root length. This means that there was a crossover effect among the cultivar tested for these parameters.

Among the genotypes, all the landraces showed the best performance in shoot length and many of them have been signaled for good performance in germination and root length (Timilia reste bianche, Timilia reste nere, Ciciredda and Cappelli).

The root number value significantly higher at 50 mM is probably due to a stimulating action of the solution to be investigated with further studies. On the other hand, the increase to the maximum concentration resulted in a statistically significant reduction of this parameter.

In conclusion, we can affirm that landraces are interesting genetic materials to be investigated and used in breeding programs for the selection of varieties better tolerant to salt stress. Timilia reste bianche, Timilia reste nere, Ciciredda and Cappelli are highlighted for the best root length performance at 100 mM.

To confirm our results and extend the study at the following growing stages, further study is needed.

Author Contributions: Conceptualization. M.V. and B.F.; methodology. S.S.; software. B.F.; validation. M.V. B.F. and S.S.; formal analysis. B.F.; data curation. B.F.; writing—original draft preparation. M.V. and B.F.; writing—review and editing. C.M.; supervision. M.V.; technical support. A.R., L.R., R.P. and M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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