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Water submersion of bean seeds in the vigour evaluation

SUMMARY

This work aimed to study the germination and vigour of seeds of two bean cultivars, submitted to different water submersion periods: 0, 4, 8, 12 and 16h at 25ºC. The following parameters were evaluated: seed moisture content, germination, first germination count, vigour classification, length and dry matter weight of hypocotyls and primary root. Results showed that germination and seed vigour decreased with the increase of seed submersion in water. There was a reduction of germination percentage in the seeds exposed to four or more hours of submersion. Vigour was evaluated by the first germination count and by tests of performance of seedlings. Germination and vigour were negatively affected. Four hours of submersion can irreversibly damage crop establishment in fewer vigorous lots, and eight hours can affect even the most vigorous lots. Four hours of submersion employed in a seed lab may be useful to discriminate vigour of bean seed lots.

Key words: flooding, *Phaseolus vulgaris* L., germination

Alagamento de sementes de feijão e avaliação do vigor

RESUMO

Este trabalho objetivou avaliar a germinação e o vigor de sementes, de dois cultivares de feijão, submetidas a diferentes períodos de submersão em água: 0, 4, 8, 12 e 16h a 25ºC. Foram avaliados os seguintes parâmetros: teor de água das sementes, germinação, primeira contagem da germinação, classificação do vigor, comprimento e massa seca do hipocótilo e da raiz primária. Os resultados demonstraram que a germinação e o vigor decresceram com aumento da submersão das sementes em água. Houve redução da porcentagem de germinação quando as sementes foram expostas a quatro ou mais horas de submersão. O vigor foi avaliado pela primeira contagem de germinação e pelos testes de desempenho de plântulas. A germinação e o vigor foram negativamente afetados. Quatro horas de submersão podem afetar o estabelecimento da cultura para lotes menos vigorosos, e oito horas afetam até os lotes mais vigorosos. Quatro horas de submersão podem ser úteis em um laboratório de sementes para classificar o vigor de lotes de sementes de feijão.

Palavras-chave: alagamento, *Phaseolus vulgaris* L., germinação

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INTRODUCTION

Plants are aerobic organisms. However, due to their inability of movement, some adaptation mechanisms were created to make them able to survive in anoxic environments (Crawford, 1992; Kolb et al., 2002), such as those occurring just after heavy rains or flooding (Dennis et al., 2000). Anaerobic conditions are a limiting factor, because seed deterioration will take place, leading to lower seedling appearance in field conditions (Duke & Kakefuda, 1981; Blom, 1999; Wuebker et al., 2001).

Excess water injury can contribute to decrease germination, because the damaged seed has low energy supply for that, reflecting low vigour (Richard et al., 1991). Several species showed a decrease in germination when submitted to flooding as barley (Narimanov & Korystov, 1998), rice (van Toai et al., 1988; Saka & Izawa, 1999), corn (Martin et al, 1991; Neumann et al., 1999; Dantas et al., 2000a, b) and navy beans (Custódio et al., 2002). This phenomenon could be driven by ready energy sources decrease, as free sugars (fructose, glucose) due to fermentation (Guglielminetti et al., 1995).

Reserve consumption for energy production and nutrient supply of embryo axis is an energy rich compound "burning" process, very dependent of free oxygen (Carvalho & Nakagawa, 2000). The under supply of that during wetting may change the biochemical pathway from respiration (glucolysis + Krebs's Cycle + respiratory chain) to fermentation (Drew, 1997), specially ethanol fermentative pathway, that would produce toxic substances for cell survival (Neumann et al, 1999; Blokhina et al., 2001; Schulüter & Crawford, 2001; Taiz & Zeiger, 2004) and germination (Zeng et al., 1999).

The germination ability of a seed lot is determined by the proportion of those that are able to produce normal seedlings in favourable conditions. In the absence of the required conditions, normally, a reduction in the germination percentage is observed, discounting the dormancy influence (Carvalho & Nakagawa, 2000).

Some species can support temporary (Souza et al., 1999) or even prolonged flooding periods, if they have the anatomical apparatus for that, as rice, where there are varietal differences in the seed survival rate after flooding (Saka & Izawa, 1999; Lenssen et al. 1999). There are indications that vigour loss, verified after controlled water submersion, can be used to discriminate lots, because vigorous seeds would be more resistant to the unfavourable condition of submersion (Dantas et al., 2000a, b; Custódio et al., 2002).

Vigour tests must be simple, objective and priceless, of fast execution and repeatability (Marcos-Filho, 1999). Some tests evaluate seed vigour through the tolerance or resistance to adverse environments (McDonald, 1975) as the cold test, accelerated ageing and controlled deterioration, which are the most useful tests.

Popinigis (1985) considered that water submersion test of non sterilized soil, as proposed by Woodstock (1973), efficient because only the most vigorous seeds would survive to pathogen presence, being a resistance or tolerance test. However, Dantas et al. (2000b) proposed a change in the procedure, named "flooding test", to be applied to corn seeds, using instead of water from soil, water with a fungicide and bactericide. In this way, it could be used as a stress test due to the low oxygen supply that would decrease navy bean germination and would cause seed deterioration.

This work aimed to evaluate the effect of seed submersion, during different periods, in the decrease of navy bean germination and the potential of this procedure to be used as a stress test.

MATERIAL AND METHODS

The experiments were carried out at the Seed Analysis Laboratory of the Agronomy Department, Universidade do Oeste Paulista (UNOESTE) at Presidente Prudente-SP, during the period of August-September of 2002.

Two cultivars of beans, 'IAPAR 65' and 'EMGOPA 201' were used in this experiment during three different years 1999, 2000 and 2001. Seeds were submersed in 100mL of water in plastic containers with 100 seeds each, with four repetitions per treatment, in the dark, at a temperature of 25ºC, during zero, 4, 8, 12 and 16 hours. To avoid fungal and bacterial proliferation, a fungicide (Carboxin 200 g/l + Thiram 200 g/l) 0.2% commercial product, and a bactericide (KILOL®) 1:125 (v:v) was added to the submersion solution.

After submersion, seeds were evaluated by the following tests:

Moisture content: To evaluate the moisture gain during the treatments, moisture determination was carried out with two repetitions per treatment in an oven at 105±3ºC, during 24 hours (Brasil, 1992).

Germination: It was carried out with four repetitions of 50 seeds per treatment sowed in a germination paper (paper roll) wetted with 2.5 times its dry weight and allowed to germinate in a 'Mangelsdorf' chamber at 25ºC . The germination counting was carried out at five and eight days after sowing and the germination percentage was calculated considering the normal seedlings (Brasil, 1992).

First germination count: Percentage of normal seedlings obtained at first count germination.

Vigour classification: It was carried out during the germination test. The seedlings were split in to; normal, strong and weak, abnormal and dead. Only the normal and strong seedlings were considered (Nakagawa, 1999).

Length and dry matter weight of the hypocotyls and primary root: The evaluation of length and dry matter of hypocotyls and primary root was carried out with four repetitions of ten seeds per treatment, in a unique line, in the first and third upper part of the germination roll, wetted with 2.2 times of its dry weight in water and germinated at 25ºC in a 'Mangelsdorf' chamber. At the fifth day, normal plants were evaluated measuring the hypocotyls and primary root length, and also the dry matter weight of hypocotyls (without the cotyledon) and root (Nakagawa, 1999).

The experimental design was completely randomized, with four repetitions of one hundred seeds each. Germination, first germination count and vigour classification, in percentage, were transformed by arcsine $(x/100)^{0.5}$. The length and dry matter weight results were not transformed. Variance was analyzed by F test, and if it was significant, the average comparison was made using Tukey's test at 5% of probability. For the submersion periods, a polynomial regression was performed. All statistical analysis was carried out using the software SANEST (Zonta et al., 1984).

RESULTS AND DISCUSSION

Results of moisture determination, before and after the submersion treatments (Table 1), showed that this parameter

Table 1. Moisture content (%) in bean seeds before and after submersion treatment

Tabela 1. *Conteúdo de umidade (%) em sementes de feijão antes e depois do tratamento de submersão*

Hours	IAPAR 65			EMGOPA		
	L99	L00	L01	L99	L00	L01
	%					
0	9,4	9,9	9,4	9,1	9,8	9,4
4	37,4	53,4	52,1	30,9	39,5	41,9
8	52,9	55,2	54,2	51,0	48,7	52,3
12	52,7	56,7	55,1	40,3	44,0	49,5
16	56,1	56,4	55,2	56,5	58,6	56,4

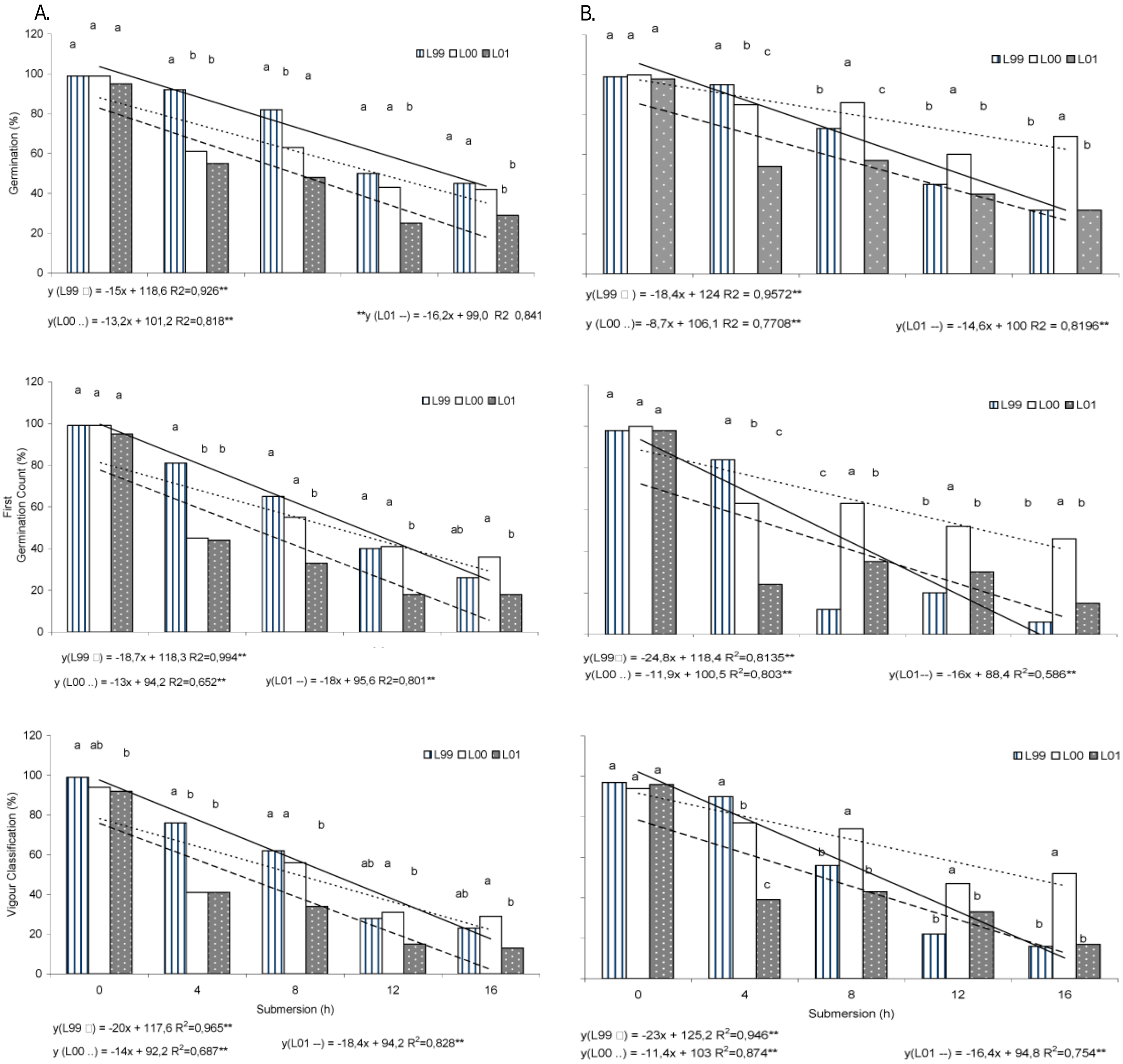

Figure 1. Germination, first germination count and vigour classification of three seeds lots of two different cultivars of beans ('IAPAR 65' (A) and 'EMGOPA-201' (B)) obtained from different periods of submersion in water. Columns are the lot response to the treatments and equal letters, per group, are not statistically significant at 5% of probability. Regression lines were the effect of the period of hypoxia over the seeds

Figura 1. *Germinação, primeira contagem de germinação e classificação de vigor de dois diferentes cultivares de feijão ('IAPAR 65' (A) e 'EMGOPA-201' (B)) obtidos de diferentes períodos de submersão em água. Colunas são as respostas ao tratamento e letras iguais, por grupo, não são estatisticamente diferentes a 5% de probabilidade. Linhas de regressão são o efeito do período de hipoxia sobre as sementes*

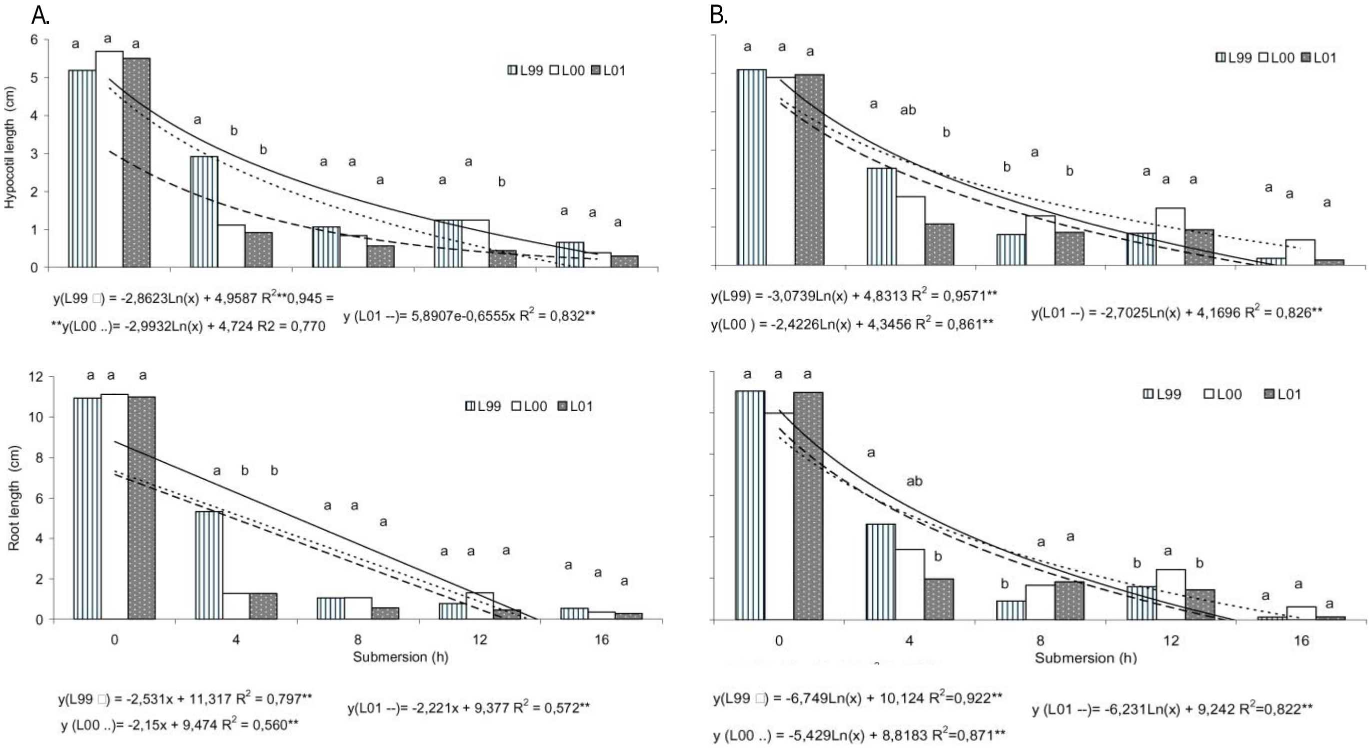

Figure 2. Length of the hypocotyls and root of three seeds lots of two different cultivars of bean ('IAPAR 65' (A) and 'EMGOPA-201' (B)) obtained from different periods of submersion in water. Columns are the lot response to the treatment and equal letters, per group, are not statistically significant at 5% of probability. Regression lines were the effect of the period of hypoxia over the seeds

Figura 2. *Comprimento de hipocótilo e raízes de dois diferentes cultivares de feijão ('IAPAR 65' (A) e 'EMGOPA-201' (B)) obtidos de diferentes períodos de submersão em água. Colunas são as respostas ao tratamento e letras iguais, por grupo, não são estatisticamente diferentes a 5% de probabilidade. Linhas de regressão são o efeito do período de hipoxia sobre as sementes*

arise from 9.1-9.9% in non treated seed to 56-58% after 16h of submersion, indicating that germination process did not proceed with the submersion (Figure 1).

Germination results for both cultivars showed a reduction in that parameter when seeds were submitted to four or more hours of submersion. Lots with germination nearly equal (zero hour), after being submitted to unfavourable condition of submersion showed a distinct pattern of germination, indicating that, four hours of submersion may be used to distinguish bean seed lots in relation to vigour (Figures 1 A and B).

There was no difference in the lot vigour in the first germination count for non treated lots for both cultivars, while vigour classification test was sufficient to differentiate lots of 'IAPAR 65' (Figures 1 A and B). However, submersion was efficient to point out the most vigorous lots, either when evaluated through germination test or by first germination count and vigour classification (Figures 1 A and B). So, the best parameter to be used after submersion for vigour evaluation will be the first germination count because the time for result obtained would be reduced.

Root length was cultivar dependent, while hipocotyl length exhibited averages that were significantly different between lots and submersion periods. Dry matter weight results of the hipocotyl and root were variable, which dry matter weight of the hipocotyl showed differences between lots of 'EMGOPA 201' but not for 'IAPAR 65' and root dry weight just the opposite.

Hipocotyl and root lengths (Figures 2 A and B) as their respective dry weight (Figures 3 A and B) were also affected by submersion, but were less effective than germination test, first germination count and vigour classification to distinguish lots.

The cause of the reduction in the germination and physiological parameters may be related with the hypoxia that seeds were submitted to. In an anaerobic environment, the metabolism turns from respiration to fermentation that has the lactic and alcoholic pathways (Zeng et al., 1999). The first one will generate lactic acid that will decrease the cell pH, which leads to a malfunction of several enzymes, from energy production to cell detoxification, what can be responsible by the cell collapse. In the alcoholic pathway, the final products are very toxic and, depending on the produced quantity, protein denaturant, also leading to a cell collapse.

The results of this work are in accordance with Woodstock (1973) and Dantas et al. (2000b) showing that water submersion is a viable alternative for vigour evaluation, exhibiting highly valuable characteristics for a vigour test at low cost, simplicity and objectiveness, with no need of sophisticated and different equipments as used in a normal seed analysis laboratory. However, the name of the test has generated conflicts in the literature. Dantas et al. (2000 a, b) na-

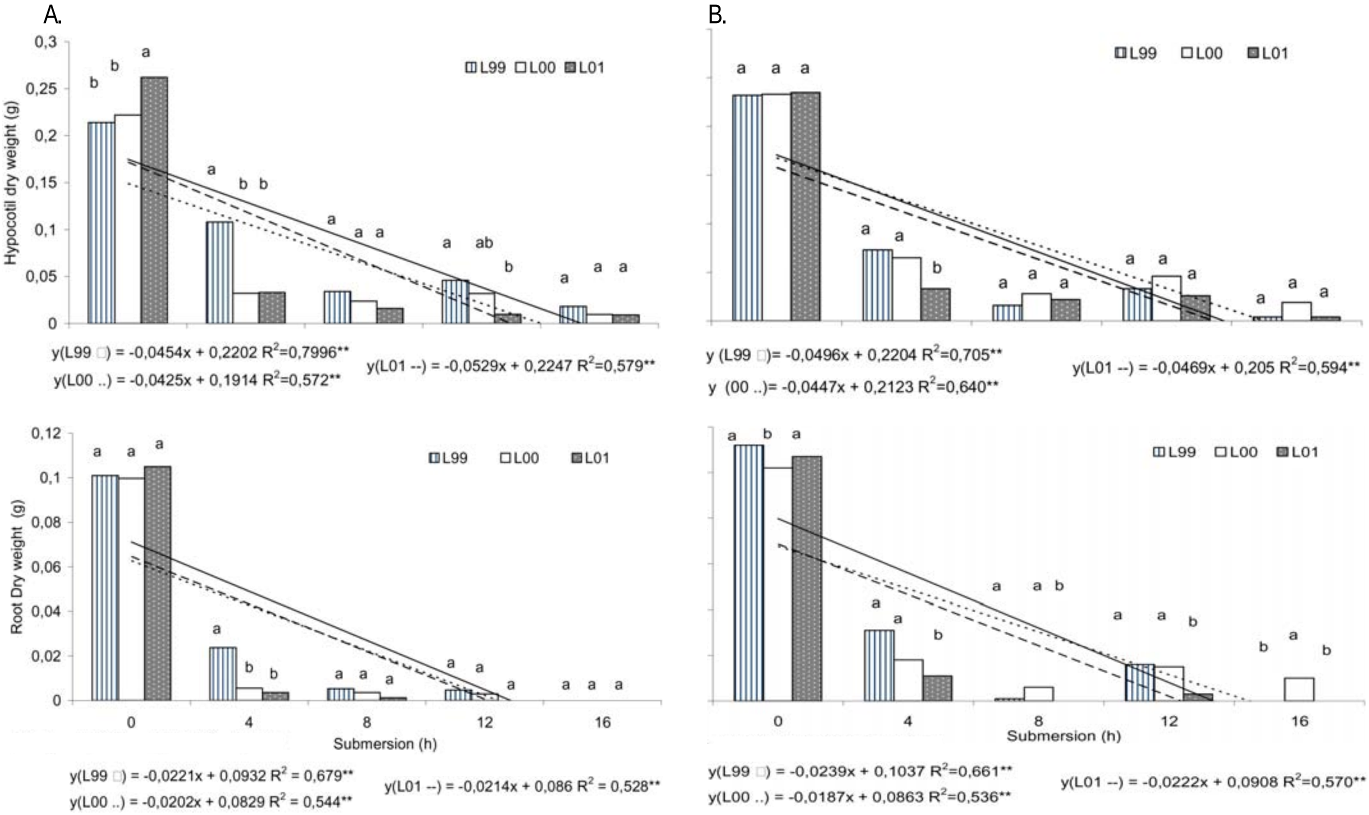

Figure 3. Dry weight matter of the hypocotyls and root of three seeds lots of two different cultivars of bean ('IAPAR 65' (A) and 'EMGOPA-201' (B)) obtained from different periods of submersion in water. Columns are the lot response to the treatments and equal letters, per group, are not statistically significant at 5% of probability. Regression lines were the effect of the period of hypoxia over the seeds

Figura 3. *Massa Seca de hipocótilo e raízes de dois diferentes cultivares de feijão ('IAPAR 65' (A) e 'EMGOPA-201' (B)) obtidos de diferentes períodos de submersão em água. Colunas são as respostas ao tratamento e letras iguais, por grupo, não são estatisticamente diferentes a 5% de probabilidade. Linhas de regressão são o efeito do período de hipoxia sobre as sementes*

med it 'flooding test' because it is less severe than the 'submersion test', since substances to control micro organisms are added to the immersion solution, in order to make it able to evaluate the vigour based in the survival to anaerobic conditions. This work followed the test name adopted by Woodstock (1973) adding the words 'in water', despite the solution used. On the other hand, the methodology proposed by Dantas et al. (2000a, b) was followed, to characterize the absence of soil. The authors of the present work considered the name 'flooding test' inadequate for a vigour test, leading to the wrong idea of soil flooding, which did not show the real methodology used in the test.

Conclusions

The vigour and germination of bean seeds were negatively affected by submersion in water. Four hours of submersion may damage without reversion the crop establishment for fewer vigorous lots and eight hours was enough to decrease germination even in the most vigorous lots.

Four hours of submersion, followed by first germination count are enough to differentiate seed lots in relation to the vigour.

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