Effect of salinity on *Echinochloa crus-galli* germination as affected by herbicide resistance

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Abstract

Salinity is one of the major abiotic stresses that may affect yield and quality of crops. Salinization, in combination with the presence of aggressive weeds, such as barnyard grass (*Echinochloa* spp.), can be considered one of the factors responsible for reducing yield in rice fields. The aims of the study were to evaluate the salt effect on germination and first seedling growth of six different Italian common barnyard grass (*E. crus-galli*) populations (three sensitive and three resistant to ALS-inhibitor herbicides) and to verify the presence of differences in salt response between populations sensitive and resistant to the ALS-inhibitor herbicides. Germination tests were conducted under nine different NaCl concentrations (from 0 mM to 400 mM). Significant differences in germination capacity were found between sensitive and resistant populations from 0 mM to 250 mM NaCl; in particular, germination capacity of the sensitive populations was higher (up to 90%) than that of the resistant ones (about 70%). The increase in salinity over 250 mM reduced progressively the germination capacity: from 300 mM onwards, no significant differences were found between sensitive and resistant populations and the germination resulted inhibited for two of them (one sensitive and one resistant). Speed of germination and root and shoot length of seedlings were also inversely related to salt concentration. Time required for achieving 50% of final germination capacity was extended from about three days at 0 mM NaCl up to about 10-12 days at 400 mM NaCl. Root length and shoot length ranged from 9.88 cm and 6.16 cm, at 0 mM NaCl, to 0.36 cm and 0.41 cm, at 400 mM NaCl. According to the results, there is no a clear evidence that response to saline conditions was related to resistance towards ALS-inhibitor herbicides, as in some cases significant differences were found between populations showing a similar herbicide sensitivity. Responses of barnyard grass to salinity are may play a role in the importance of this weed in future scenarios of salt intrusion: for example, a lower speed of germination at increasing salt levels could suggest a delayed emergence of this weed during crop establishment and first growth. To evaluate the real consequences in terms of competitions towards the crop, future studies are needed for assessing the response to salinity of the main rice varieties cultivated in the environment in which the *E. crus-galli* populations tested in this study were collected.

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Introduction

Salinity represents one of the major limitations for yield and quality of a number of different crops (Maggio *et al.*, 2011). According to the estimates of the Food and Agriculture Organization of the United Nations, about 20% of irrigated land worldwide is affected by the increase of the salinity level (Rozema and Flowers, 2008). This phenomenon is accentuated by the competition for fresh water between agricultural and civil uses, which is worsened by climate changes, growing population (Maggio *et al.*, 2011), socio-economic development and water contamination (Balia and Viezzoli, 2015). Salinity conditions are relevant not only to arid and semiarid environments and to the southern regions of the world, but also to the Mediterranean coastal areas. In Europe, 26 countries (Maggio *et al.*, 2011), in particular Spain, Portugal, Italy, Greece (Ghiglieri *et al.*, 2012) and France (Puard *et al.*, 1999) are interested by salinization phenomena (Maggio *et al.*, 2011). In the coastal areas, salinization of aquifers is usually caused by saltwater intrusion (Mongelli *et al.*, 2013) as a result of groundwater overexploitation (Balia and Viezzoli, 2015).

In Italy, this phenomenon is found in various regions, such as Sardinia (Capaccioni *et al.*, 2005), the Catania Plain (Capaccioni *et al.*, 2005), Tuscany (Barrocu, 2003), the Tiber Delta (De Luca *et al.*, 2005), Campania, Calabria (Barrocu, 2003) and the Adriatic coast (Ghiglieri *et al.*, 2012), especially the Po Plain (Antonellini *et al.*, 2008).

Some of these areas, in particular the Po Plain and the Oristano province (Sardinia), are used for rice cultivation. In these areas, the process of salinization may contribute to reduce crop yield, together with the presence of highly problematic weeds. In fact, salinity represents one of the environmental conditions that affect seed germination and plant growth (Sadeghloo *et al.*, 2013), both in weeds and in crops. The knowledge of the ability of seeds to germinate in different environmental conditions, including salinity, is considered of fundamental importance not only for crop establishment, but also for estimating weed development in agricultural ecosystems (Koger *et al.*, 2004; Benvenuti, 2011).

Among the main weed species that infest rice fields, barnyard grasses (several species of the genus *Echinochloa* P. Beauv.), weedy rice (*Oryza sativa* L.), and sedges (mainly the genus *Cyperus* L.) are some of the most troublesome, able to cause significant yield losses (Panozzo *et al.*, 2013).

In particular, *Echinochloa* spp. have been key weeds in almost all rice systems worldwide, including European and Italian rice fields. These weeds are characterized by C4 photosynthetic pathway and some species are able to grow both in dry and flooded conditions (Vidotto *et al.*, 2007). *Echinochloa* spp. exhibit great competition effects towards rice, especially during early stages of cultivation. Gibson *et al.* (2002), for example, found that the competition established by *Echinochloa* spp. is significantly lower if a rice field is maintained free from these weeds during the first 30 days after seeding.

Echinochloa spp. can be distinguished in *red* or *white* biotypes on the basis of the different pigmentation at the basal sheaths of the plant. It has been established that this different plant pigmentation could reflect differences in herbicide sensitivity of the weed (Tabacchi *et al.*, 2006). Problems associated with *Echinochloa* spp. management have worsened in the last decade, due to the selection of populations resistant to different herbicides applied in rice field, in particular ALS and ACC-ase inhibitors (Panozzo *et al.*, 2013). In the search of alternative and effective techniques for controlling these weeds, several methods have been tested, including biocontrol agents (Hershenhorn *et al.*, 2016) and the use of rice varieties tolerant to herbicides (Kraehmer *et al.*, 2017).

Even though the area of rice cultivation affected by salinization is increasing at global level, the response of rice weeds to salt conditions and the potential influences on weed ecology and competition effects have been poorly investigated so far. The stress due to the presence of salt causes physiological and biochemical alterations and tolerance to salinity in plants is related to the synthesis (induced by the stress) of several compounds, including abscisic acid (ABA), glycinebetaine, organic acids, proline, polyamine and polyols (Cowan *et al.*, 1992). The functions of these compounds in response to salt stress seem to be those of chemical signals, osmotic adjustment, free radical scavenging, preservation of proteins and membrane integrity. As concerns the grass weeds, it was found that the tolerance to saline conditions is due to their capacity in uptake and translocation of Na^+, maintaining K^+, and osmotic regulation through the accumulation of proline and glycinebetaine, even though salt tolerance in *E. crus-galli* seems to be not related to Na^+ adsorption changes (Yamamoto *et al.*, 2003).

In the case of *Echinochloa* spp., in particular, it is not yet entirely clear what is the effect of salt stress on their behaviour and physiological pathways. In these terms, a potential delay in germination or a negative influence on seedling establishment and first growth due to salinity could potentially alter the role of these weeds in rice production systems. In addition, the knowledge of the interactions between response to salinity and other agronomical relevant traits, such as herbicide resistance, is essential to estimate potential future impacts of salinity on *Echinochloa* spp. management in rice. In particular, it is not clear if there is interaction between herbicide resistance and salt stress and what could be the biochemical mechanisms involved.

This study aimed to evaluate the effect of salinity on germination of different common barnyard grass (*E. crus-galli* (L.) P. Beauv.) populations collected in the Italian rice area and to verify the presence of differences in terms of salt sensitivity between populations that are susceptible or resistant to ALS-inhibitor herbicides.

Materials and methods

Seed collection

Seeds of six common barnyard grass populations (*E. crus-galli*) were used during the trials. The seeds were collected between 2010 and 2012 in Italian rice fields, which underwent repeated applications of penoxsulam since many years. Penoxsulam is a broad-spectrum ALS-inhibitor herbicide used in rice to control common barnyard grass, water-plantain (*Alisma plantago-aquatica* L.), red stem (*Ammannia coccinea* Rottb.) and other weeds. After collection, the seeds were let dry at room temperature for about one week and then stored at +5°C. At different timings, seedlings from an aliquot of the collected seed lots were tested for resistance to ALS-inhibitor herbicides *via* a greenhouse bioassay and a molecular study that confirmed the presence of target site resistance in three populations (labelled *r1*, *r2*, and *r3*) due to Trp-574-Leuc point mutation in the ALS gene (unpublished data). Thus, the other three populations (*s1*, *s2*, and *s3*) were considered sensitive to penoxsulam. For the present study, seeds of the six populations were taken for the same collected seed lots.

Effect of water salinity on germination

In order to evaluate the effect of water salinity on the germination, 20 seeds for each population were placed in Petri dishes (9 cm diameter) lined with one filter paper imbibed with 5 mL of deionized water or saline solution.

Nine different salt concentrations were applied. Salt solutions were prepared by dissolving NaCl in deionized water at the following concentrations: 0 mM, 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM, 350 mM, and 400 mM. These nine salt concentrations were selected among those applied in previous studies (Chauhan and Johnson, 2009; Sadeghloo *et al.*, 2013; Opeña *et al.*, 2014) and according to the water salinity levels found in some European rice cultivation areas (Isla *et al.*, 2003; Gay *et al.*, 2010; Moret-Fernández and Herrero, 2015) in order to be able to compare the results of our trial with those found in other studies carried out in analogous conditions and in order to use saline concentrations that can be found in the area from which the six common barnyard grass populations tested came from. The number of doses was selected following the guidelines provided by Holland-Letz and Kopp-Schneider (2015).

Three replicates for each salt treatment were used. Petri dishes were sealed with Parafilm to avoid drying and contamination. Afterward, they were incubated in a growth chamber at a constant temperature of 25°C and arranged in a randomized complete block design.

Seed germination was recorded every day for 15 days. Moreover, at the end of the incubation period (15th day), length of roots and shoots of a sample of 10 seeds for each Petri dish was determined.

Two runs of the entire experiment were carried out.

Statistical analyses

ANOVA was carried out on data of germination capacity (total of number of seeds germinated by the end of germination test), speed of germination and length of roots and shoots in order to test the effects of population, NaCl concentration and experiment run. The test was conducted using the open source programme and environment *R* (R Core Team, 2016)

A regression analysis was performed on data of germination capacity. The fitted model was the following 3-parameter log-logistic regression model (Streibig *et al.*, 1993; Knezevic *et al.*, 2007; Vidotto *et al.*, 2013):

$$Y = \frac{d}{1+\exp[b(\log(x)-\log(e))]} \quad (1)$$

where *Y* is the germination capacity, *x* is the NaCl concentration in mM, *d* is the upper limit, and *b* is the relative slope at the point of inflection *e*.

Model fitting was performed using the function *drm* of the add-on package *drc* of the programme *R* (Ritz and Streibig, 2012; Ritz *et al.*, 2015).

The same model was also used to describe the effect on speed of germination, as the relationship between duration of germination test and cumulated number of germinated seeds. The analysis was conducted separately for each salt concentration with the time (days) as the parameter *x* in Eq. (1) and cumulated germination as the dependent variable *Y*. Furthermore, the same analysis was applied to model the relationship between NaCl concentrations (independent variable) and either the length of roots or length of shoots (dependent variables).

The effective concentrations required to reduce by 50% either germination capacity or shoot or root length in comparison to the values obtained at 0 mM salt concentration (EC_{50}) were estimated from the fitted models using the function *ED* of the package *drc*. In the case of cumulated germination, the number of days required to obtain 50% of final total cumulated germination was calculated. The values of EC_{50} were used to perform pair-wise comparisons between populations, or between averages among resistant or susceptible populations, by calculating a Sensitivity Index (*SI*):

$$SI = \frac{EC_{50}(A)}{EC_{50}(B)} \quad (2)$$

where A and B refer to two generic populations under comparison. *SI* was also calculated by considering $EC_{50}(A)$ and $EC_{50}(B)$ in Eq. 2 as estimated by pooling data of resistant or susceptible populations, respectively.

The significance of *SI* of each comparison was calculated by using the function *EDcomp* of the package *drc* of the *R* programme.

Results and discussion

Germination capacity

The results showed that the germination of the tested weed biotypes was affected by the salt treatments (Figure 1).

The analysis of variance showed significant differences in terms of germination capacity between resistant and sensitive populations from 0 mM NaCl to 250 mM NaCl (Table 1). At these saline doses, the germination capacity of the sensitive populations ranged from about 84% to 94% and resulted greater than that of the resistant ones, which ranged from about 60% to 71%.

Seed germination remained quite stable up to a saline concentration of 250 mM, with values slightly above 60% and 80% for resistant and sensitive populations, respectively. These germination values were similar to those recorded in previous studies on

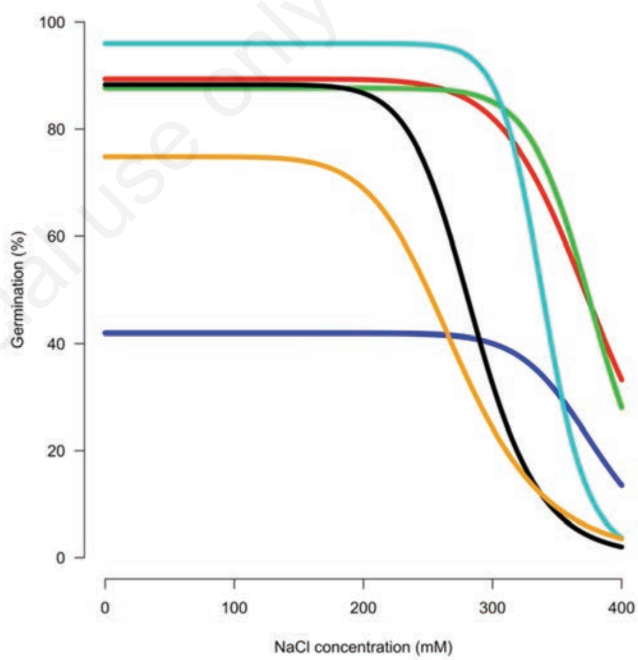

Figure 1. Seed germination (%) of the tested barnyard grasses populations as function of NaCl concentration, as fitted by the following log-logistic model *Y = d/{1+exp[b(log(x) – log(e))]}*. Blue line: population *r1*; orange line: *r2*; green line: *r3*; cyan line: *s1*; black line: *s2*; red line: *s3*.

Table 1. Seed germination capacity (expressed as percentage of germinated seeds) at 15 days of resistant (R) and sensitive (S) populations from 0 mM to 400 mM NaCl concentrations. R and S are averages from ALS-herbicide resistant (*r1, r2,* and *r3*) and sensitive (*s1, s2* and *s3*) populations, respectively.

Population	0 (mM)	50 (mM)	100 (mM)	150 (mM)	200 (mM)	250 (mM)	300 (mM)	350 (mM)	400 (mM)
R	69.44	71.11	69.44	67.50	60.55	63.33	50.55	36.11	13.89
S	91.11	93.89	90.55	92.78	87.78	83.89	69.44	34.44	12.78
t-test significance	*	*	*	*	*	*	ns	ns	ns

R, populations resistant to ALS-inhibitor herbicides (*r1*, *r2*, and *r3*); S, populations susceptible to ALS-inhibitor herbicides (*s1, s2,* and *s3*); *P≤0.05; ns: P>0.05.

E. glabrescens Munro ex Hook in which at a salt concentration of 200 mM the germination was more than 60% and averaged about 73% at 250 mM NaCl (Opeña *et al.*, 2014); similar germination level (68%) was also found on *E. crus-galli* at a salt concentration of 225 mM (Sadeghloo *et al.*, 2013). Conversely, in trials carried out by Chauhan and Johnson (2009), germination of *E. colona* (L.) Link was totally inhibited at the salt concentration of 200 mM. In our study, increasing salt concentration above 250 mM reduced seed germination, until about 13% germination at 400 mM NaCl, with no differences between resistant and sensitive populations. At 400 mM NaCl germination was inhibited for the populations *s2* and *r2*, while it ranged from 5% to 33% for the populations *s1* and *s3*, respectively (Figure 1). At the same NaCl concentration Sadeghloo *et al.* (2013) found that germination was completely inhibited. Previous studies on *E. colona* and *E. glabrescens* also found a linear decrease of germination with the increase of salt concentration (Chauhan and Johnson, 2009; Opeña *et al.*, 2014).

The EC_{50} for germination capacity was the lowest for the resistant population *r2* (EC_{50} 274.47 mM), while the highest for the sensitive population *s3* (EC_{50} 380.73 mM) (Table 2). The pairwise comparison between populations showed significant differences between population *s2* and population *r3* and between population *r2* and *s3* (Table 3). Furthermore, significant differences were also found between the resistant populations *r2* and *r3*. According to these results, there is no a clear evidence that response to saline conditions was influenced by sensitivity towards ALS-inhibitor herbicides, as in some cases significant differences were found between populations showing a similar herbicide sensitivity (such in the case of *s2 vs s3* and *r2 vs r3*).

Speed of germination

The results obtained showed that the appearance of the first germinated seeds delayed with the increase of NaCl concentration (Figure 2). An analogous trend was found by Hakim *et al.* (2011) during a germination test conducted on different weeds, including *E. crus-galli* and *E. colona*. At 0 mM NaCl concentration the seeds began to germinate between the second and the third day from the start of germination test and reached their maximum germination between the fourth and the ninth day. Furthermore, at 0 mM NaCl only populations *s1* and *r2* showed significant differences in the time required for achieving 50% of their final germination capacity: in particular, population *s1* required 2.48 days while population *r2* needed 3.84 days (Table 4; Figure 2).

For the resistant populations, the time required to reach 50% of germination capacity ranged, on average, from a minimum of 2.85 days at 50 mM NaCl to a maximum of 12.42 days at 400 mM NaCl; averaging among sensitive populations, EC_{50} ranged from a minimum of 3.42 days to a maximum of 10.26 days, respectively, at 50 mM and 400 mM salt concentration (Figure 2). At the higher saline concentrations, the barnyard grass populations tested in our trials showed a speed of germination greater than that found by Hakim *et al.* (2011): in fact, in their study, the authors observed that *E. crus-galli* and *E. colona* began to germinate after 12-15 days. Pairwise comparisons showed the presence of significant differences between resistant and sensitive populations at 50 mM, 200 mM, 250 mM and 300 mM NaCl concentrations (Table 4). Furthermore, significant differences were found within resistant and within sensitive populations at salt concentrations of 50 mM (*s1 vs s2* and *r2 vs r3*), 200 mM (*r2 vs r3*), 250 mM (*r1 vs r2*) and 300 mM (*s1 vs s2*). As observed for germination capacity, also in the case of speed of germination there is no a clear evidence that sensitive and resistant populations react differently to increasing NaCl concentrations.

Table 2. NaCl concentration required to reduce by 50% seed germination capacity (EC_{50}) of the tested populations. Values in brackets are the standard error.

Population code	EC_{50}
r1	377.46 (58.18)
r2	*274.47 (45.63)*
r3	379.91 (28.25)
s1	339.61 (19.02)
s2	286.21 (27.66)
s3	380.73 (35.94)

Table 3. *SI* values of germination capacity (ratio between EC_{50} of two populations) of the pairwise comparisons between tested populations. SI values are tested against the hypothesis that they are not dissimilar to 1 and the P values of this test are reported.

Compared populations	*SI*	P value
r1/s1	1.11	ns
r1/s2	1.32	ns
r1/r2	1.37	ns
r1/s3	0.99	ns
r1/r3	0.99	ns
s1/s2	1.19	ns
s1/r2	1.24	ns
s1/s3	0.89	ns
s1/r3	0.89	ns
s2/r2	1.04	ns
s2/s3	0.75	*
s2/r3	0.75	*
r2/s3	0.72	*
r2/r3	0.72	*
s3/r3	1.00	ns

*P≤0.05.

Table 4. *SI* values of speed of germination (ratio between EC_{50} of two populations) of the pairwise comparisons between tested populations. SI values are tested against the hypothesis that they are not dissimilar to 1 and the P values of this test are reported. Only comparisons giving P values ≤0.05 are included.

NaCl concentrations (mM)	Compared populations	SI	P value
0	*s1/r2*	0.64	*
50	*s1/s2*	0.78	*
	s1/r2	0.78	*
	s1/r3	1.47	*
	s2/r3	1.89	*
	r2/r3	1.89	*
200	*s2/r3*	1.73	*
	r2/r3	1.61	*
250	*r1/s2*	0.64	*
	r1/r2	0.60	*
	s1/r3	1.43	*
	s2/r3	1.79	*
300	*r1/s2*	0.55	*
	s1/s2	0.69	*
	s2/r3	1.94	*

*P≤0.05

Length of roots and shoots

The results showed that, in all the tested populations, the length of roots decreased with increasing NaCl concentrations (Figure 3). Root length ranged, on average, from 9.88 cm at 0 mM NaCl to 0.36 cm at 400 mM NaCl. A similar trend was found by Hakim *et al.* (2011) on *E. crus-galli* and *E. colona*, in which a progressive reduction of root length with the increase of saline concentration was observed. The value of EC_{50} for root length in resistant populations averaged 162.60 mM, with a minimum of 96.36 mM for the population *r2* and a maximum of 230.98 mM for

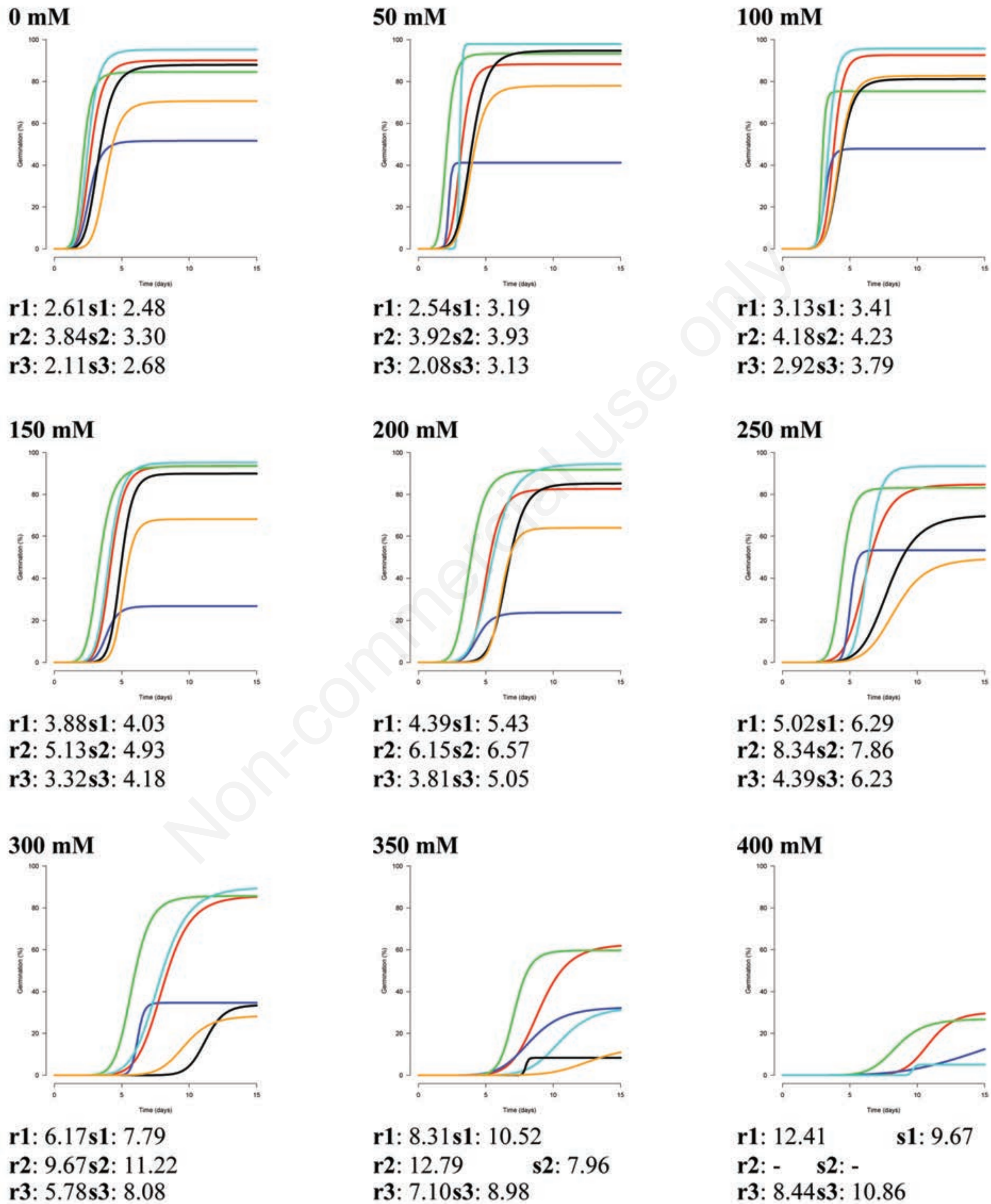

Figure 2. Cumulated percent of germinated seeds as function of duration of germination test, as fitted by the following log-logistic model $Y = d/\{1+exp[b(log(x) - log(e))]\}$ (graphs) and EC_{50} values (days) of the different populations at the tested NaCl concentrations. Blue line: population *r1*; orange line: *r2*; green line: *r3*; cyan line: *s1*; black line: *s2*; red line: *s3*.

the population *r3*. In the case of the sensitive populations, EC_{50} for root length averaged 159.76 mM, with a minimum of 114.81 mM for the population *s2* and a maximum of 210.10 mM for the population *s3* (Table 5).

The pairwise comparisons between populations underlined the presence of significant differences in root length among some of the resistant and sensitive populations (Table 6).

Regarding the length of shoots (Figure 4), a similar behaviour was observed, with shoot length decreasing with the increase of salt concentration. Shoot length ranged, on average, from 6.16 cm at 0 mM NaCl to 0.41 cm at 400 mM NaCl. Hakim *et al*. (2011) obtained analogous results on different species, including *E. crus-galli* and *E. colona*. In general, the values of EC_{50} for shoot length were higher than those recorded for root length, indicating that shoot growth was apparently less affected by salinity than root growth. In resistant populations, EC_{50} for shoot length was on average 246.10 mM, with a minimum of 226.47 mM for the population *r2* and a maximum of 261.67 mM for the population *r1* (Table 5). In the case of sensitive populations, EC_{50} for shoot length was 217.00 mM, with a minimum of 199.23 mM for the population *s2* and a maximum of 249.67 mM for the population *s3*. The pairwise comparisons between populations underlined the presence of significant differences in shoot length among some of the resistant and sensitive populations (Table 6). As observed for germination capacity and speed of germinations, there are no clear trends of different tolerance to salinity conditions in the tested populations that could be attributable to sensitivity to ALS-herbicides. Nevertheless, a similar behaviour was observed in terms of response of shoot length to salinity within resistant populations.

Conclusions

The results obtained in this study suggest that in the tested *E. crus-galli* populations, seed germination capacity, speed of germination, root and shoot growth were affected by saline conditions.

A remarkable barnyard grass tolerance to moderate salinity levels was observed: from 0 mM to 250 mM NaCl the seed germination capacity was up to 90% in the sensitive populations while it was about 70% in the resistant ones. Moreover, one of the populations (*s3*) showed a good tolerance to salinity, with a percentage of germination equal to 33%, even at 400 mM NaCl. Although the resistant tested populations exhibited an intrinsically lower germination capacity, as shown by the lower values recorder in non-saline conditions (0 mM NaCl), the response to salinity is similar to that observed in sensitive populations.

Table 5. NaCl concentration required to reduce of 50% root and shoot length (EC_{50}) of populations *r1, r2, r3, s1, s2* and *s3*, average EC_{50} for resistant and sensitive populations and their comparison. Values in brackets are the standard error.

Population code	EC_{50} root length	EC_{50} shoot length
r1	160.47 (13.47)	261.67 (12.57)
r2	96.36 (12.24)	226.47 (11.61)
r3	230.98 (7.59)	250.15 (8.49)
s1	154.38 (9.29)	202.09 (11.56)
s2	114.81 (9.06)	199.23 (6.77)
s3	210.10 (10.24)	249.67 (6.38)

These results are interesting both from an ecological and agronomic perspective. For example, the reduction of speed of germination at increasing salt levels could suggest a reduced competitive activity of barnyard grass. Moreover, these results could be poten-

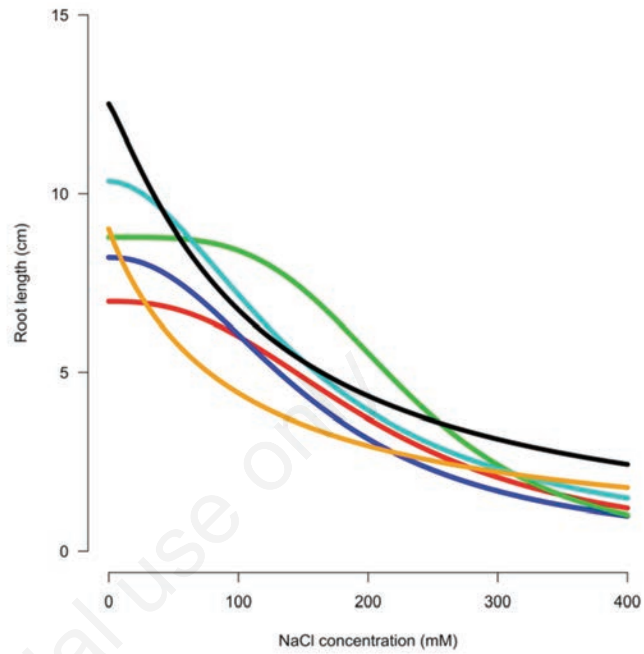

Figure 3. Root length of the different populations at the tested NaCl concentrations, as fitted by the following log-logistic model *Y = d/{1+exp[b(log(x) – log(e))]}*. Blue line: population *r1*; orange line: *r2*; green line: *r3*; cyan line: *s1*; black line: *s2*; red line: *s3*.

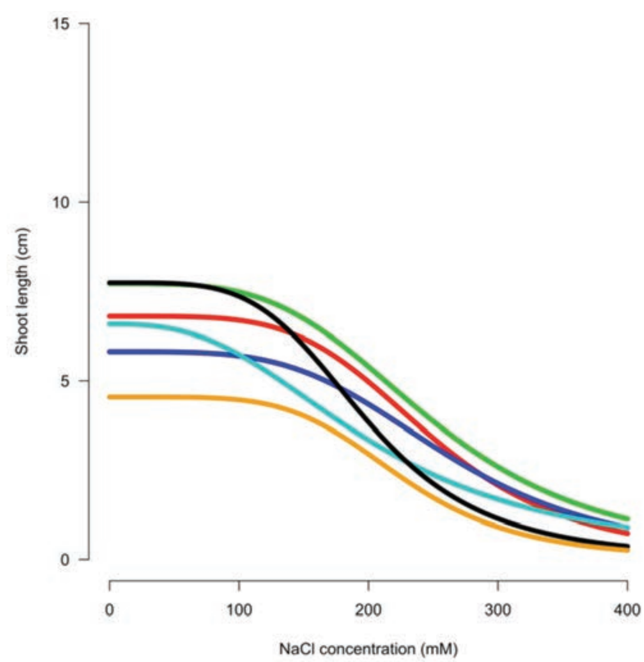

Figure 4. Shoot length of the different populations at the tested NaCl concentrations, as fitted by the following log-logistic model *Y = d/{1+exp[b(log(x) – log(e))]}*. Blue line: population *r1*; orange line: *r2*; green line: *r3*; cyan line: *s1*; black line: *s2*; red line: *s3*.

Table 6. Comparison between *r1, r2, r3, s1, s2* and *s3* populations for NaCl concentration required to reduce by 50% (SI) root length and shoot length.

Compared populations	Root length		Shoot length	
	SI	P value	SI	P value
r1/s1	1.04	ns	1.29	*
r1/s2	1.40	*	1.31	*
r1/r2	1.67	*	1.15	ns
r1/s3	0.76	*	1.05	ns
r1/r3	0.69	*	1.05	ns
s1/s2	1.34	*	1.01	ns
s1/r2	1.60	*	0.89	ns
s1/s3	0.73	*	0.81	*
s1/r3	0.67	*	0.81	*
s2/r2	1.19	ns	0.88	*
s2/s3	0.55	*	0.80	*
s2/r3	0.50	*	0.80	*
r2/s3	0.46	*	0.91	ns
r2/r3	0.42	*	0.90	ns
s3/r3	0.91	ns	1.00	ns

*P≤0.05; ns: P>0.05.

tially exploited for predicting weed emergence dynamics through modelling, also in crops different from rice (Masin *et al.*, 2010, 2012).

The real consequences in terms of competitions towards the crop should be evaluated also taking into consideration the negative impact that salinity could have also on germination and first growth of the crop itself. Future studies are then needed for assessing the response to salinity of the main rice varieties cultivated in the environment in which the *E. crus-galli* populations tested in this study were collected.

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