COMMUNICATIONS IN PLANT SCIENCES

RESEARCH ARTICLE

Characterization of herbicide tolerant rice genotypes under hydroponic culture

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OPEN ACCESS

doi: 10.26814/cps2018014

Received on August 31, 2018
Accepted on October 17, 2018
Early View on October 22, 2018
Revised on October 23, 2018

Authors declare no conflict of interest

The use of nutrient solution combined with different herbicide concentrations allows the evaluation and discrimination of tolerant and non-tolerant genotypes to imidazolinone herbicides. The objectives of this research were to identify possible morphological characteristics to be used as markers of resistance to imidazolinones, as well as the identification of herbicide doses and evaluation time to discriminate sensitive and tolerant genotypes of rice under hydroponic systems. Six rice genotypes four characterized as sensitive, BRS Querência, BRS Border, BRS Atalanta and BRS Pampa and two as tolerant, BRS Sinuelo CL and PUITA INTA CL to imidazolinones were tested in a hydroponic system. Four doses of herbicide were tested, which were evaluated at 7, 14, 21 and 28 days after transplantation. The insertion of the first leaf was the most responsive variable and can be used as morphological marker in experiments for selecting rice genotypes tolerant to imidazolinones. The best time to discriminate genotypes is the 7th day of development. The concentration of herbicide that enables better discrimination between tolerant and sensitive genotypes is 25 µg L^{-1}, according to the methods described in this bioassay.

Highlighted Conclusions

The insertion of the first leaf can be used as morphological marker for selecting rice genotypes tolerant to imidazolinones at 7th day of development.

Rice is an important agricultural product that serves globally as a basic diet for mankind. Brazil is among the ten largest rice producers in the world, currently producing 12 million tons and grain yield is estimated at 6.1 tons ha^{-1} (Conab 2018).

Plant breeders develop strategies in order to increase productivity and meet the growing demand for rice. Grain yield increases are limited by the inappropriate crop management, the use of uncertified seed, and mainly unsatisfactory weed control (Noldin et al. 1999). The high incidence of red rice (*Oryza sativa* L.) stands out as the most limiting factor for rice production potential in most regions of the world (Webster 2000, Burgos et al. 2008) and also in Brazil (Noldin et al. 2004). The red rice control by selective herbicides is hampered by the similarity between the cultivated rice and red rice, since they belong to the same species. Further, controlling red rice by other methods have limited efficiency, particularly in large cultivated areas. The characterization of the growth and development of different rice and red rice genotypes may be important for the establishment of strategies to control red rice, and to assist in breeding rice cultivated to increase its competitiveness against this weed (Kwon et al. 1992, Noldin et al. 1999, Bosco et al. 2006).

The evaluation of seedlings grown hydroponically under different abiotic stresses allows greater experimental control, and has some advantages over the conventional method such as: achieving results in short time, reducing operating costs, easier evaluation and greater efficiency (Furlani et al. 2000). The nutrient solution employed combined with different herbicide concentrations allows the evaluation and discrimination of tolerant and non-tolerant genotypes to the herbicide imidazolinone (Fonseca 2011).

Imidazolinone group herbicides belong to the acetolactate synthase (ALS) inhibitors that disrupts the synthesis of valine, leucine and isoleucine. The Clearfield® technology of rice production, which uses tolerant genotypes to imidazolinones, is an effective strategy for red rice control in irrigated rice fields (Steele et al. 2002, Ottis et al. 2003, Villa et al. 2006). Only® is the herbicide recommended for this technology in Brazil, and is formulated based on imazethapyr + imazapic at concentrations of 75 and 25 g ai L^{-1}, respectively (Sosbai 2010).

Due to the high occurrence of imidazolinone resistant red rice plants is necessary develop methods for providing fast and correct identification of resistant individuals. The objectives were to identify possible morphological characteristics to be used as markers of resistance to the imidazolinones, as well as the identification of herbicide doses and time of evaluation to discriminate sensitive and tolerant genotypes under hydroponic systems.

MATERIAL AND METHODS

Plant Material. Rice genotypes were tested in a hydroponic culture under controlled environmental conditions in the Double-haploid and Hydroponics Laboratory belonging to the Plant Genomics and Breeding Center (CGF), Federal University of Pelotas (UFPel), Capão do Leão - RS, Brazil. In this work, four sensitive genotypes (BRS Querência, BRS Fronteira, BRS Atalanta and BRS Pampa) and two tolerant (BRS Sinuelo CL and Puitá INTA CL) to imidazolinones were evaluated. The genotypes used belong to the Embrapa germplasm bank, except the cultivar Puitá INTA CL (BASF S.A.), were chosen because they have great agronomic and scientific interest.

Growth conditions. The seeds were previously treated with carboxin and thiran (Vitavax-Thiran 200 SC®, 200+200 g ai L^{-1}, Brazil) in concentration 250-300 g of commercial product to 100 kg seeds, and germinated in germinating chamber (BOD type) at 25 °C with a photoperiod of 16 hours and 100% of relative humidity, according to the criteria established by the Rules for Seed Analysis (Brasil 2009).

Seedlings with uniform root length (about 5 mm) were placed in polyethylene nets on top of five gallon capacity plastic containers, allowing the support of seedlings and the growth of roots in growth medium, the root keeping in constant contact with the standard nutrient solution for rice (Yoshida et al. 1976) containing: 40 mg L^{-1} [$(NH_4)_2SO_4$], 10 mg L^{-1} (KH_2PO_4), 40 mg L^{-1} (KNO_3), 40 mg L^{-1} ($CaNO_3$), 40 mg L^{-1} ($MgSO_4$ $7H_2O$), 0.5 mg L^{-1} ($MnSO_4H_2O$), 0.05 mg L^{-1} (Na_2MO_4 $2H_2O$), 0.58 mg L^{-1} (NaCl), 0.2 mg L^{-1} (H_3BO_3), 0.01 mg L^{-1} ($ZnSO_47H_2O$), 0.01 mg L^{-1} ($CuSO_4H_2O$), 2 mg L^{-1} ($FeSO_47H_2O$) and the herbicide treatment.

The herbicide concentrations added to the nutrient solution were: 0 (control), 0.5, 1.0 and 1.5 L ha^{-1} of Only®. After preparation of the nutrient solution a pH adjustment to 4.5 was made with H_2SO_4. The herbicide concentrations used were previously reported (Magalhães Júnior et al. 2000, Agostinetto et al. 2001).

The containers were placed in a hydroponic tank with water at 25 ± 1 ºC, with a photoperiod of 16 h light/8h dark. The nutrient solution was not changed, and only distilled water was added to compensate for losses due to evaporation.

Traits available. Number of roots (NR) and tillers (NT), length (cm) of roots (RL), shoots (SL), the first (LFL) and second (LSL) leaves, as well as the insertion of the first leaf (IFL) of seedlings were evaluated at 7, 14, 21 and 28 days after transplanting (DAT). Subsequently, tissue samples were placed in an oven with air circulation at 65 °C until constant weight for subsequent determination of dry matter (g) of roots (DMR) and shoots (DMS).

Design experimental and statistical analysis. The experimental design was completely randomized in a factorial scheme (4 herbicides doses × 6 rice genotypes) with three replications of ten plants. Data were subjected to variance analysis (ANOVA) ($p \leq 0.05$) and when significant effects were found, polynomial regression models were tested with the statistical program Winstat (Machado and Conceição 2003). The selection of models was based on the statistical significance (F test) and fitting of the coefficient of determination (R^2). Data were also subjected to a Pearson correlation test with the aid of the SAS software (SAS 2002), and were plotted in Microsoft® Office Excel 2007 software.

RESULTS AND DISCUSSION

ANOVA revealed differences between the rice, BRS Querência, BRS Fronteira, BRS Atalanta, BRS Pampa, BRS Sinuelo CL and Puitá INTA CL genotypes and herbicide doses for all variables at 7 DAT (Table 1), indicating that genotypes differ at distinct magnitudes to the herbicide doses used. The identification of herbicide resistance in the early stages of plant development can speed decision-making in crops suspected of infestation with resistant red rice. Rice genotypes showed differential responses to the effect of doses in all variables, confirming the results obtained in the ANOVA.

Table 1. Summary of the analysis of variance analysis of six genotypes of rice, valued at four herbicide concentrations the 7 and 14 days of growing hydroponically.

SV	DF	Mean Square							
		7 days							
		NR	RL	SL	INSFL	LFL	LSL	DMS	DMR
Dose(D)	3	76.78*	840.29*	3652.32*	99.35*	78.83*	2152.76*	0.0006*	0.0002*
Genotype(G)	5	369.48*	451.27*	1542.41*	19.4*	5.28*	392.46*	0.0003*	9.27*
DxG	15	39.58*	25.29*	270.73*	8.72*	17.46*	120.89*	$4.78.1^{-5}$*	$3.71.1^{-5}$*
Residue	696	3.30	1.30	4.5	0.29	1.37	2.36	$2.42.1^{-6}$	$3.06.10^{-6}$
Average	-	6.27	4.02	7.71	1.98	1.79	3.55	0.0046	0.0024
CV (%)	-	28.99	28.39	27.51	27.0	65.34	43.28	33.45	72.66
SV	DF	14 days							
		NR	RL	SL	INSFL	LFL	LSL	DMAP	DMR
Dose(D)	3	179.47*	1746.69*	6070.32*	141.8*	33.98*	2090.71*	0.0011*	0.0002*
Genotype(G)	5	545.71*	849.52*	4717.7*	30.67*	13.35*	360.66*	0.0011*	0.0002*
D x G	15	82.37*	81.82*	327.24*	11.7*	4.01*	127.5*	$8.95.10^{-5}$*	$8.15.10^{-5}$*
Residue	696	6.68	1.83	4.47	0.36	0.31	1.38	$1.59.10^{-6}$	$6.95.10^{-6}$
Average	-	7.36	4.98	9.73	1.89	1.53	3.33	0.0060	0.0032
CV (%)	-	35.11	27.19	21.73	31.67	36.39	35.28	21.00	83.55

Number of roots (NR), root length (RL), shoot length (SL), insert the first leaf (INSFL), length of the first leaf (LFL), length of the second leaf (LSL), dry matter of shoot (DMS) and dry matter of root part (DMR) * Significant values at 5% error probability by F test. DF: degrees of freedom. MS: mean square. CV: coefficient of variation.

A different performance for the rice genotypes can be seen for NR (Figure 1a). Only BRS Sinuelo CL (tolerant), showed a quadratic regression fit indicating a critical dose (x_V = -b/2c), and an increase in the NR average up to a dose of 35 µg L^{-1} (0.75 L^{-1} ha^{-1}), with further decrease. However, the genotype Puitá INTA CL (also tolerant), showed similar performance to sensitive genotypes, fitting to a linear regression, showing even greater sensitivity as herbicide doses increased than BRS Pampa (sensitive). This can be observed by the analysis of the b parameter of the equation (Puitá INTA CL; b= -0.016 and BRS Pampa; b= -0.012).

Rice genotypes showed a decrease of RL when the herbicide dose was increased, fitting a quadratic regression, except for the cultivar Puitá INTA CL (linear regression). BRS Sinuelo CL (tolerant) presented performance similar to herbicide sensitive genotypes. The sensitive genotypes and BRS Sinuelo CL showed a marked decrease in RL as herbicide dose increased, showing sensitivity. This sensitive-like response of BRS Sinuelo CL may was due to its leak imidazolinone resistance, since it belongs to a first-generation group of mutants. The joint analysis of traits NR and RL revealed a different performance of tolerant rice genotypes, and allowed a better separation between sensitive and tolerant ones (Figure 1a and b). Among the tolerant genotypes, BRS Sinuelo CL showed higher NR and Puitá INTA CL, longer roots than the other rice genotypes.

The variable shoot length is shown on Figure 1c. A better fitting to a quadratic regression model can be observed. It can be inferred that Puitá INTA CL showed tolerance features to the herbicide, showing increase in shoot length up to the dose 55 µg L^{-1}, with a further decrease. The other genotypes showed sensitivity to phytotoxic effects caused by the herbicide, including the tolerant cultivar BRS Sinuelo CL who was harmed by a dose of 50 µg L^{-1} or higher.

For the variable IFL (Figure 1d), a linear regression was observed for the tolerant genotypes (BRS Sinuelo CL; b= -0.007 e Puitá INTA CL; b= - 0.006). On the other hand, sensitive cultivar responses fitted to a quadratic regression, with more steep reductions in the morphological trait, allowing a better differentiation among sensitive and tolerant genotypes.

The results for the variable LFL (Figure 1e) suggest that BRS Atalanta, BRS Sinuelo CL and Puitá INTA CL did not fit a polynomial regression model, with averages of 1.68; 1.49 and 1.99 cm, respectively. The other genotypes showed slight decrease on the traits evaluated, except for BRS Querência with the highest reduction in the LFL.

For the morphological character LSL (Figure 1f) you can check that BRS Pampa, BRS Querência, BRS Fronteira (sensitive) showed no second leaf development in the presence of the herbicide, only in the control treatment. However, BRS Atalanta presented development of second leaf, with maximum reduction in the dose 47.5 µg L^{-1} similar to BRS Sinuelo CL with maximum reduction in dose 52.5 µg L^{-1}.

Considering the performance of each individual genetic constitution, for the variable DMS (Figure 1g), one can infer that the genotypes set the quadratic regression model. Exceptions were observed for BRS Querência that fitted a linear regression expressing a reduction of 0.00008 g for each increase in the herbicide dose and Puitá

INTA CL that did not fit to a polynomial regression model with an average reduction of 0.0067 g. The genotypes showed similar performance, not allowing a discrimination between sensitive and tolerant genotypes.

The results for DMR (Figure 1h) indicate that the data set fits a linear model, except for BRS Fronteira that did not fit a regression model, with an average of 0.0023 g. Also, Puitá INTA CL had the lowest linear regression coefficient, showing increased tolerance to the herbicide, followed by genotypes BRS Pampa, BRS Sinuelo CL, BRS Querência and BRS Atalanta.

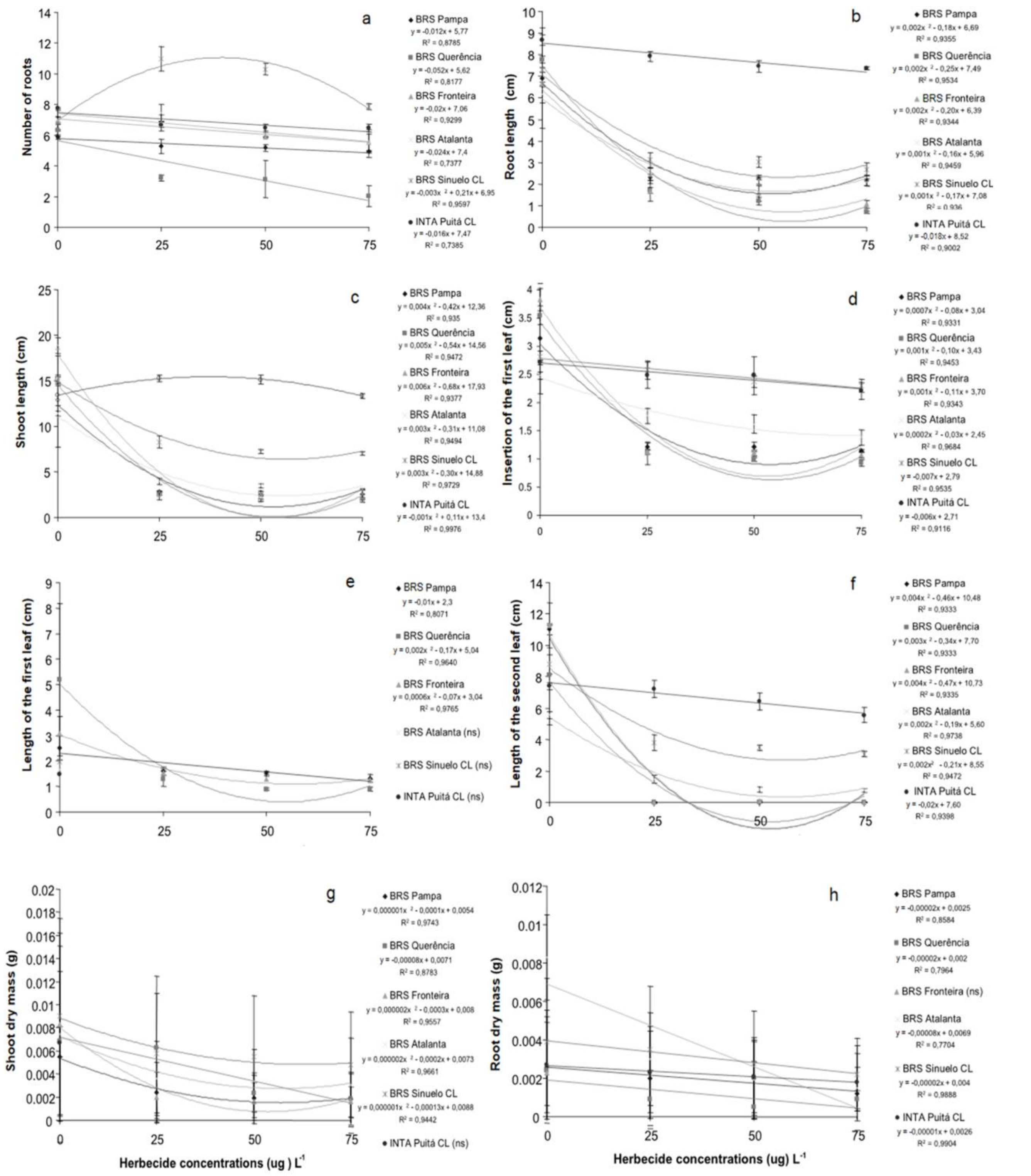

Figure 1. Parameters of regression equations and their graphical representations of the variables: a - number of roots (NR), b - root length (RL) c – shoot length (SL), d - insertion of the first leaf (INSFL) e - length of the first leaf (LFL), f - length of the second leaf (LSL), g - dry matter of shoot (DMAP), h - dry matter of root (DMR), six genotypes of rice, valued four herbicide concentrations (Only®) (0, 25 µg L^{-1}, 50 µg L^{-1} and 75 µg L^{-1}) at seven days of cultivation in a hydroponic system. Bars represent mean standard error, and (ns) no significant polynomial regression equation.

The degree of correlation between the variables can be shown on Table 2, where significant and positive correlations for almost all variables can be seen. Only one of the combinations (LFL with NR) showed no significant correlation ($p \leq 0.05$), indicating that responses of these variables are independent of each other. The significant good extent correlation found between the variables RL, SL and LSL with IFL suggests the possibility of indirect selection for herbicide tolerance using IFL. Correlation analyses are very important for the indirect selection of genotypes when the target trait is difficult to measure (Carvalho et al. 2004).

Table 2. Pearson correlation coefficient between the variables of six rice genotypes evaluated in four herbicide concentrations after seven days.

Variables	NR	RL	SL	INSFL	LFL	LSL	DMS	DMR
NR	1	0.26*	0.35*	0.47*	0.23	0.31*	0.34*	0.69*
RL		1	0.94*	0.82*	0.36*	0.84*	0.72*	0.39*
SL			1	0.91*	0.39*	0.90*	0.77*	0.45*
INSFL				1	0.48*	0.87*	0.70*	0.56*
LFL					1	0.24*	0.65*	0.28*
LSL						1	0.58*	0.45*
DMS							1	0.40*
DMR								1

Number of roots (NR), root length (RL), shoot length (SL), insert the first leaf (INSFL), length of the first leaf (LFL), length of the second leaf (LSL), dry matter of shoot (DMS) and dry matter of root part (DMR), * Significant at the 0,05 level of probability by t test.

The results obtained in the evaluation at seven days (Figure 1) suggest that the IFL was the best variable to identify imidazolinone chemical group herbicide tolerant genotypes, needing a minimum dose of 25 µg L^{-1} herbicide for the best discrimination between sensitive and tolerant genotypes. However, significant correlations (Table 2) indicate that the variables RL, SL and the SLL may be used as secondary criteria for selection.

In addition, bioassay reports on rice seedlings, indicated the variable RL as an appropriate variable to identify the sensitivity to imidazolinone chemical group herbicides (Roso et al. 2010). Other bioassay reports were also performed for ACCase inhibitor resistance in *Eleusine indica* and for ALS inhibitor resistance to *Bidens pilosa* and *Euphorbia heterophylla* (Portes 2005).

Significant interaction effects between genotype and dose was found for all variables at 14 days (Table 1), indicating that genotypes showed differential responses to the used herbicide doses (Figure 2).

For the variable NR, a different behavior for sensitive and tolerant genotypes was observed (Figure 2a). BRS Sinuelo CL and CL Puitá INTA characterized as herbicide tolerant showed better fit to the polynomial model, displaying an increase in the average NR in the doses 36.67 and 32.5 µg L^{-1}, respectively. Despite showing a decrease up to the highest dose of herbicide, the maintained average was higher than the one found in the control, suggesting a resistance feature. The sensitive genotypes reduced the average NR with increased herbicide doses, fitting to a linear regression. Among the sensitive genotypes, BRS Pampa (b= -0.07) and BRS Querência (b= -0.05) showed greater sensitivity to the herbicide that the genotypes BRS Fronteira (b= -0.04) and BRS Atalanta (b= -0.03) observed through the parameter b of the equation.

The variable RL, measured at 14 days of development, reveals a differential behavior among sensitive and tolerant genotypes, forming a group to set the polynomial model, except the cultivar Puitá INTA CL that presented the best fit linear regression model (Figure 2b). The tolerant 'BRS Sinuelo CL' showing quadratic regression differs from other genotypes sensitive to present length reduction to the dose 70 µg L^{-1}. With regard to sensitive genotypes observed a marked reduction of the dose from 25 µg L^{-1} to doses ranging between 47.5 e 70 µg L^{-1}.

For shoot length, tolerant and sensitive genotypes differed in their fitting to the polynomial model. Puitá INTA CL displayed tolerance until dose 20 µg L^{-1}, with subsequent slight decrease. The other genotypes probably had sensitivity to phytotoxic effects caused by the herbicide. BRS Sinuelo CL (tolerant) showed less reduction than the sensitive cultivar up to the 67.5 µg L^{-1} dose (5.36 cm for each increment of herbicide concentration unit, considering the linear term, without changes in the quadratic term), demonstrating higher tolerance to herbicide than sensitive genotypes. These showed a decrease ranging from 0.51 to 0.76cm in the average shoot length per herbicide concentration unit, up to doses ranging from 51 to 57.5 µg L^{-1}.

For the variable IFL, two groups are observed in Figure 2d, allowing the discrimination of sensitive and tolerant genotypes. The first group, formed by the tolerant genotypes, were fitted to a linear regression (BRS Sinuelo CL; b= -0.01 and Puitá INTA CL; b= -0.005) presents a less steep decrease with the addition of an herbicide concentration unit. The second group comprises the genotypes sensitive to the herbicide, that had as the best fit a quadratic regression, with greater reduction in the morphological trait analyzed, with a decrease until doses ranging from 55 to 66.67 µg L^{-1}.

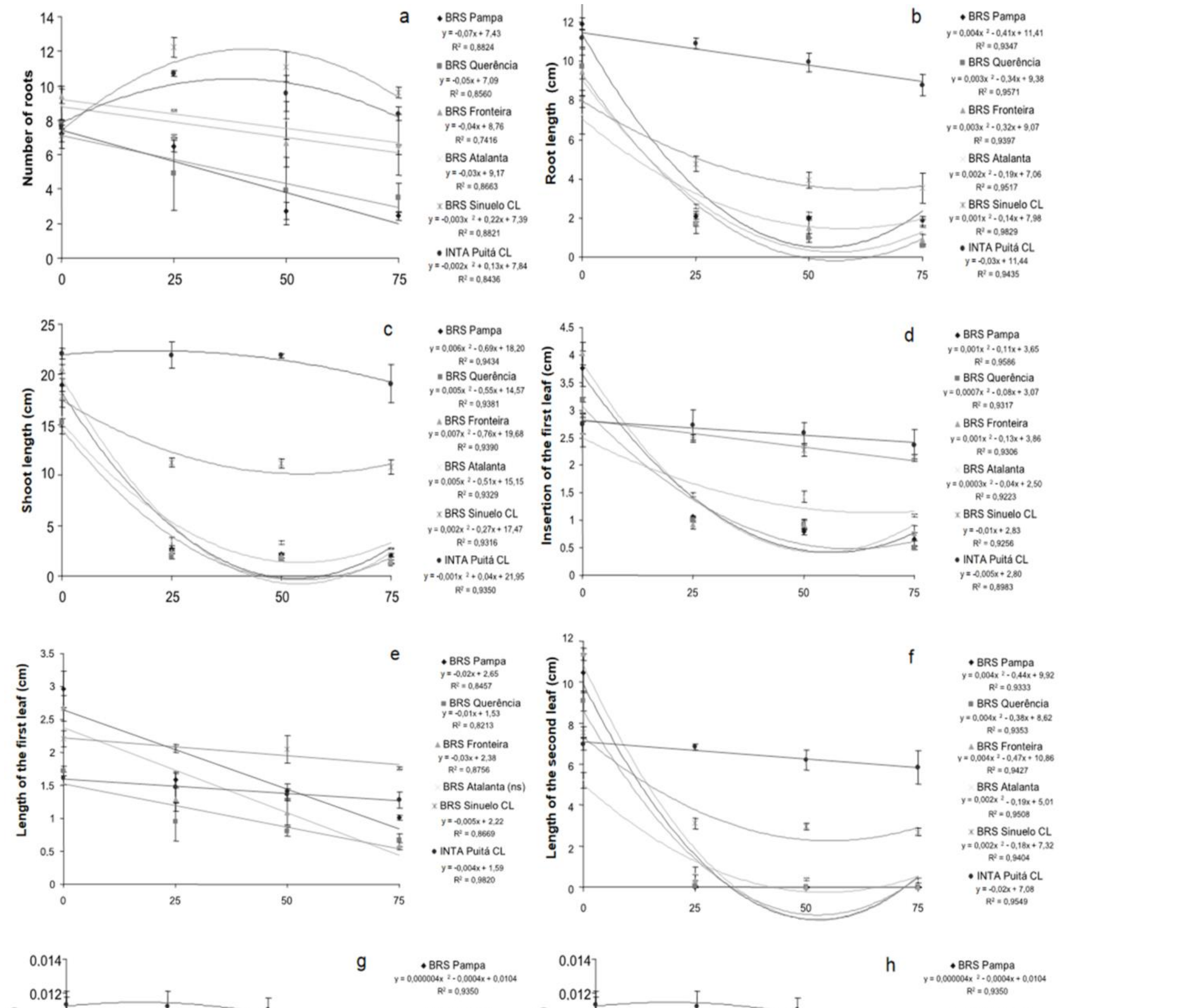

Figure 2. Parameters of regression equations and their graphical representations of the variables: a - number of roots (NR), b - root length (RL) c –shoot length (SL), d - insertion of the first leaf (INSFL) e - length of the first leaf (LFL), f - length of the second leaf (LSL), g - dry matter of shoot (DMS), h - dry matter of root part (DMRP), six genotypes of rice, valued four herbicide concentrations (Only®) (0, 25 µg L^{-1}, 50 µg L^{-1} and 75 µg L^{-1}) at 14 days of cultivation in hydroponic system. Bars represent mean standard error, and (ns) no significant polynomial regression equation.

The analysis of the variable LFL (Figure 2e) indicated that BRS Atalanta did not fit a polynomial regression model, showing an average of 1.53 cm. The response of other genotypes was fitted to a linear model. A small decrease on the trait evaluated for tolerant genotypes and a greater decrease for BRS Querência, BRS Pampa and BRS Fronteira (sensitive) can be observed through the regression coefficients.

For the trait LSL (Figure 2f), sensitive genotypes BRS Pampa, BRS Querência, BRS Fronteira showed no development of the second leaf in the presence of the herbicide, only in the control treatment. However, BRS

Atalanta presented development of second leaf, with a reduction to the dose 47.5 µg L^{-1} similar to BRS Sinuelo CL (45 µg L^{-1}). Puitá INTA CL response was fit to a linear regression model demonstrating increased tolerance to the herbicide toxicity effect, a decrease of 0.02 cm each concentration unit increased herbicide.

Considering the performance of each individual genotype, for the variable DMS (Figure 2g) a quadratic regression model was observed, except for BRS Sinuelo CL which presented a linear regression. Puitá INTA CL expressed his characteristic tolerance to the herbicide and presented an increase in the variable DMS up the dose 21.43 µg L^{-1}, with a subsequent decrease. Sensitive genotypes (BRS Pampa, BRS Fronteira, BRS Querência and BRS Atalanta) showed marked reduction in the variable evaluated at 50 µg L^{-1}, showing greater sensitivity to the toxic effect of the herbicide. This variable allows discrimination between sensitive and tolerant genotypes.

For the variable DMR (Figure 2h), BRS Fronteira, BRS Atalanta and Puitá INTA CL did not fit a polynomial regression model and showed the following average 0.0029, 0.0032 and 0.003 g respectively. The genotypes BRS Pampa and BRS Querência are suited to a linear model with a decrease in 0.00003 g and 0.00002 g. BRS Sinuelo CL was also sensitive for this trait, showing a quadratic response.

Variables had positive and significant correlations with each other, except for the variable DMR with variables NR, RL, SL and LSL (Table 3). The results for the dose-response curves suggest that the variables NR, RL, IFL, LAP, LFL and DMAP are indicated to select promising genotypes for tolerance to the herbicide. Significant correlations indicate that the variables present including high correlation magnitude, except LFL which has an average magnitude of correlations.

Table 3. Pearson correlation coefficient between the variables of six rice genotypes evaluated in four herbicide concentrations after 14 days

Variables	NR	RL	SL	INSFL	LFL	LSL	DMS	DMR
NR	1	0.43*	0.53*	0.55*	0.44*	0.41*	0.61*	0.18
RL		1	0.95*	0.88*	0.57*	0.93*	0.87*	0.20
SL			1	0.89*	0.58*	0.91*	0.91*	0.23
INSFL				1	0.78*	0.95*	0.85*	0.26*
LFL					1	0.69*	0.55*	0.25*
LSL						1	0.80*	0.23
DMS							1	0.27*
DMR								1

Number of roots (NR), root length (RL), shoot length (SL), insert the first leaf (INSFL), length of the first leaf (LFL), length of the second leaf (LSL), dry matter of shoot (DMS) and dry matter of root part (DMR), * Significant at the 0,05 level of probability by t test.

The results of the evaluated at 14 days (Figure 2) suggest that the variables NR, RL, SL, IFL, LFL and DMAP can be shown to discriminate genotypes tolerant and sensitive to herbicide imidazolinones chemical group, an approximate dose is required 25 µg L^{-1} of herbicide to occur discrimination of sensitive and tolerant genotypes.

Significant interaction effects between the genotype and dose factors for all variables were observed at 21 days (Table 4).

Table 4. Summary of the analysis of variance of six genotypes of rice, valued at four herbicide concentrations at 21 and 28 days of cultivation in hydroponic system.

SV	DF	Mean Square							
		21							
		NR	RL	SL	INSFL	LFL	LSL	DMS	DMR
Dose	3	392.27*	2948.08*	8088.85*	129.89*	27.83*	2454.74*	0.0033*	0.0003*
Genotype	5	558.68*	1540.05*	5800.86*	27.30*	9.68*	353.61*	0.0028*	0.0002*
D x G	15	38.19*	128.07*	412.90*	12.83*	2.52*	178.45*	0.0002*	0.0001*
Residue	696	5.86	3.04	5.15	0.25	0.24	16.19	$7.65.10^{-7}$	$1.31.10^{-5}$
Average	-	7.96	6.16	10.73	1.91	1.56	3.57	0.0081	0.0041
CV (%)	-	30.4	28.29	21.14	26.39	31.60	112.66	10.82	88.14
SV	DF	28 (7)							
		NR	RL	SL	INSFL	LFL	LSL	DMS	DMR
Dose(D)	3	489.44*	3787.98*	8447.25*	161.77*	70.98*	2366.15*	0.0053*	8.74*
Genotype(G)	5	1046.01*	2446.39*	6220.35*	44.47*	21.98*	721.42*	0.0047*	0.0001*
D x G	15	100.29*	134.34*	418.91*	10.38*	3.91*	114.64*	0.0001*	$8.02.10^{-6}$*
Residue	696	6.60	4.45	6.22	0.60	1.06	3.42	$1.16.10^{-6}$	$2.75.10^{-7}$
Average	-	8.71	7.08	10.97	2.02	1.68	3.70	0.0096	0.0036
CV (%)	-	29.48	29.78	22.73	38.27	61.20	50.02	11.24	14.65

Number of roots (NR), root length (RL), shoot length (SL), insert the first leaf (INSFL), length of the first leaf (LFL), length of the second leaf (LSL), dry matter of shoot (DMS) and dry matter of root part (DMR). *Significant values at 5% error probability by F test. DF: Degrees of freedom. MS: mean. Square. CV: coefficient of variation.

For the variable NR, differences between sensitive and tolerant genotypes were observed. BRS Sinuelo CL and Puitá INTA CL (tolerant) showed better fit to the polynomial model, an increase in the average NR to the dose 45 µg L^{-1}. These genotypes showed a subsequent slight decrease, indicating tolerance. The sensitive cultivar responses fitted to a linear regression, reducing the average NR with increasing of herbicide doses (Figure 3a).

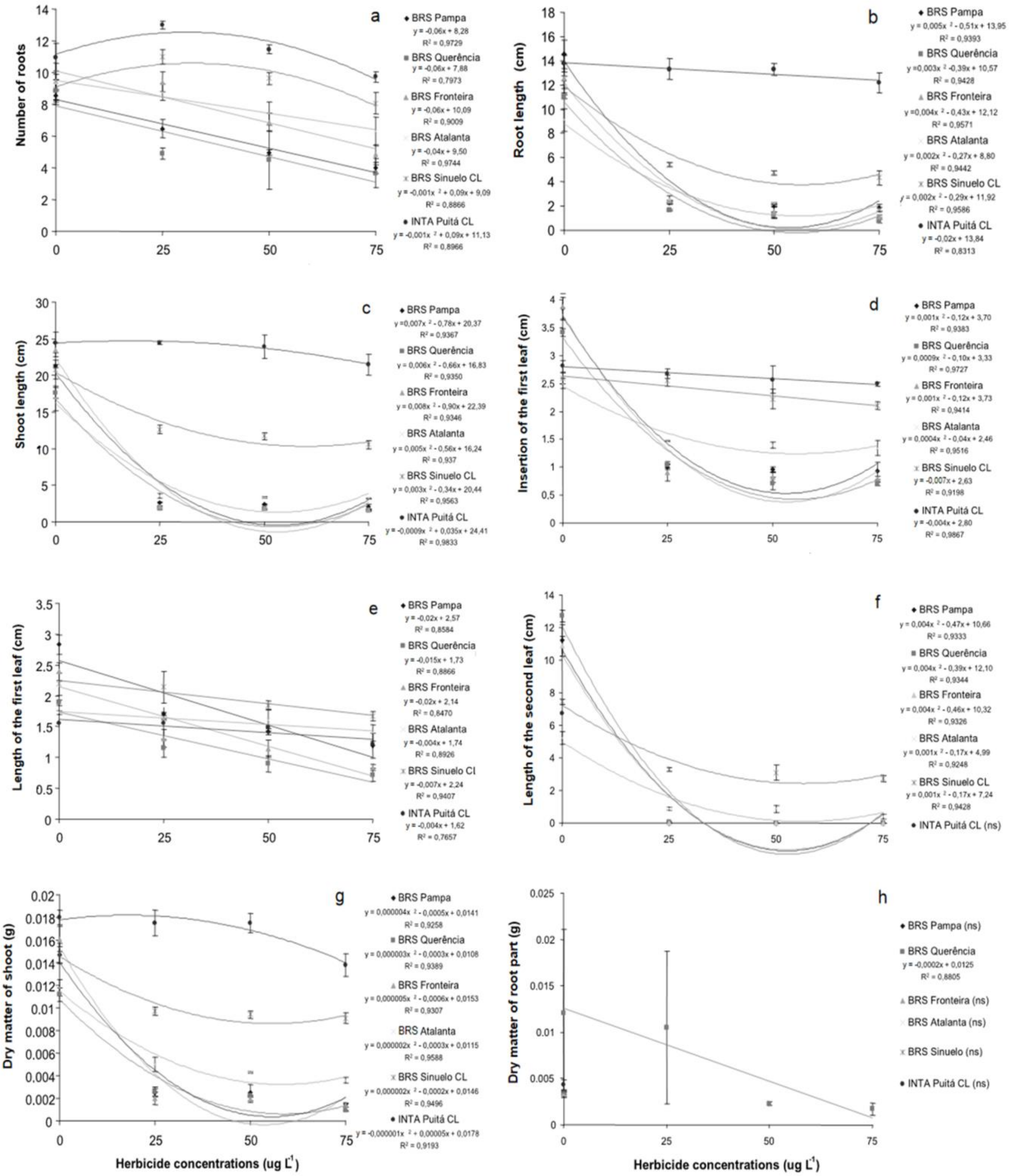

Figure 3. Parameters of regression equations and their graphical representations of the variables: a - number of root (NR), b - root length (RL) c – shoot length (SL), d - insertion of the first leaf (INSFL) e - length of the first leaf (LFL), f - length of the second leaf (LSL), g - dry matter of shoot (DMS), h - dry matter of root part (DMRP), six genotypes of rice, valued in four herbicide concentrations (Only®) (0, 25 µg L^{-1}, 50 µg L^{-1} and 75 µg L^{-1}) at 21 days of cultivation in hydroponic system. Bars represent mean standard error, and (ns) no significant polynomial regression equation.

For the variable RL measured at 21 days of development (Figure 3b), genotypes displayed a range of performances, forming a polynomial model response, except for Puitá INTA CL (linear response). BRS Sinuelo CL (tolerant), showed a reduction in length to the dose 72.5 µg L^{-1}, similar to BRS Atalanta (67.5 µg L^{-1}). Other sensitive genotypes also suffered severe reductions at doses ranging from 51 and 65 µg L^{-1}.

For SL (Figure 3c), the genotype responses were fitted to a quadratic regression model. Puitá INTA CL showed characteristic of tolerance to the herbicide showing increase to the dose 19.44 µg L^{-1}, with subsequent decrease. BRS Sinuelo CL showed lower reduction than sensitive genotypes to the dose 56.67 µg L^{-1} (0.34cm for each increase in herbicide concentration unit, considering the linear term without changes in the quadratic term), demonstrating increased tolerance to the herbicide when compared to sensitive genotypes, which showed a reduction ranging from 0.90 to 0.56 cm in average shoot length at herbicide doses ranging from 55 to 56.25 µg L^{-1}.

For the variable IFL, two groups were formed, allowing the discrimination of sensitive and tolerant genotypes. The first group was formed by tolerant genotypes, fitting to a linear regression (BRS Sinuelo CL, b= - 0.007; Puitá INTA CL, b= - 0.004), showing a slight decrease with respect to the addition of an herbicide concentration unit. The second group was formed by sensitive genotypes, showing as best fit a quadratic regression.

The responses for the variable LFL resulted in a linear model for all genotypes. A less severe response was detected in BRS Atalanta (sensitive), demonstrating increased tolerance to the herbicide, and a more severe response for BRS Querência, BRS Pampa and BRS Fronteira (sensitive).

For LSL (Figure 3f) one can observe that Puitá INTA CL did not fit a regression model, with an average of 6.73 cm. Sensitive genotypes BRS Pampa, BRS Querência and BRS Fronteira showed no development of the second leaf in the presence of the herbicide, only in the control treatment. However, BRS Atalanta BRS Sinuelo CL did show some development of second leaf. This trait did not allow genotypes discrimination to the herbicide tolerance.

Considering the performance for the variable DMS (Figure 3g), the genotypes followed a quadratic regression model. Puitá INTA CL expressed an increase in the variable up the dose 25 µg L^{-1}, with a subsequent decrease. BRS Sinuelo CL decreased until the dose 50 µg L^{-1}. The sensitive genotypes (BRS Pampa, BRS Fronteira, BRS Querência and BRS Atalanta) expressed a larger reduction for the variable assessed until dose ranging from 50 to 75 µg L^{-1} demonstrating greater sensitivity to the toxic effect of the herbicide. The variable DMS allowed discrimination between sensitive and tolerant genotypes.

For the variable DMR (Figure 3h), the genotypes BRS Pampa, BRS Fronteira, BRS Atalanta, BRS Sinuelo CL and Puitá INTA CL did not follow a polynomial regression model and showed the following average 0.0035, 0.0034, 0.0033, 0.0035 and 0.0043 g, respectively. BRS Querência was fitted to a linear model showing a decrease in the variable dry matter at each increment of herbicide.

Variables had positive and significant correlations with each other, except for the variable DMR that showed no significant correlation ($p \leq 0.05$) with the other variables (Table 5). The results for the dose-response curves suggest that the variables NR, APL, IFL and DMAP are indicated to select promising genotypes for tolerance to the herbicide, at a dose of 25 µg L^{-1}. Significant correlations indicate that the variables have high correlation between them and average magnitude. The results obtained were similar to those observed at fourteen days of development.

Table 5. Pearson correlation coefficient between the variables of six rice genotypes evaluated in four herbicide concentrations after 21 days.

Variables	NR	RL	SL	INSFL	LFL	LSL	DMS	DMR
NR	1	0.69*	0.74*	0.64*	0.47*	0.61*	0.77*	0.05
RL		1	0.97*	0.88*	0.58*	0.94*	0.95*	0.14
SL			1	0.89*	0.55*	0.92*	0.98*	0.09
INSFL				1	0.74*	0.95*	0.88*	0.17
LFL					1	0.68*	0.56*	0.04
LSL						1	0.89*	0.16
DMS							1	0.07
DMR								1

Number of roots (NR), root length (RL), shoot length (SL), insert the first leaf (INSFL), length of the first leaf (LFL), length of the second leaf (LSL), dry matter of shoot (DMS) and dry matter of root part (DMR), * Significant at the 0.05 level of probability by t test.

At 28 days the interaction effects between genotype and dose factors were significant for all variables (Table 4; Figure 4).

For the variable NR, differences were observed for sensitive and tolerant genotypes. BRS Sinuelo CL and Puitá INTA CL characterized as tolerant to the herbicide showed better fit to the polynomial model, showing an increase in the average NR up to the doses 35 and 32.5 µg L^{-1}. For genotypes BRS Sinuelo CL and Puitá INTA CL, respectively, with little subsequent sharp decline, showing characteristic tolerance. The susceptible genotypes, by adjusting the linear regression showed sensitivity, reducing the average of NR with increased herbicide doses.

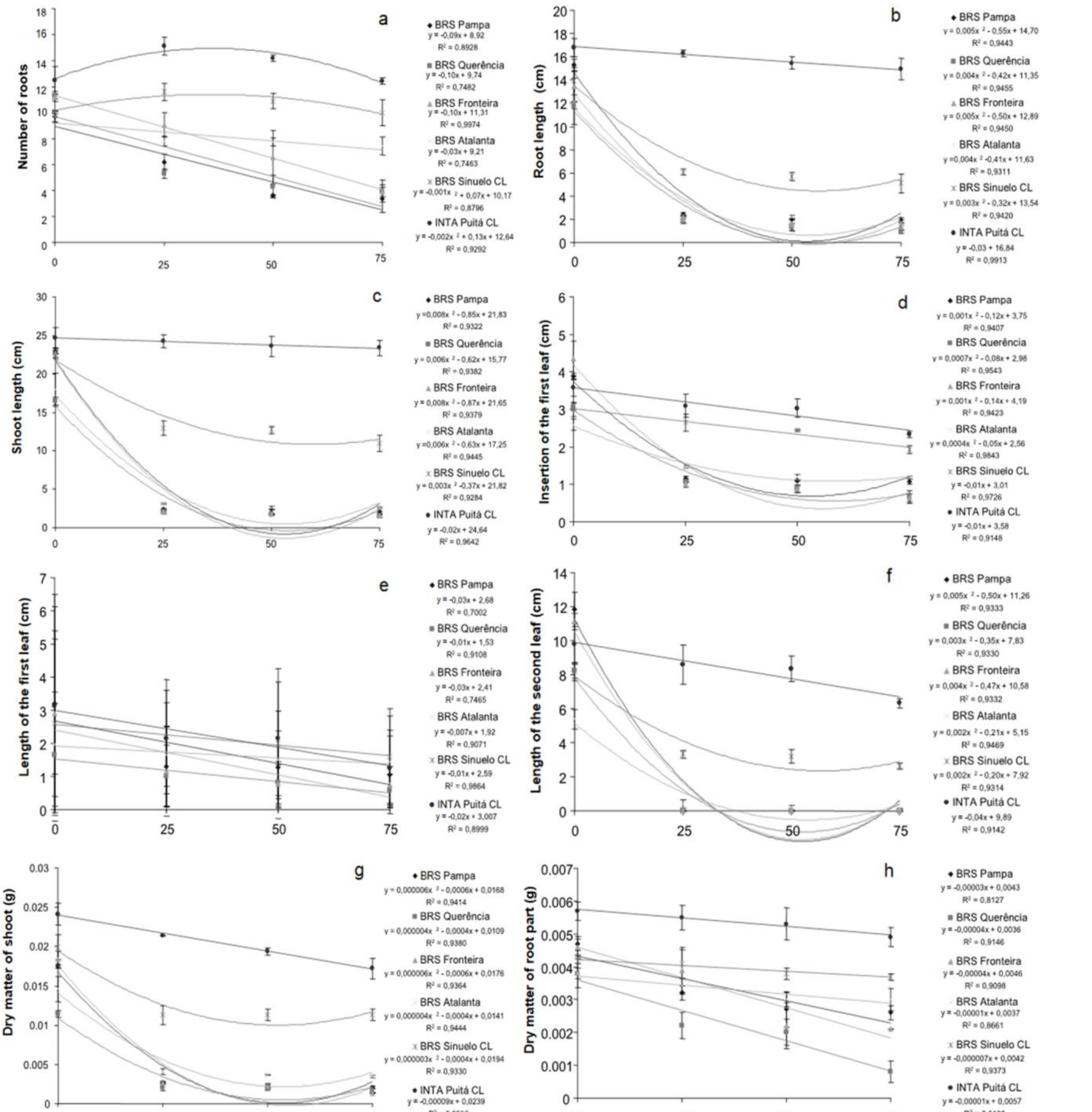

Figure 4. Parameters of regression equations and their graphical representations of the variables: a - number of roots (NR), b - root length (RL) c – shoot length (SL), d - insertion of the first leaf (INSFL) e - length of the first leaf (LFL), f - length of the second leaf (LSL), g - dry matter of shoot (DMS), h - dry matter of root part (DMRP), six genotypes of rice, valued four herbicide concentrations (Only®) (0, 25 µg L^{-1}, 50 µg L^{-1} and 75 µg L^{-1}) at 28 days of cultivation in hydroponic system. The bars represent the means of standard error and (ns) no significant polynomial regression equation.

The variable RL, according to Figure 4b, shows a differential behavior among genotypes, forming a group fit to a polynomial model, except for the cultivar Puitá INTA CL, who presented the best fit in the linear regression model. The cultivar BRS Sinuelo CL presented length reduction up to a dose 53.33 µg L^{-1} (0.32cm for each increase in herbicide concentration unit, without considering the change in the linear quadratic term). Considering the other sensitive genotypes, a marked reduction from the dose 25 µg L^{-1} until doses ranging between 50 and 55 µg L^{-1} observed.

Evaluating the variable length of shoot (Figure 4c), it can be inferred that the genotypes have adapted to the quadratic regression model except for the cultivar Puitá INTA CL, which showed a tolerance behavior, fitting a linear regression model, with a reduction of 0.02 cm at each unit increase of herbicide concentration. BRS Sinuelo

CL showed a lower decrease in the character than the sensitive genotypes to the critical dose 61.67 µg L^{-1} (0.37 cm for each unit increase in herbicide concentration, considering the linear term without changes in the quadratic term), demonstrating behavior greater tolerance to the herbicide that sensitive genotypes.

For the variable IFL, it is observed in Figure 4d the formation of two groups, discriminating genotypes in sensitive and tolerant. The first group, formed by the tolerant genotypes fitted a linear regression response (BRS Sinuelo CL; b= -0.01 and Puitá INTA CL; b= -0.01), showing a less steep slope with the addition of herbicide. The second group comprises the genotypes sensitive to the herbicide, that best fit a quadratic regression. The behavior of genotypes for the variable IFL was similar to other evaluated periods.

For the variable LFL (Figure 4e), a lower decrease for BRS Atalanta (sensitive, b= -0.007) can be seen, demonstrating more tolerance to the herbicide. All the other genotypes showed higher decreases.

For LSL (Figure 4f), Puitá INTA CL showed a linear decrease of 0.04cm for each unit increased of herbicide. Sensitive genotypes BRS Pampa, BRS Querência, BRS Fronteira showed no development of the second leaf in the presence of the herbicide, only in the control treatment. Maximum reduction was seen at 52.5 µg L^{-1} (BRS Atalanta), and 50 µg L^{-1} (BRS Sinuelo CL).

For DMS (Figure 4g), a quadratic regression tendency was seen for most genotypes, except Puitá INTA CL (linear). BRS Sinuelo CL showed decrease until the dose 66.67 µg L^{-1} (reducing 0.0004 g for each increment of herbicide concentration unit).

On the other hand, sensitive genotypes (BRS Pampa, BRS Fronteira, BRS Querência and BRS Atalanta) expressed greater reduction for the variable evaluated until the dose 50 µg L^{-1} showing greater sensitivity to the toxic effect of the herbicide. DMS allows to discriminate sensitive and tolerant genotypes, the example of the results obtained for the other evaluation periods.

For DMR (Figure 4h) a linear response was observed. BRS Pampa, BRS Querência and BRS Fronteira showed the highest linear regression coefficients, expressing greater sensitivity to the herbicide. BRS Atalanta and Puitá INTA CL showed greater tolerance to the toxic effect of the herbicide.

All variables showed positive and significant correlations with each other, after 28 days of development (Table 6). The results for the dose-response curves suggest that the variables NR, RL, SL, IFL, LSL and DMS are indicated to select promising genotypes for tolerance to the herbicide imidazolinone chemical group. A dose of 25 µg L^{-1} of the herbicide is needed to occur discrimination between sensitive and tolerant genotypes at seven, fourteen and twenty-one days of growth. Significant correlations indicate that the variables showed high correlation magnitude with each other. At 28 days, plant death could be observed in some genotypes (BRS Querência and BRS Fronteira).

Table 6. Pearson correlation coefficient between the variables of six rice genotypes evaluated in four herbicide concentrations after 28 days

Variables	NR	RL	SL	INSFL	LFL	LSL	DMS	DMR
NR	1	0.77*	0.82*	0.75*	0.62*	0.72*	0.83*	0.82*
RL		1	0.97*	0.89*	0.71*	0.94*	0.96*	0.79*
SL			1	0.90*	0.72*	0.94*	0.98*	0.80*
INSFL				1	0.87*	0.95*	0.90*	0.75*
LFL					1	0.81*	0.76*	0.67*
LSL						1	0.91*	0.73*
DMS							1	0.82*
DMR								1

Number of roots (NR), root length (RL), shoot length (SL), insert the first leaf (INSFL), length of the first leaf (LFL), length of the second leaf (LSL), dry matter of shoot (DMS) and dry matter of root part (DMR), * Significant at the 0,05 level of probability by t test.

An appropriate variable would be the one that has higher magnitude of change, or the most responsive variable, and also presents a significant effect for genotype x dose interaction, since efficiently discriminates the genotype responses facing the doses used (Camargo and Oliveira 1981, Camargo and Ferreira 1992, Freitas 2003).

Another important observation obtained in this study was the independent variable number of tillers, which was expressed only in the presence of the herbicide: at seven days of development in the genotypes BRS Fronteira and BRS Atalanta; at 14 days of development, in the genotypes BRS Querência, BRS Fronteira, BRS Atalanta and BRS Sinuelo CL; at 21 days in the genotypes BRS Pampa, BRS Querência, BRS Fronteira, BRS Atalanta and BRS Sinuelo CL; and at 28 days in the genotypes BRS Pampa, BRS Querência, BRS Fronteira and BRS Sinuelo CL. The cultivar Puitá INTA CL showed no tillers in this experiment. The tillering stage on rice usually starts after 30 days of development, therefore the appearance of tillers was a defense mechanism developed by the genotypes to survive in the presence of herbicide.

According to Roso et al. (2010), seedling bioassays offer great potential for differentiation between cultivated rice biotypes resistant and susceptible to imidazolinone chemical group herbicides. It also stresses that the seedling stage is, in general, when there is a greater need of diagnosis of resistance in real time, because it represents the herbicide application stage in commercial rice crops.

The bioassays with rice seedlings sensitive and tolerant to imidazolinone herbicides presented in this study show allow to discriminate tolerant and sensitive genotypes. This could be a useful tool to determine the tolerance in red rice populations during crop development, allowing the adoption of measures that can maintain the sustainability of red rice control in herbicide tolerant rice cropping systems.

The variable IFL may be considered a suitable variable for studies because it discriminates more effectively, the different responses of genotypes to the doses used and for periods of development, and can be indicated as morphological markers. They also enable the discrimination of genotypes in shorter time and can be seen at 7 days of seedling development. The concentration of herbicide that enables better discrimination between tolerant and sensitive genotypes is 25 µg L^{-1}, according to the methods described in this bioassay.

The most responsive variable was IFL, showing significant effect for genotype x dose at seven days of seedling development. At 14 days, the variables NR, RL, SL, IFL. LFL and DMS were the most responsive. At 21 days, the best variables were NR, SL, IFL and DMS. At 28 days, NR, RL, SL, IFL, LSL and DMS.

Acknowledgements

The authors are thankful to CNPq, CAPES, FAPERGS and Embrapa for the grants and fellowships.

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