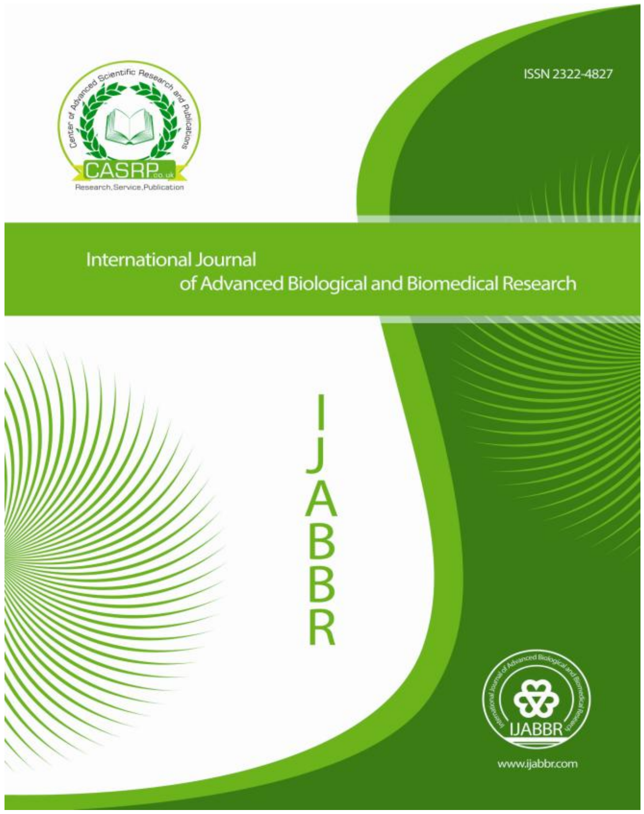
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Salt and water stress of ACCase herbicides resistant and susceptible populations of rigid ryegrass *(Lolium rigidum)*

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Abstract

Laboratory studies were conducted to compare the relative fitness of clodinafop-propargyl herbicide resistant *Lolium rigidum* accessions with their clodinafop-propargyl susceptible counterparts in 2014. Factors evaluated were *Lolium* populations at constant salinity stress (0, 5, 10, 15, 20 and 25 dS cm^2) and water stress (0, -3, -6, -9, -12 and -15 bar). The maximum germination in salinity stress was among three biotypes regarding susceptible biotype with 93.62 percent. The concentration of salinity which 50% seeds germination reached S and Semi-R biotypes to the is more than Rbiotype. In salinity stress the highest rate of germination in the S biotype of 8.66 (number of germinated seeds in days) at a concentration of 17 dS RG reaches 50% in S and Semi-R biotypes and in R biotypes in salinity 14 dS cm^2. The results of the application of various potential water showed that the more negative water potential, germination all biotypes (R, Semi-R and S) dropped and in potential -15 bar PG in R and Semi-R biotypes to less than 15 percent. Coefficients from the regression equation sigmoid three parameter to

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data PG resistant and susceptible biotypes showed that Semi-R biotype Compared to R and S were more tolerant of water stress and in water potential 7.5, it PG Reduced to 50 percent. In examining the relative fitness of ryegrass biotypes water and salinity stress showed that Semi-R and R biotypes the cost of considerable fitness, But the most results cost fitness no significant.

Keywords: Biotype, Fitness, *Lolium rigidum*, Salinity stress, Water stress.

1. Introduction

Weed resistance to herbicides arise usually due to continuous use of the same herbicides or herbicides with the same action (Heap, 2013). Also continuous use Acetyl-CoA Carboxylase inhibiting herbicides causes resistance in more than 46 narrow-leaf weed species in many countries of the world (Heap, 2015; De Prado, 2000). Acetyl-CoA carboxylase inhibitors are a group of commercial herbicides that are able to effectively manage weed grasses. This chemical herbicides belonging to three families: phenoxy aryloxyphenoxypropionate (AOPP), cyclohexane-dione (CHDs) and Phenylpyrazolin (PPZ) are (Zand et al., 2009). The aryloxyphenoxypropionate herbicides inhibit acetyl CoA carboxylase (ACCase), catalyzes the first committed step in fatty acid biosynthesis which leads to inhibition of acyl lipid biosynthesis, and eventually results in death of the plant (Burton et al., 1991). Monocotyledons contain the eukaryotic form of ACCase in their plastids, which is sensitive to these herbicides (Konishi and Sasaki, 1994).

Ryegrass with the scientific name *Lolium rigidum* Guad of poaceae and is one of the most important weeds in wheat (Styles, 1986; Monaghan, 1980). In Iran, chemical control, the main approach to weed control in wheat. The resulting reports the results of research projects carried out in Iran suggest that herbicides diclofop methyl, clodinafop-propargyl, pinoxaden, mesosulfuron-methyl + idosulfuron methyl, sulfosulfuron + metsolfuron, fenoxaprop-P-ethyl and isoproton + diflufenican ryegrass weed, depending on the type of herbicide Good to fairly good range control (Baghestani et al., 1386; Montazeri et al., 1384; Zand et al., 2007).

Predictions based on evolutionary theory suggests that evolution of resistant alleles may fitness the cost of the so-called "cost of compliance" are called, are compromised (Vila-Aiub et al., 2009). Fitness is ability to deploy, survival and successful reproduction weed in the absence of herbicide (Alcocer-Ruthling et al., 1992; Anderson et al., 1996). In most cases when mutation occurs in gene encoding the enzyme or protein that plays a fundamental role in plant survival, makes the plant resistant less fitness regarding to plant sensitive deserve to be in the absence of herbicide (Joseph and Bernard, 2000) .

In most species of monocots (not dicotyledonous) ACCase herbicides are strong inhibitors of key enzymes plastid, acetyl coenzyme A carboxylase (ACCase). It is important, understanding the results fitness of herbicide-tolerant alleles contained and the absence of herbicide to predict the evolution of resistance to herbicide (Neve et al., 2003). This is also effectin in the understanding of strategies that can be managed by the resistance (Beckie, 2006; Walsh and Powles, 2007).

Dastoori et al (2009) in their study observed that with increasing salinity and water was reduced germination percentage and rate germination in resistant and susceptible ryegrass to ACCase inhibitor herbicides that this the reduction in the resistant biotype than the susceptible biotypes is further. Bena Kashani et al (2011) in their study found that increasing water stress percentage and rate germination weed wild oat herbicides inhibiting ACCase-resistant and susceptible reduced that this reduction in the resistant biotype is more than susceptible biotypes.

2. Materials and methods

2.1. The plant material

Tests on 29 biotype suspected of planting ryegrass resistance to herbicides inhibiting the enzyme acetyl-Co A carboxylase and alsoa biotype as a susceptible biotype were collected of areas that have not had any history of chemical control of ryegrass from the wheat fields of Khuzestan.

2.2. Test screening

Early detection of resistant biotypes to clodinafop-propargyl herbicide experiment based on randomized complete block design with four replications (for each pot sprayed a pot control was considered as control). Four weeks after planting, at the three- to four-leaf stage, the plants were treated with a commercial formulation of Aryloxyphenoxypropionates herbicides using a laboratory sprayer (MATABI® Elegance plus) equipped with a flat-fan nozzle (8001) calibrated to deliver 400 L ha^{-1} of spray solution at 200 kPa. Clodinafop propargyl were applied at recommended rates of 64g ai ha-1. Plants were harvested 4 weeks after herbicide application and the dry weight of foliage was recorded following drying for 48 h at 75 0C, also the number of dead and surviving plants was scored. To examine the differences between seed populations, data were expressed as percentage of untreated control. Moss (1999), in the United Kingdom to determine the difference in the degree of resistance based on plant response to a single dose used in the screening of resistant biotypes of the star rating system. In this system, biotype with loss fresh weight more than 81% as a susceptible biotype (S) considered and loss fresh weight than the susceptible and zero to 5 parts (of a star to 5 stars) was divided. At the end of the screening tests three biotypes (AH3) while maintaining 75.0 weight percent as compared to susceptible biotypes resistant biotype, biotype semiresistant (HAM6) while maintaining 44.53 weight percent as semiresistant and susceptible biotypes (S) to test relative fitness assess the biotpyes of susceptible and resistant to water and salinity stress were selected.

2.3. The test salinity on germination resistant and susceptible populations

To evaluate the germination of susceptible populations, semiresistant and resistant ryegrass in salinity, a factorial experiment in a completely randomized design with 4 replicates will be done. 20 number of seeds was inside Petri Dishes 9 cm containing Whatman filter paper number one. For the salinity will be used of sodium chloride. Per petri dish 8 mm of water with different osmotic potential (distilled water, 5, 10, 15, 20 and 25 dS m) were added and in the growth chamber. Count of germinated seeds as once every 24 hours for two consecutive weeks. The radicle of 2 mm is seeds, germinated were considered. The final percentage of germination and germination rate (the time required for germination 50%) is done on the basis of viable seeds. The germination percent and germination rate are calculated by the following equations (Hartman et al., 1990):

Equation (1) $PG= n_i/N\times100$

In this equation,: PG: germination percent, ni: number of germinated seeds until the day i and N: is the number of seeds.

Equation (2) $GR=\sum_{i=1}^{n} Gi/Ti$

In this equation, GR: germination rate, G: number of germinated seeds per day; T: the number of days elapsed since the start of the experiment.

Statistical analysis was performed by regression analysis and application Sigmaplot.

In this test of the software used for charting Sigmaplot and then fit the data, three parameter logistic model was the most appropriate model equation is as follows:

Equation (3) $Y=a/1+(x/x0)^b$

In this equation, a: maximum germination, b: the slope and x0: the concentration of salt that germination percentage of 50 percent.

2.4. Evaluation of water stress on the germination of resistant and susceptible populations

To evaluate the germination of R, semi-R and S biotypes ryegrass to water, a factorial experiment in a completely randomized design with four replications. 20 seeds per petri dish were placed on Whatman filter paper number one that had been moistened with 8 ml of distilled water and different potentials (-3, -6, -9, -12, 15 bar) added in a growth chamber in the 12/12 hour light-dark / light at 15/25 ° C night/ day time required (Seeds were allowed to germinate for 14 day). To apply the stress was used of polyethylene glycol 6000 as a solution in distilled water. Count of germinated seeds as is done every 24 hours for two weeks. The radicle of 2 mm is seeds, germinated considered. The final percentage of germination and germination rate is determined on the basis of viable seeds. Germination rate based on the equation (2) was calculated.

In this test of the software used for charting Sigmaplot and then fit the data, sigmoid three parameter model was the most appropriate model equation is as follows:

Equation (4) $f = a/(1+\exp(-(x-x_0)/b))$

In this Equation, Y: germination at each potential, a: maximum germination, x: water potential, b: the slope and x_0: water potential which Caused 50% germination.

3. Results and discussion

3.1. Evaluation of salinity stress on the germination of resistant and susceptible populations

As you can see in the table 1 the maximum germination was in all biotypes regarding susceptible biotype with 93.62 percent. The concentration of salinity which 50% seeds germination reached S and Semi-R biotypes to the is more than R, hence S and Semi-R concentration of 21 dS cm^2 and R biotype at a concentration lower levels of salinity (19 dS cm^2) to 50% germination (x_0) reaches (Table1).

Table 1
Parameters obtained from the Equation fitted to data PG ryegrass biotypes in different levels of salinity stress.

Biotype	a	b	X_0	R^2
S	93.6218	8.4115	21.9105	0.98
SemiR	84.0252	10.8572	21.8862	0.96
R	89.2524	4.6018	19.2171	0.96

Germination percent of 15 dS cm^2 biotype S to follow a fixed linear process, but with increasing concentrations of salt, then showed a sharp decrease. If Semi-R and R biotypes to concentration of 5 dS PG follow a fixed linear trend, but the decline was 10 dS concentration lower than the S biotype that this showed a greater drop (fig.1). Hill slope S and Semi-R biotypes and resistant 8.4 and 10.8 and R biotype was 4.6 (Table1). The results, the Germination percent S biotype was more than Compared to Semi-R and R biotypes.

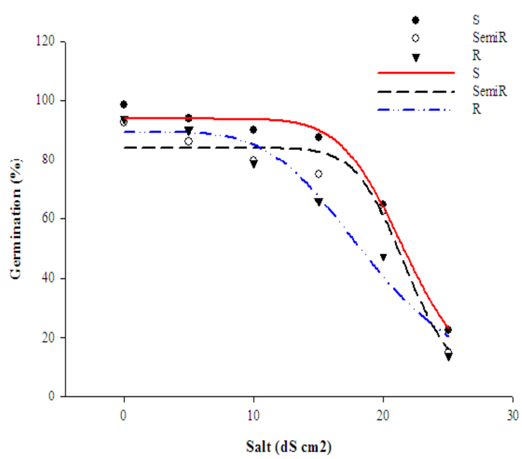

Fig. 1. Percentage Emergence Pattern of Resistant, Semi- Resistant and Susceptible Biotypes of *Lolium rigidum* Seeds at Different concentration Salinity.

According to the table2 with the highest rate of germination in the S biotype of 8.66 (number of germinated seeds in days) at a concentration of 17 dS cm^2 RG reaches 50% in S and Semi-R biotypes. In Semi-R and R biotypes to the highest RG was 7.5 and 7.9. In R biotypes RG in salinity 14 dS cm^2 reduced to 50% (Table2).

Table 2
Parameters obtained from the Equation fitted to data RG ryegrass biotypes in different levels of salinity.

Biotype	a	b	X_0	R^2
S	8.6609	4.4279	17.1611	0.97
SemiR	7.5471	4.4154	17.1438	0.96
R	7.9038	3.4738	14.5875	0.97

All three biotypes S, Semi-R and R to RG in concentration 5 dS cm^2 salinity of a trend of gradual decline was observed (fig. 2). This reduction in R biotype Significant decrease. In other words, R biotype with speed descending curve slope or gradient Hill (3.4) compared to S and Semi-R populations (4.4) showed a greater decline (table2).

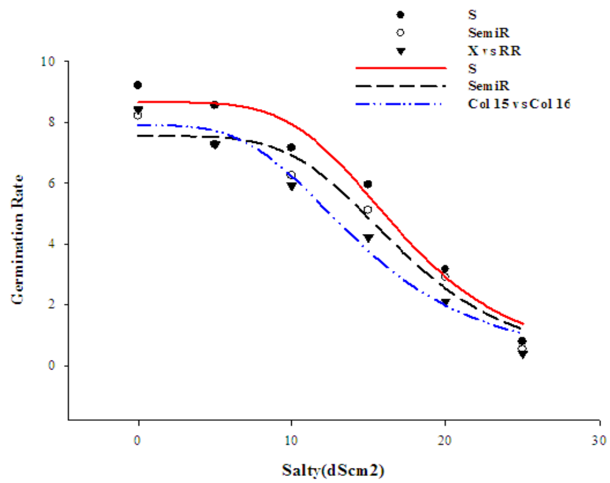

Fig. 2. Rate germination pattern of resistant, semi- resistant and susceptible biotypes of *Lolium rigidum* seeds at different concentration salinity.

3.2. Evaluation of water stress on the germination of resistant and susceptible populations

The results of the application of various potential water showed that the more negative water potential, germination all biotypes (R, Semi-R and S) dropped and in potential -15 bar PG in R and Semi-R biotypes to less than 15 percent (fig. 3).

Table3
Parameters obtained from the Equation fitted to data PG ryegrass biotypes in different levels of water stress.

Biotype	a	b	X_0	R^2
S	114.3254	3.9710	-7.9518	0.98
SemiR	108.7120	4.2250	-7.5328	0.97
R	100.9254	3.9343	-7.7969	0.97

Coefficients from the regression equation sigmoid three parameter to data PG resistant and susceptible biotypes showed that Semi-R biotype Compared to R and S were more tolerant of water stress and in water potential 7.5, it PG Reduced to 50% (see table3). But R and S biotypes puts less stress and more water potential (less negative) to 50% germination (Table3). Reduction process R, Semi-R and S Biotypes to negative water potential, as shown in the Fig3 .

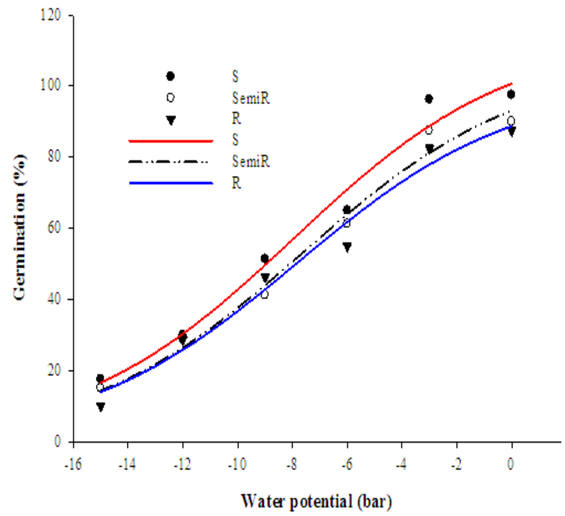

Fig. 3. Percentage Emergence Pattern of Resistant, Semi- Resistant and Susceptible Biotypes of *Lolium rigidum* Seeds at Different concentration water stress**.**

Also, by reducing the osmotic potential, germination rate began to rise with steep at all biotype biotype, that this increase in potential -10 bar S more than Semi-R and R. Chart fitted GR is shown in (Fig.4) That this results is consistent of the study Dastoori et al (2009) and BenaKashani et al (2011).

Table 4
Parameters obtained from the Equation fitted to data RG ryegrass biotypes in different levels of water stress.

Biotype	**a**	**b**	X_0	R^2
S	12.2202	3.5320	-4.4883	0.98
SemiR	10.2396	3.9381	-4.6167	0.97
R	9.7674	3.8635	-4.6252	0.97

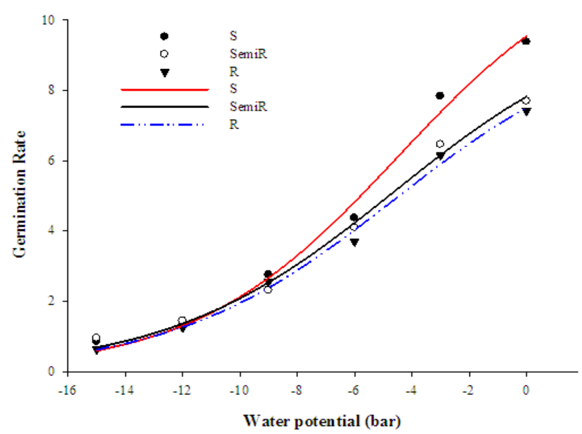

Fig. 4. Rate germination pattern of resistant, semi- resistant and susceptible biotypes of *Lolium rigidum* seeds at different concentration water stress**.**

4. Conclusion

Overall assessment of the relative fitness of weed species resistant and sensitive resistance is an important factor to predict the evolution resistance. If you remove the selection pressure herbicide if fitness resistant plants is less sensitive plants, with time sensitive plants replace resistant plants In this case, since is hope the population of weed resistance to reduce resistance there,that results in examining showed of S, Semi-R and R ryegrass biotypes the cost of considerable fitness, but the most results cost fitness no significant that if the the differences are not significant, the frequency of resistant plants in the population likely will not decrease, then the resistant plants need to adopt long-term management strategies that reduced the severity of selection for resistant plants is desirable and also integrate other management strategies.

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