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Response of oat seedlings to stress caused by acetic and butyric acids

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Abstract

White oat is an excellent alternative winter crop for crop rotation systems. The decomposition of organic matter retained on the soil in no-till conditions associated with poor drainage or excessive moisture increase the formation of organic acids, including acetic and butyric acids, with high phytotoxic potential. This study aimed to evaluate the effect of acetic and butyric acids on germination and early seedling development of different oat cultivars. The experiment was a completely randomized block design, where seeds of the cultivars Afrodite, Albasul, Brisasul, FAEM 06, IAC 7, URS 21 and URS Taura, were subjected to four levels of acetic (0, 4, 8 and 12 mM) and butyric (0, 3, 6 and 9 mM) acids. Seeds were kept in a germination chamber for ten days. The traits evaluated were: shoot length (SL), root length (RL), and germination percentage (% GER). Butyric acid was more toxic than acetic acid, impairing more root development than shoot length. The cultivars IAC 7 and Brisasul were more sensitive and the cultivars URS Taura and Afrodite were tolerant to the organic acids studied. In the control and in the treatment with 12 mM acetic acid, we found correlation between RL and %GER. There is a large variation among cultivars in response to acetic and butyric acids.

Key words: phytotoxicity, *Avena sativa,* germination, root, shoot.

Resposta de plântulas de aveia ao estresse por ácidos acético e butírico

Resumo

A aveia branca é uma excelente opção como cultura de inverno em sistemas de sucessão de culturas. A decomposição do material orgânico, mantido na superfície do solo no plantio direto, associado a condições de má drenagem ou umidade excessiva, acentua a formação de ácidos orgânicos. Dentre estes, os ácidos acéticos e butírico apresentam grande potencial fitotóxico. O objetivo do trabalho foi avaliar o efeito dos ácidos acético e butírico na germinação e no desenvolvimento inicial de plântulas de diferentes cultivares de aveia branca. O experimento foi conduzido em blocos completamente casualizados, onde sementes das cultivares Afrodite, Albasul, Brisasul, FAEM 06, IAC 7, URS 21 e URS Taura foram embebidas em quatro doses de ácido acético (0, 4, 8 e 12 mM) e butírico (0, 3, 6 e 9 mM). As sementes foram mantidas em câmera de germinação por dez dias. Foram avaliados os caracteres comprimento da parte aérea (CPA), comprimento de raiz (CR) e a porcentagem de germinação (% GER). O ácido butírico demonstrou-se mais fitotóxico que o ácido acético, sendo o desenvolvimento das raízes mais afetado que a parte aérea. As cultivares IAC 7 e Brisasul demonstraram-se mais sensíveis e as cultivares URS Taura e Afrodite demonstraram-se menos afetadas pelos ácidos orgânicos em estudo. Sem a adição de ácidos e na dose de 12 mM de ácido acético, verificou-se a correlação entre % GER e CR. Existe grande variabilidade entre as cultivares estudadas, em resposta aos ácidos acético e butírico.

Palavras-chave: fitotoxidez, *Avena sativa,* germinação, raiz, parte aérea.

1. INTRODUCTION

White oat (*Avena sativa* L.) is grown for grain production for human food and animal feed, for pasture and/or as ground cover for the no-tillage system, being a viable alternative to crop rotation (CBPA, 2006; Kopp et al., 2009a).

It is an annual crop with great importance as an alternative winter crop, especially in southern Brazil (Crestani et al., 2010), also reaching areas in the Central-West region. In the areas of expansion of oats cultivation, the main goal is to maintain ground cover for the formation of biomass and grain production in no-tillage system (Castro et al., 2012).

The no-tillage production system is characterized by retaining crop residues on the soil surface. During the decomposition of these residues there is increased production of organic acids (Camargo et al., 2001). Among the short-chain aliphatic acids formed, acetic and butyric acids are those found in higher concentrations, wherein the acetic acid is present at higher concentrations than butyric acid, ca. 10x (Bohnen et al., 2005).

The Southern Region of Brazil is composed of an area of 6.8 million hectares with hydromorphic soils, characterized by poor natural drainage (Pinto et al., 2004). These soils, when combined with vegetable residues resulting from the no-tillage management, favor the production of organic acids (Kopp et al., 2009b; Sousa and Bortolon, 2002). Even in non-hydromorphic soils, under excessive moisture upon planting in the no-till system, the wet straw can be allocated in the same depth of the seed, and during its decomposition, the formation of acids can affect the development of seedlings (Lynch, 1986).

For some oat genotypes, toxicity caused by organic acids have been observed in the early stages of plant development, reducing germination, root growth and seedling height (Kopp et al., 2009a; Lynch, 1978; Tunes et al., 2008). These symptoms are associated with respiration inhibition, with consequent changes in cell division and membrane degradation (Camargo et al., 2001).

Organic acids interfere with vital plant processes, responsible for energy production in the root system as respiration and oxidative phosphorylation, and act as inhibitors of mitochondrial functions (Bortolon et al., 2009).

Understanding the behavior of oat genotypes in the presence of organic acids, as well as identifying the genetic variability of cultivars in response to them is of utmost importance for breeding programs aiming to provide the marked with cultivars better adapted to their respective growing regions. In this context, this study evaluated the effect of acetic and butyric acids on germination and early seedling development of different oat cultivars.

2. MATERIAL AND METHODS

Two experiments were performed simultaneously; one with acetic acid and the other with butyric acid. Each experiment was conducted in a completely randomized block design, with a simple factorial (cultivar x level). We used seeds of seven oat cultivars, which are: Afrodite, Albasul, Brisasul, FAEM 06, IAC 7, URS 21 and URS Taura. The levels evaluated were 0 (control), 4, 8 and 12 mM for acetic acid and 0 (control), 3, 6 and 9 mM for butyric acid.

To induce stress, seeds were imbibed in solutions with the respective concentrations of the studied acids for 90 minutes. After the soaking period, seeds were allowed to germinate on a paper towel (germitest), moistened with distilled water at a ratio of 2.5 times the weight of the dry paper (BRASIL, 2009). The rolls were stored in a BOD germination chamber, 12 hours photoperiod, at 20°C for ten days.

On the tenth experimental day, we evaluated the length of shoots (SL) and roots (RL), measured in centimeters (cm), and germination (% GER), expressed as a percentage of normal seedlings obtained.

For % GER, four replications were evaluated; each experimental unit consisted of a roll with 50 seeds. For SL and RL, we analyzed three replications; for each replication we measured ten normal seedlings each which were arranged in the upper third of the germination paper.

Data were subjected to analysis of variance and polynomial regression. A Pearson correlation test was run between the variables measured, within each level tested. The statistical software used was Winstat (Machado and Conceição, 2002).

3. RESULTS AND DISCUSSION

The analysis of variance (Table 1) evidenced a significant interaction between the main factors, cultivar and level for all traits. The response of cultivars was verified by polynomial regression. The graphs also illustrate the bars of

Table 1. Analysis of variance for the variables percentage of germination (% GER), shoot length (SL) and root length (RL) of oat cultivars subjected to stress by different levels of acetic and butyric acids

SV	DF.	Mean square		DF	Mean square			
		Acetic Acid	Butiric Acid		Acetic Acid		Butiric Acid	
		%GER			RL	SL	RL	SL
Genotype	6	449.58*	694.48*	6	16.11*	19.25*	5.52*	11.12*
Level	3	20.61*	280.67*	3	9.39*	0.90*	23.94*	1.83*
G x D	18	17.66*	111.77*	18	2.49*	0.39*	2.72*	3.41*
Residue	84	5.61	5.90	56	0.12	0.15	0.13	0.18
Mean		91.62	87.94		6.89	13.82	5.96	13.84
CV (%)		2.58	2.76		5.05	2.84	5.99	3.08

*Significant at ($p<0.05$) by F-test. DF= degrees of freedom.

standard deviation. For all analyzes, we verified the power of the test, and rejected the presence of type II error.

The performance of each cultivar in response to the addition of acetic acid for % GER is illustrated in figure 1. We observed that the increase in levels did not alter the % GER of the cultivars Afrodite, Albasul, FAEM 06 and IAC 7. In an experiment adopting the same methodology used herein, the authors verified that the percentage of germination of the cultivar UPF 18 was not affected by the stress caused by acetic acid (Tunes et al., 2013). In the field cultivation, seed germination is the most sensitive stage, possibly because the seedling needs to break a barrier formed by soil and plant residues rich in organic acids, which cover the seed layer.

The cultivar Brisasul had its % GER best explained by a quadratic equation. Although the F test had detected significant changes, based on the standard deviation, it should be noted that the more restrictive level differed only from intermediate levels of 4 and 8 mM, which promoted a small increase in the percentage of germination. The cultivars URS Taura and URS 21 behaved similarly, and were represented by a linear equation, with a small increase of 6.43% and 4.60% in % GER, respectively, at the maximum level of acetic acid. A study on other organic acids (succinic, fumaric, malic and lactic) indicated their ability to stimulate % GER in oat until certain concentrations, by directly or indirectly promoting the absorption of oxygen (Adkins et al., 1985).

Figure 2 shows the response of cultivars, in % GER, to the addition of butyric acid. Only the cultivars Albasul and FAEM 06 kept unchanged their % GER. Cultivars Afrodite, URS Taura and URS 21 showed a better fit to a quadratic equation, with slight increases in germination at intermediate levels of 3 and 6 mM. For the cultivar URS 21, the level of 9 mM was restrictive to % GER, causing a significant reduction when compared to the control. Brisasul was also fitted to a quadratic equation, but it was possible to observe its sensitivity to butyric acid, sharply reducing the % GER yet in initial levels. The critical level for this cultivar was 6.55 mM butyric acid, reaching % GER of 70%.

Unlike that observed for acetic acid, for butyric acid, the IAC 7 showed a linear reduction in % GER, with acid addition. In fact, butyric acid is more phytotoxic than acetic acid (Camargo et al., 2001). At the maximum level (9 mM), % GER was 13.7% lower than observed in the control, reaching only 79% GER. Reductions in % GER by adding butyric acid have been previously observed in oats and rice; for oats the difference was significant, however less than 5% (Tunes et al., 2008; 2013). It is worth mentioning that small differences in% GER, although statistically significant, may not result in yield loss, given the tillering ability of oats.

The addition of acetic acid had no influence on the SL of the cultivars Afrodite, FAEM 06, URS Taura and URS 21 (Figure 3). The cultivars Albasul and IAC 7 were represented by a quadratic equation, and their critical levels were 3.89 and 5.46 mM, respectively. Differently, the cultivar Brisasul increased its SL in response to acetic acid. Although significant, the increase at the maximum level was only 1.04 cm.

Studies with rice (Neves et al., 2010) and wheat (Neves et al., 2009) demonstrated increases in shoot length after soaking seeds in less concentrated levels of acetic acid. The authors suggest it is a result of an increase in translocation of nutrients from the seed endosperm through an increase in hydrolase enzyme activity. Neves and Moraes (2005) found that the total α-amylase activity was increased with increasing concentrations of acetic acid.

Regarding the addition of butyric acid (Figure 4), the SL of URS Taura was not changed, just as was observed with the addition of acetic acid. The cultivar IAC 7 had SL linearly reduced, reaching 11.34 cm SL at 9 mM of butyric acid, equivalent to 71% of the length observed in the control. The cultivars Brisasul and URS 21 showed a better fit to a quadratic equation, with a significant increase at the initial level of 3 mM butyric acid. This was also the degree of the polynomial representativeness of the cultivars Albasul and FAEM 06, however the curve was concave, and the variations were of low magnitude. The performance of the cultivar Albasul was very similar to that presented with addition of acetic acid.

The root length according to increasing levels of acetic acid is illustrated in figure 5. This variable remained constant in the cultivars Albasul and URS Taura. None of the studied acids altered the RL of the cultivar URS Taura (Figure 5 and 6). A quadratic regression represented the performance of cultivars Afrodite, IAC 7 and URS 21, and between levels of 4 mM and 8 mM, there was a significant increase in root length. For the URS 21, the level of 12 mM was sufficient to reduce the RC compared with the control. As for the cultivars Afrodite and IAC 7, this level caused no significant reduction compared with the control, indicating the tolerance of these cultivars to the levels tested. The cultivars Brisasul and FAEM 06 showed a linear decrease in RL, especially the cultivar Brisasul, which presented a RL of 9.02 cm in the control and 5.12 cm at the maximum level (12 mM). Root growth of rice seedlings was inhibited by acetic acid (Armstrong and Armstrong, 2001; Sousa and Bortolon, 2002). Another study with rice also showed that with higher levels of acetic acid reduces the RL, but low levels (<6.8 mM) allowed a slight increase (Neves et al., 2010).

Butyric acid has been shown to be very phytotoxic to root development (Figure 6), resulting in a linear decrease in RL in the cultivars Albasul, Brisasul, FAEM 06 and IAC 7 In a hydroponic experiment, with a mixture of acetic, propionic and butyric acids, the cultivars Albasul and IAC 7 linearly reduced their root length (Kopp et al., 2009a).

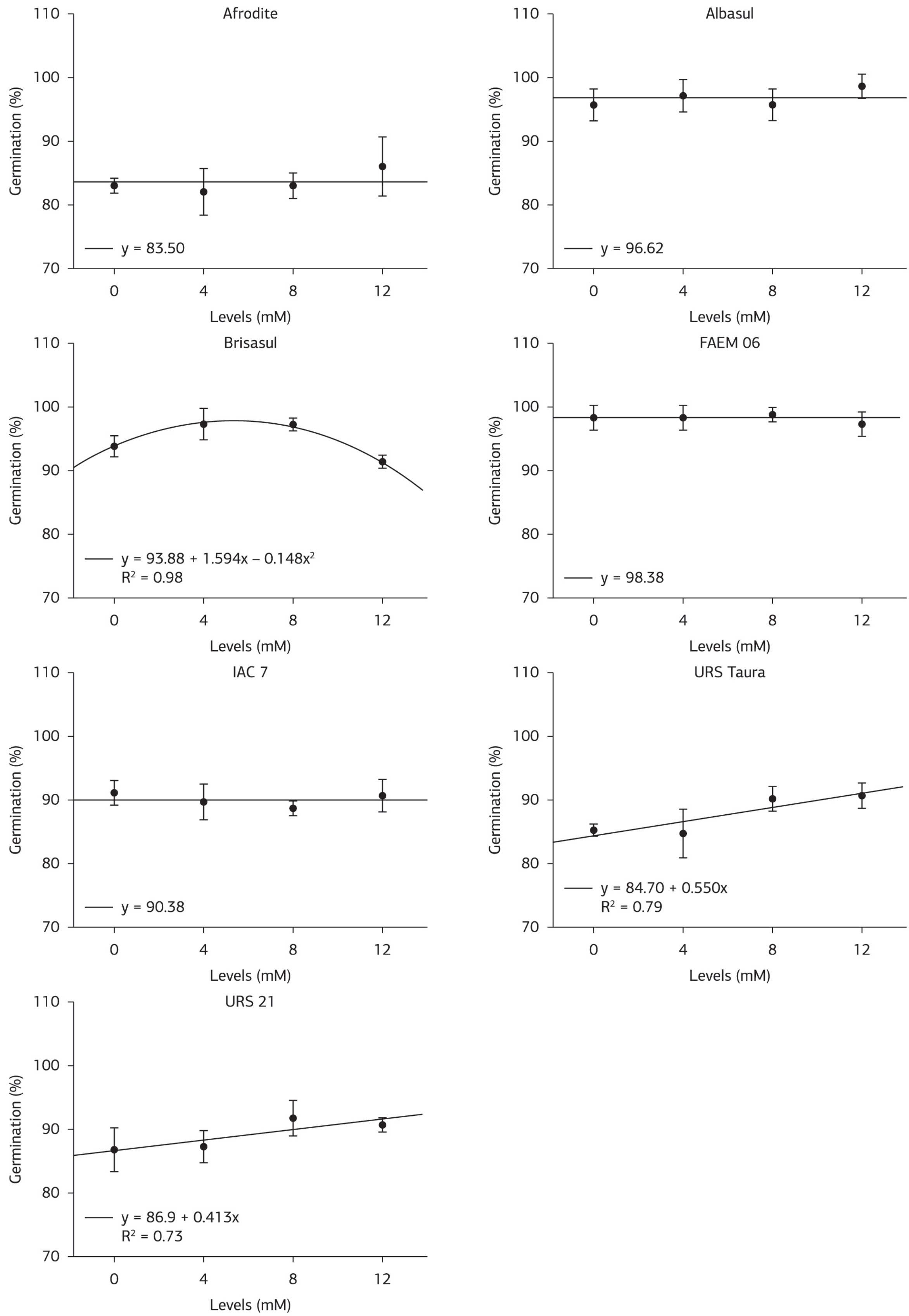

Figure 1. Graphical representation. Fit of the regression equations and coefficients of determination (R^2) of percentage of germination for oat cultivars subjected to stress by different levels of acetic acid.

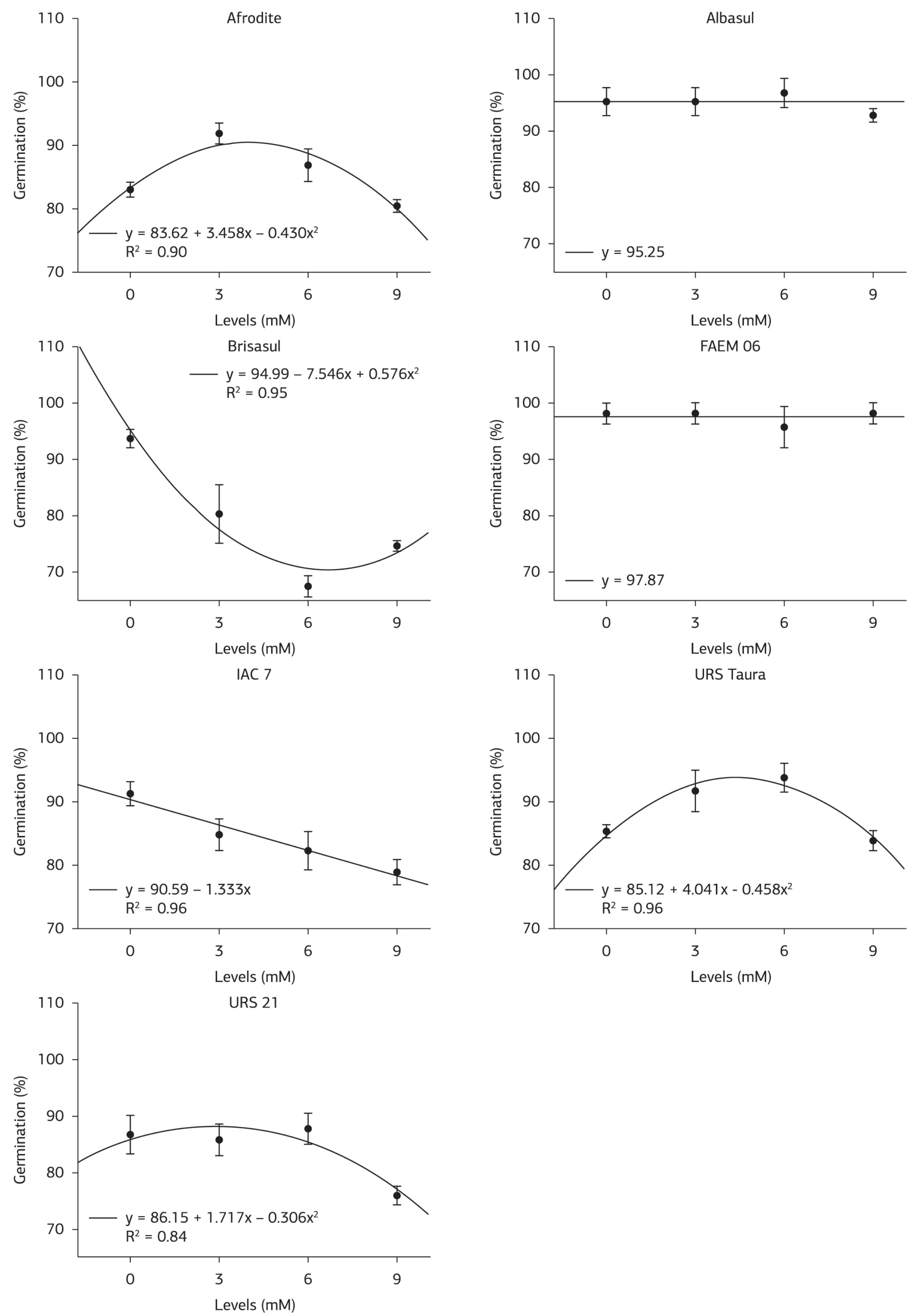

Figure 2. Graphical representation. Fit of the regression equations and coefficients of determination (R^2) of percentage of germination for oat cultivars subjected to stress by different levels of butiric acid.

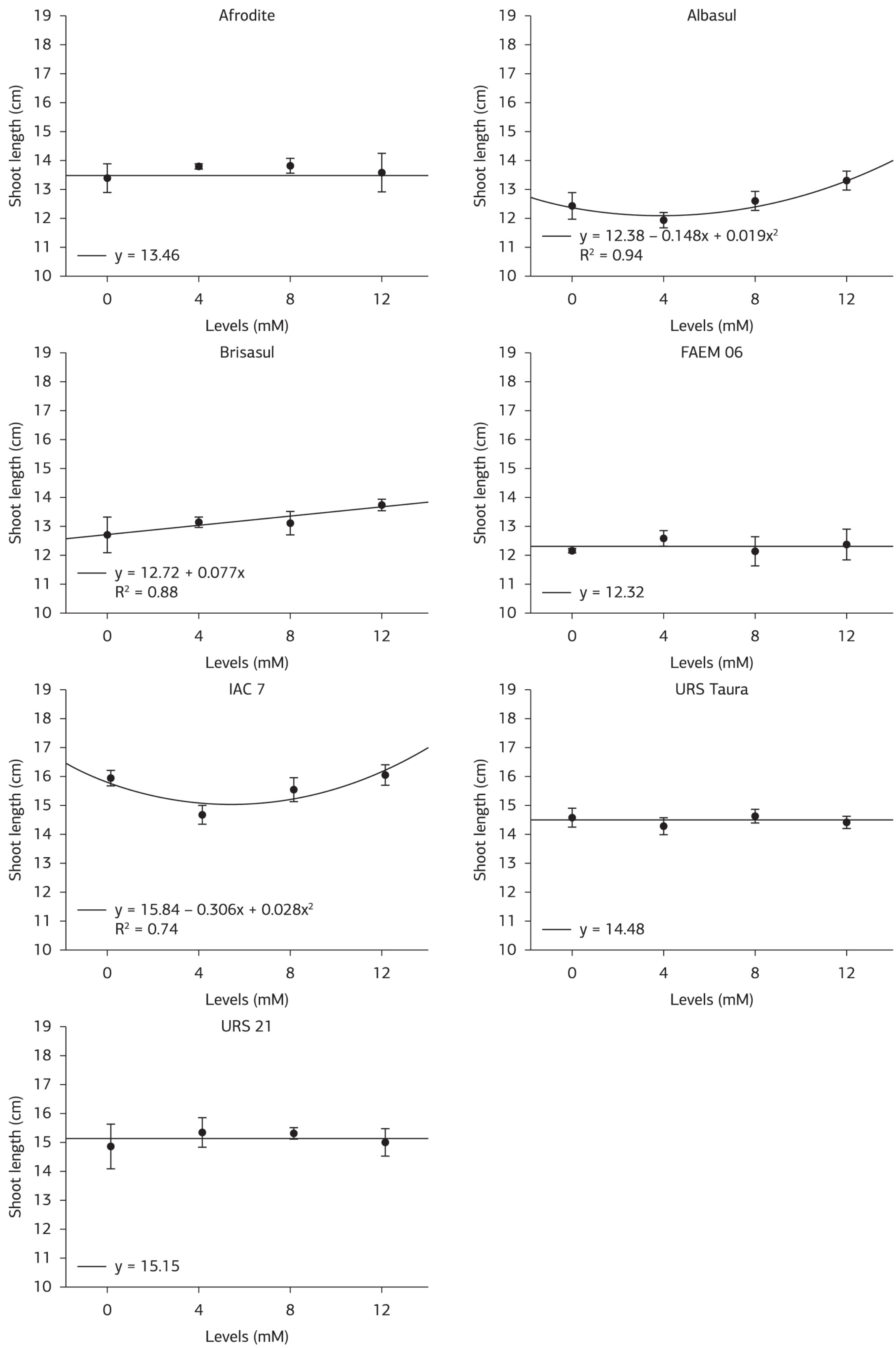

Figure 3. Graphical representation. Fit of the regression equations and coefficients of determination (R^2) of shoot length for oat cultivars subjected to stress by different levels of acetic acid.

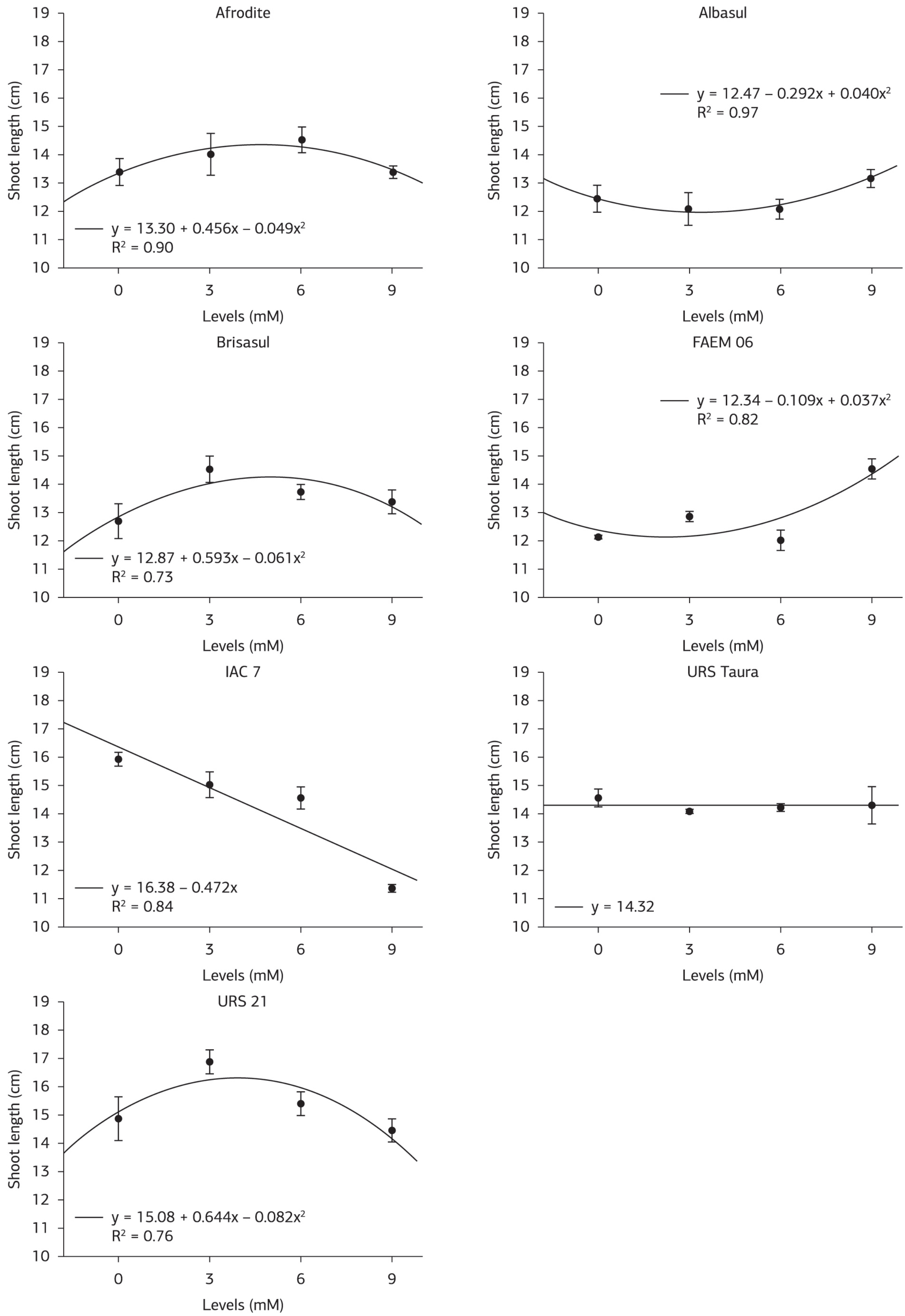

Figure 4. Graphical representation. Fit of the regression equations and coefficients of determination (R^2) of shoot length for oat cultivars subjected to stress by different levels of butiric acid.

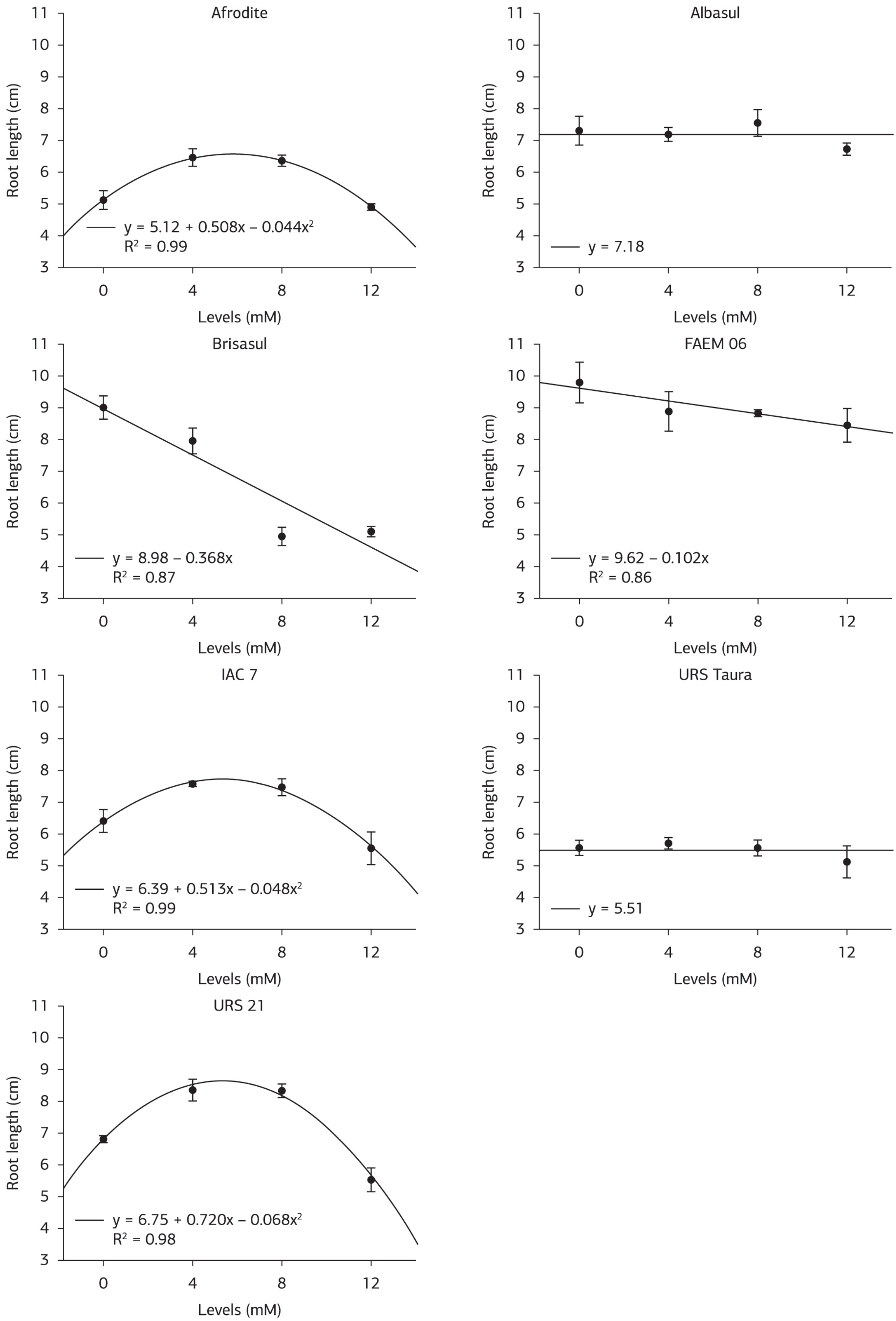

Figure 5. Graphical representation. Fit of the regression equations and coefficients of determination (R^2) of root length for oat cultivars subjected to stress by different levels of acetic acid.

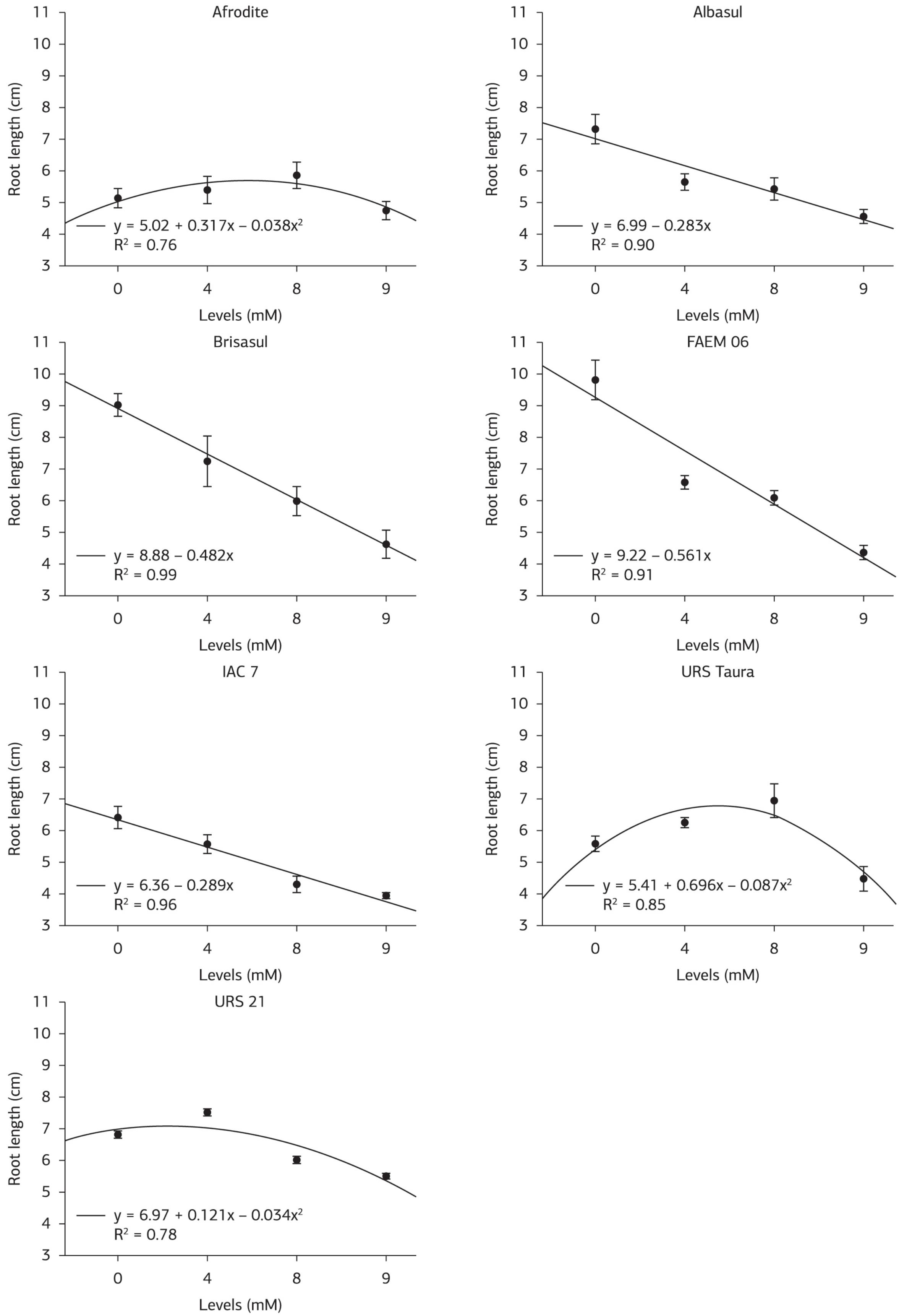

Figure 6. Graphical representation. Fit of the regression equations and coefficients of determination (R^2) of root length for oat cultivars subjected to stress by different levels of butiric acid.

Similar results were found in this work, only for butyric acid, suggesting a strong contribution of this acid to the decrease in RL.

Indeed, despite at a lower concentration in the soil solution, butyric acid has high potential to cause damage to seedling establishment, because the larger the size of the carbon chain of the acid the higher is its phytotoxicity (Angeles et al., 2005; Rao and Mikkelsen, 1977). Studies with rice and oats confirm the greater phytotoxic effect of butyric acid on root length (Bortolon et al., 2009; Kopp et al., 2010; Schmidt et al., 2007; Tunes et al., 2008; 2013).

Moreover, the other cultivars showed better fit to quadratic equation, with reductions in root development at higher levels. Among these cultivars, URS Taura and URS 21 reached 80% of RL, at the maximum level of 9 mM. At this level, the cultivar Afrodite maintained 92% of RL obtained in the control, suggesting a lower sensitivity to butyric acid.

In general, there was a great variability among cultivars studied in response to acetic and butyric acids. This variation should be explored when seeking cultivars tolerant to the phytotoxic effect of organic acids.

Among the cultivars analyzed, the cultivar Brisasul was sensitive to the action of organic acids, with the greatest reduction in RL (21%) at the maximum level of acetic acid (12 mM) and linear and sharp reductions in RL and% GER as a function of levels of butyric acid. The cultivar IAC 7 was sensitive to butyric acid with linear and pronounced reduction in RL, SL and % GER in response to different levels of this acid.

The cultivar URS Taura stood out as potential tolerance to acids studied. % GER did not reduced with acetic acid and reduced only 2% at the highest level of the butyric acid and both acids did not interfere with the SL. The RL was altered only by butyric acid where the highest level (9 mM) resulted in 80% of the performance of this variable. The cultivar Afrodite, also deserves mention, as despite the effects caused by acetic and butyric acids, there was no significant reduction in % GER in RL and SL; the maximum observed reduction was 8% for the RL trait, compared to a maximum level of butyric acid.

The correlation analysis showed that when there was no addition of any acids, only the variables % GER and RL and remained highly positively correlated (0.88, Tables 2 and 3). The presence of correlation indicates that changes suffered by a variable are accompanied by changes in the other (Carvalho et al. 2004). Considering the acetic acid at 12 mM, a significant correlation was observed, again between the variables RL and % GER (0.83; Table 2). Significant correlations were registered between RL and SL in oats and rice seedlings subjected to stress with a mixture of acetic, butyric and propionic acids in hydroponic system (Kopp et al., 2009b; 2012). In the present study these variables were not correlated. Using levels of butyric acid, there was no association between the variables measured (Table 3). The lack of correlation reflects the variability between cultivars with different responses to the addition of the acid.

Table 2. Estimation of correlations between percentage of germination (% GER), shoot length (SL) and root length (RL) of oat cultivars subjected to stress by different levels of acetic acid

Levels Variables	-----------------------0 mM-----------------------				-----------------------8 mM-----------------------		
	% GER	SL	RL		% GER	SL	RL
% GER	-	–0.53	0.88**	% GER	-	–0.59	0.21
SL	–0.56	-	–0.60	SL	–0.47	-	–0.06
RL	0.65	–0.02	-	RL	0.83*	–0.59	-
Levels	-----------------------4 mM-----------------------				-----------------------12 mM-----------------------		

* and **Significant by t-test. p<0.01 and p<0.05 respectively. In the upper diagonals, correlations are calculated for the levels 0 to 8 mM, and in the lower diagonals, correlations are obtained for levels of 4 and 12 mM.

Table 3. Estimation of correlations between percentage of germination (% GER), shoot length (SL) and root length (RL) of oat cultivars subjected to stress by different levels of butiric acid

Levels Variables	-----------------------0 mM-----------------------				-----------------------6 mM-----------------------		
	% GER	SL	RL		% GER	SL	RL
% GER	-	–0.53	0.88**	% GER	-	–0.37	0.20
SL	–0.71	-	–0.60	SL	0.28	-	0.01
RL	–0.42	0.54	-	RL	–0.34	0.60	-
Levels	-----------------------3 mM-----------------------				-----------------------9 mM-----------------------		

**Significant by t-test. p<0.01. In the upper diagonals, correlations are calculated for the levels 0 and 6 mM, and in the lower diagonals, correlations are obtained for levels of 3 and 9 mM.

4. CONCLUSION

Butyric acid is the most phytotoxic to the early development of seedlings of oat cultivars studied, mainly limiting the root length. The cultivars IAC 7 and Brisasul are more sensitive and the cultivars URS Taura and Afrodite are more tolerant to the organic acids. There is a positive correlation between percentage of germination and root length in the control and at the level of 12 mM acetic acid. There is large variation among cultivars in response to acetic and butyric acids.

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