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Enhancement of Soybean Seed Vigour as Affected by Thiamethoxam Under Stress Conditions

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1. Introduction

Cruiser ® (thiamethoxam), developed and registered by Syngenta, is a chloronicotinic insecticide, belonging to the class of neonicotinoids for seed treatment and has long residual control for a wide range of chewing and sucking insects present in seeds, soil and leaves (Maienfisch et al., 2001).

Thiamethoxan acts by contact and ingestion and the insect stops eating within 24 h after contact with the insecticide. The primary mode of action involves interference with, or by binding to nicotinic acetylcholine receptors (Maienfisch et al., 2001).

Surprisingly, it has been noticed that the treatment of soybean seeds with Cruiser results in a "stand" more uniform, vigorous and more productive, thus acting on germination.

However, seed germination and seedling development of crops are negatively affected by adverse conditions, such as drought (Davidson & Chevalier, 1987; Passioura, 1988, Soltani et al., 2004), salinity (Hampson & Simpson, 1990; Ramoliya & Pandey, 2003, Soltani et al., 2004, Luo et al., 2005; Athar et al., 2008) and high concentrations of soluble forms of aluminum (Matsumoto, 2000; Echart & Cavalli-Molina, 2001, Rout et al., 2001).

A common characteristic of various stress types is the increased production of reactive oxygen species (ROS), which are generally considered harmful to plant cells (Alscher et al. 1997; Smirnov, 1993, Richards et al., 1998). The ROS include superoxide radical ($O_2^{\bullet-}$) and hydroxyl ($\bullet OH$), hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2). There are evidences that increased production of ROS under environmental adversities may induce oxidative stress in plants. It has been reported the induction of oxidative stress under conditions of water stress (Smirnov, 1993; Alscher et al., 1997), salinity (Rio-Gonzalez et al. 2002; Bor et al., 2003; Athar et al., 2008) and excessive concentrations of aluminum in soils (Tamás et al., 2004).

For protection against ROS, plant cells contain an antioxidant system, including various enzymes, among which, superoxide dismutase (SOD) and peroxidase (POD) (Fridovich, 1978, Bowler et al., 1992, Foyer et al., 1994; Cataneo et al., 2005; Ferreira et al., 2010). SOD and

POD are metalloenzymes acting in the elimination of, respectively, $O_2^{\bullet -}$ radical and H_2O_2 produced in stress conditions. Peroxidases are active in many physiological and development processes and are involved both in consumption, as in the production of H_2O_2 and other ROS (Silva et al. 1994; McQueen-Mason & Cosgrove, 1994; McQueen-Mason, 1995, Bacon et al. 1997; Amaya et al. 1999; Passardi et al., 2004).

Thus, the aim of this study was to evaluate the effect of Cruiser on the enzymes involved in protection against oxidative stress (SOD and POD) caused by drought, salinity and presence of high concentrations of aluminum during soybean germination.

2. Methods

2.1 Plant material and conduction of experiments

In this study were used seeds from two different cultivars of soybean (*Glycine max* L.): Pintado, representative of the Brazilian Midwest region, characterized by the predominance of the Brazilian savanna (cerrado) features and BRS 133, representative of the South region, with features adapted to the soil and climate of this geography.

Three experiments were carried out in the Xenobiotic Lab from Department of Chemistry and Biochemistry, Institute of Biosciences, UNESP, Botucatu, in a germination chamber at 25°C in the dark.

Seeds were germinated on filter paper rolls moistened with distilled water or with different solutions. The volume of such solutions used in the treatments was 2.5 mL X g filter paper weight. The germination rolls were placed into plastic containers, each with a perforated lid. In the germination evaluations, seeds presenting root length equal to or greater than to 2 mm were considered germinated (Duran & Tortosa, 1985).

In the three experiments were adopted the experimental design completely randomized, with four replicates and twenty-five seeds per plot. The results were subjected to analysis of variance. The treatments were compared by Tukey test at 1% probability. The experiments were conducted in three phases.

2.2 First experiment

Seeds of two soybean cultivars were treated with the recommended level of Cruiser 350 FS - D1 - (100 mL f.p./100Kg seed), with twice the recommended level of Cruiser 350 FS - D2 - (200 mL f.p./100Kg seeds) and the control seeds were treated only with distilled water - D0. The counting of germinated seeds of the three treatments was performed at 24, 36, 48, 60 and 72 h of imbibition.

2.3 Second experiment

Seeds of two soybean cultivars were treated with the recommended level of Cruiser 350 FS - D1 - (100 mL f.p./100Kg seed) and the control seeds were treated only with distilled water - D0.

2.3.1 Presence of heavy metal – aluminum

Followed by treatment with the levels D0 and D1 of Cruiser, germination paper leaves were moistened with solutions of aluminum sulphate at concentrations of 0; 5; 10 and 15 mmol L⁻¹. Germination evaluations were performed at 24, 36, 48, 60 and 72 h of imbibition in the solutions of different concentrations of aluminum sulfate. At the end of the experiment (72 h) the embryo axis were removed and weighed.

2.3.2 Salinity – NaCl

Followed by treatment with the levels D0 and D1 of Cruiser, germination paper leaves were moistened with solutions of sodium chloride at concentrations of 0; 25; 50; 100 and 150 mmol L⁻¹. Germination evaluations were performed at 24, 36, 48, 60, 72 and 84 h of imbibition in the solutions of different concentrations of NaCl. At the end of the experiment (84 h) the embryo axis were removed and weighed.

2.3.3 Water deficit

Treated seeds with levels D0 and D1 of Cruiser were germinated on filter paper rolls moistened with solutions of polyethylene glycol 6000 (PEG) that simulate different situations of water deficit. PEG solutions at the water potentials -0.1; -0.2 and -0.3 MPa were prepared according to Michel & Kaufmann (1973). Distilled water was used in the control. Germination evaluations were performed at 24, 36, 48, 60, 72 and 84 h of imbibition in the solutions of different concentrations of PEG. At the end of the experiment (84 h) the embryo axis were removed and weighed.

2.4 Third experiment

To develop the third experiment, were chosen for each cultivar, the concentrations of the solutions of aluminum sulfate, NaCl, PEG and the period of imbibition that provided the biggest differences between the treatment with Cruiser and control, from the second study. Seeds of two soybean cultivars were treated with the recommended level of Cruiser 350 FS - D1 - (100 mL f.p./100Kg seed) and the control seeds were treated only with distilled water - D0. The concentrations of the solutions and the periods of imbibition used in the different treatments are shown in the Table 1.

Seed treatment	Concentration of solutions (* chosen from second experiment)		Periods of Imbibition (h)	
	cv. BRS 133	cv. Pintado	cv. BRS 133	cv. Pintado
H ₂ O (D0)	Distilled H ₂ O	Distilled H ₂ O	24 and 36	24 and 36
Cruiser (D1)	Distilled H ₂ O	Distilled H ₂ O	24 and 36	24 and 36
H ₂ O (D0)	Al sulfate 10 mmol.L ⁻¹	Al sulfate 10 mmol.L ⁻¹	24 and 36	36 and 48
Cruiser (D1)	Al sulfate 10 mmol.L ⁻¹	Al sulfate 10 mmol.L ⁻¹	24 and 36	36 and 48
H ₂ O (D0)	NaCl 50 mmol.L ⁻¹	NaCl 100 mmol.L ⁻¹	24 and 36	36 and 48
Cruiser (D1)	NaCl 50 mmol.L ⁻¹	NaCl 100 mmol.L ⁻¹	24 and 36	36 and 48
H ₂ O (D0)	PEG -0,3 MPa	PEG -0,3 MPa	60 and 72	72 and 84
Cruiser (D1)	PEG -0,3 MPa	PEG -0,3 MPa	60 and 72	72 and 84

Table 1. Concentration of solutions (*) used in third experiment – aluminum (Al sulfate), salinity (NaCl) and water deficit (PEG) and periods of imbibition in which were collected the samples of Embryo Axis of soybean cv. BRS 133 and Pintado.

For each treatment and imbibition period described in Table 1, were collected samples of embryo axis in two imbibition periods to determine activity of the antioxidant enzymes, peroxidase (POD) and superoxide dismutase (SOD).

Enzymatic extracts used for determination of SOD and POD activities were obtained according to the method described by Ekler et al. 1993. POD and SOD activities were assayed according to the method described by Teisseire & Guy (2000) e Bor et al. (2003), respectively.

3. Results

3.1 First experiment: Action of cruiser on the germination of soybean seeds

In the cultivar BRS 133 the treatment with Cruiser used in the recommended level (D1) and at twice the recommended level (D2) accelerated the germination in the first 24 h of imbibition (Figure 1). The increase in germination was higher at D2 treatment.

In the cultivar Pintado (Figure 2) Cruiser caused acceleration of germination until 36 h of imbibition, being observed that at 24 h of imbibition the increase in germination was higher at the twice-recommended level of Cruiser and at 36 hours of imbibition, germination did not differ statistically between the two levels of Cruiser. Germination in both cultivars did not differ significantly between the control seeds (D0) and seeds treated with two levels of Cruiser (D1 and D2) between 48 and 72 h of imbibition.

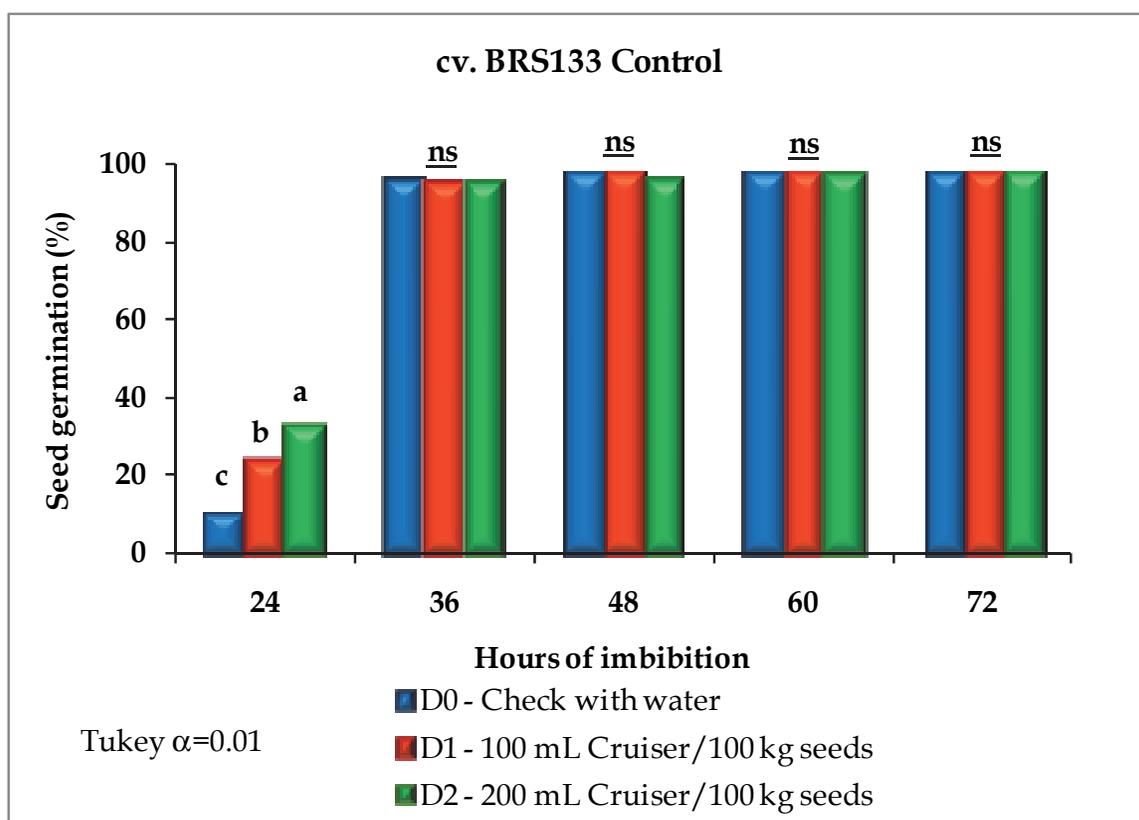


Fig. 1. Soybean germination percentage cv. BRS 133 treated at recommended dose of Cruiser (D1), double of recommended dose (D2) and check (D0). Average followed by the same letter did not differ significantly for each imbibition period. ns: not differ significantly for each imbibition period.

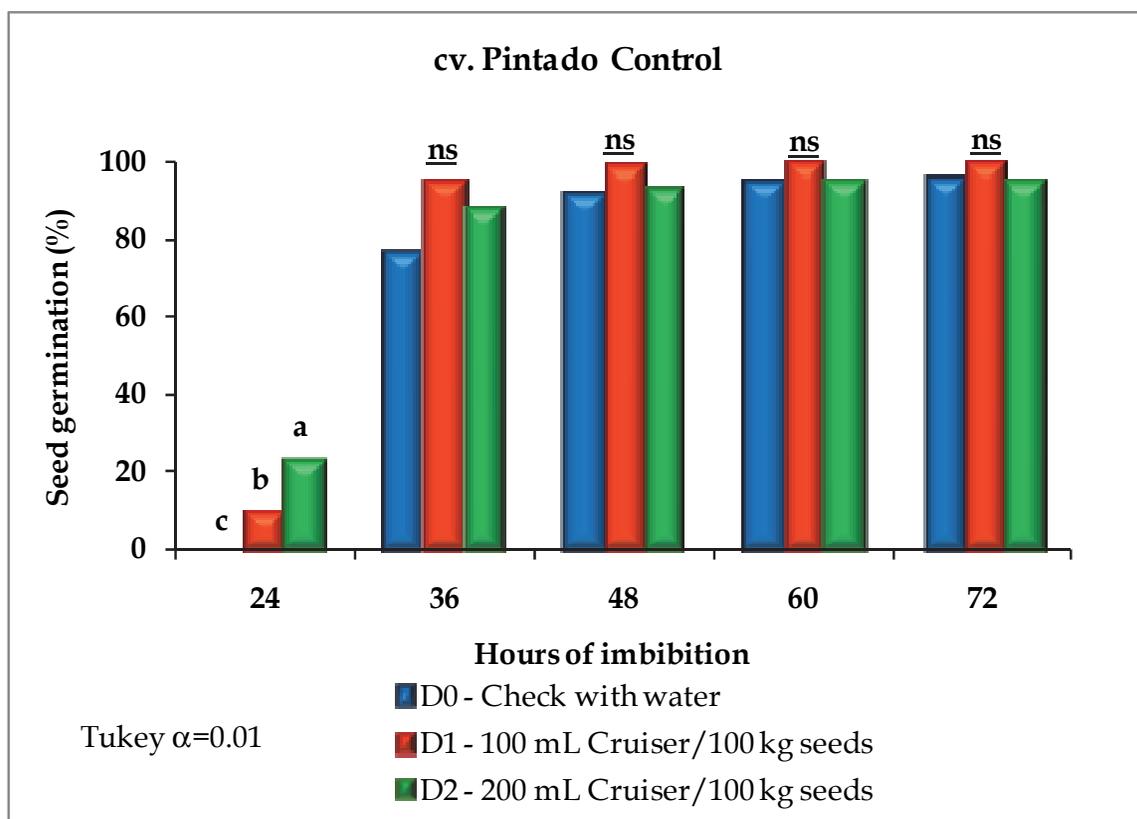


Fig. 2. Soybean germination percentage cv. Pintado treated at recommended dose of Cruiser (D1), double of recommended dose (D2) and check (D0). Average followed by the same letter did not differ significantly for each imbibition period. *ns*: not differ significantly for each imbibition period.

3.2 Second Experiment: Cruiser action on the germination of soybean seeds subjected to stress conditions induced by heavy metal (aluminum), salinity (NaCl) and water deficit

In the presence of aluminum in different concentrations (Figures 3 to 5), the treatment of soybean seeds of cultivar BRS 133 with the recommended level of Cruiser (D1) caused acceleration of germination, when compared with the control (D0), up to 36 h of imbibition. In the soybean seeds of cultivar Pintado, the same pattern of cultivar BRS 133 was observed within 36 h of imbibition in aluminum concentration of 5 mmol L⁻¹ (Figure 3) and up to 48 h of soaking in aluminum concentrations of 10 and 15 mmol L⁻¹ (Figures 4 and 5, respectively). In the Figure 6 is shown comparisons of the effect of Cruiser on the germination of cultivar BRS 133 in the different concentrations of aluminum, at 24 and 36 h of imbibition. In the cultivar Pintado comparisons were performed at 36 and 48 h of imbibition (Figure 7). Analyzing the results can be considered that: a) aluminum delays germination in both cultivars studied; b) in the two soybean cultivars, the increase in aluminum concentration caused a decrease in germination; c) Cruiser increase germination in aluminum stress conditions and d) on cultivar BRS 133 at 36 h of imbibition (Figure 6) and on cultivar Pintado at 48 h of imbibition (Figure 7) greater the stress by the presence of aluminum, greater was the effect of Cruiser. Therefore, the results of soybean germination in response to treatment of seeds with Cruiser, under stressful aluminum conditions, indicate that the insecticide acts by reducing the toxic effect of aluminum on germination.

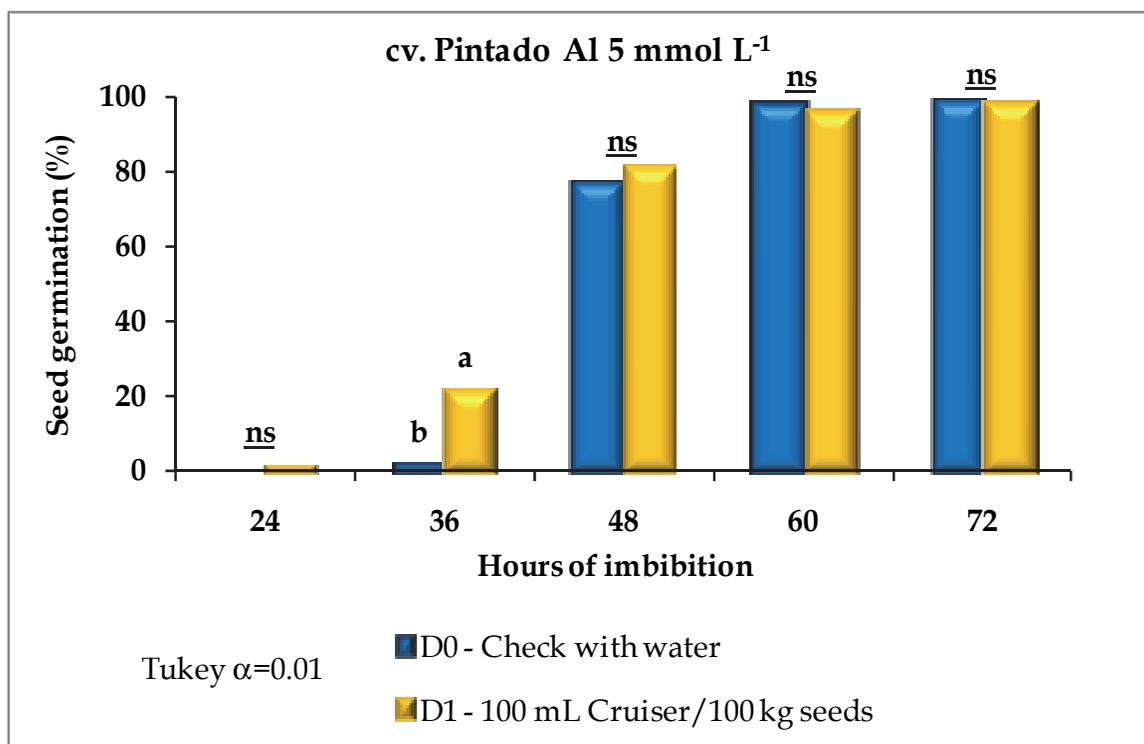
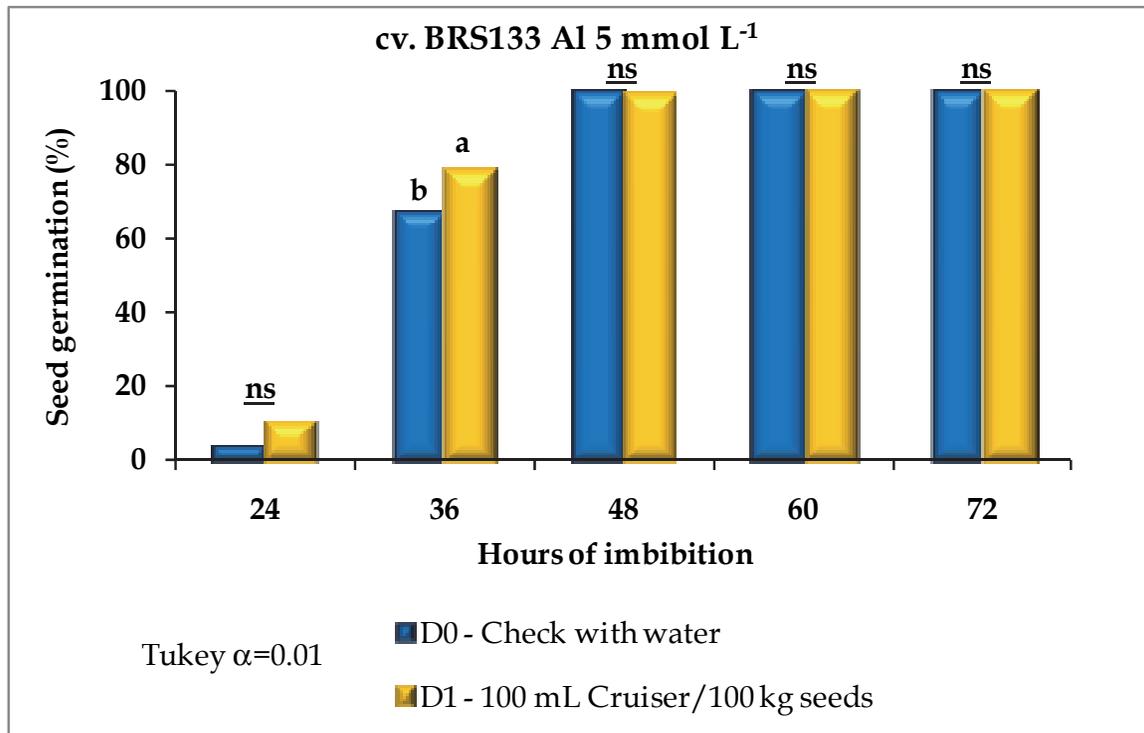


Fig. 3. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under aluminum sulfate 5 mmol L⁻¹. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

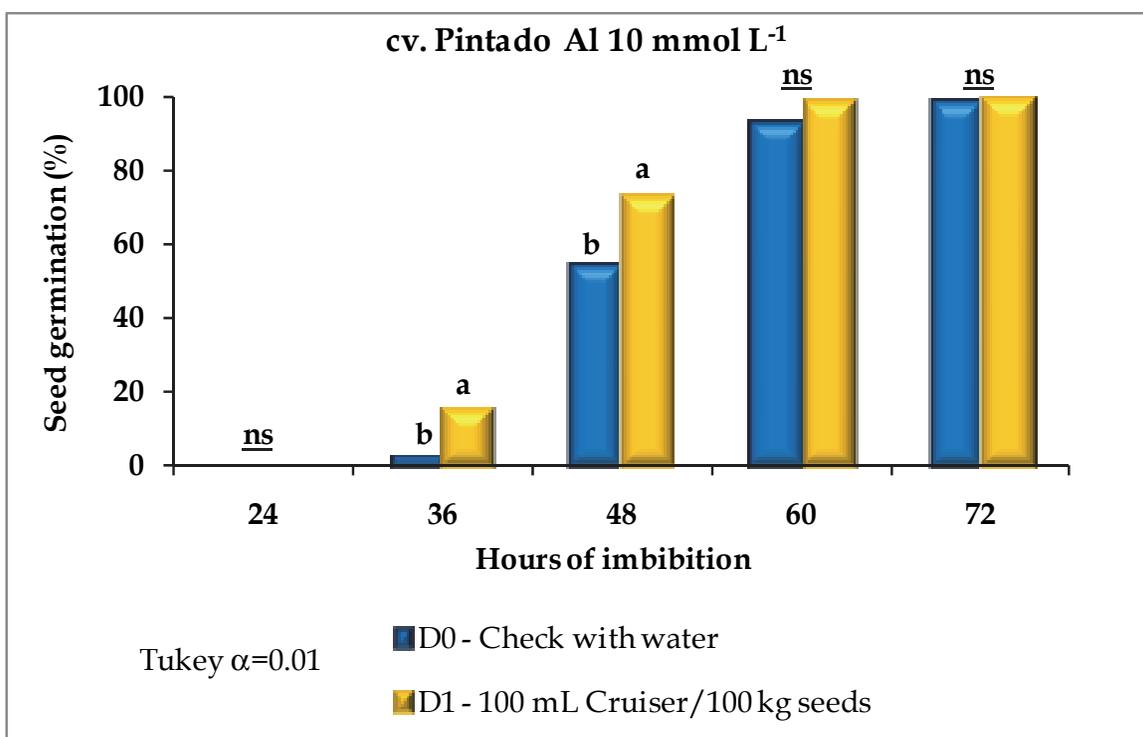
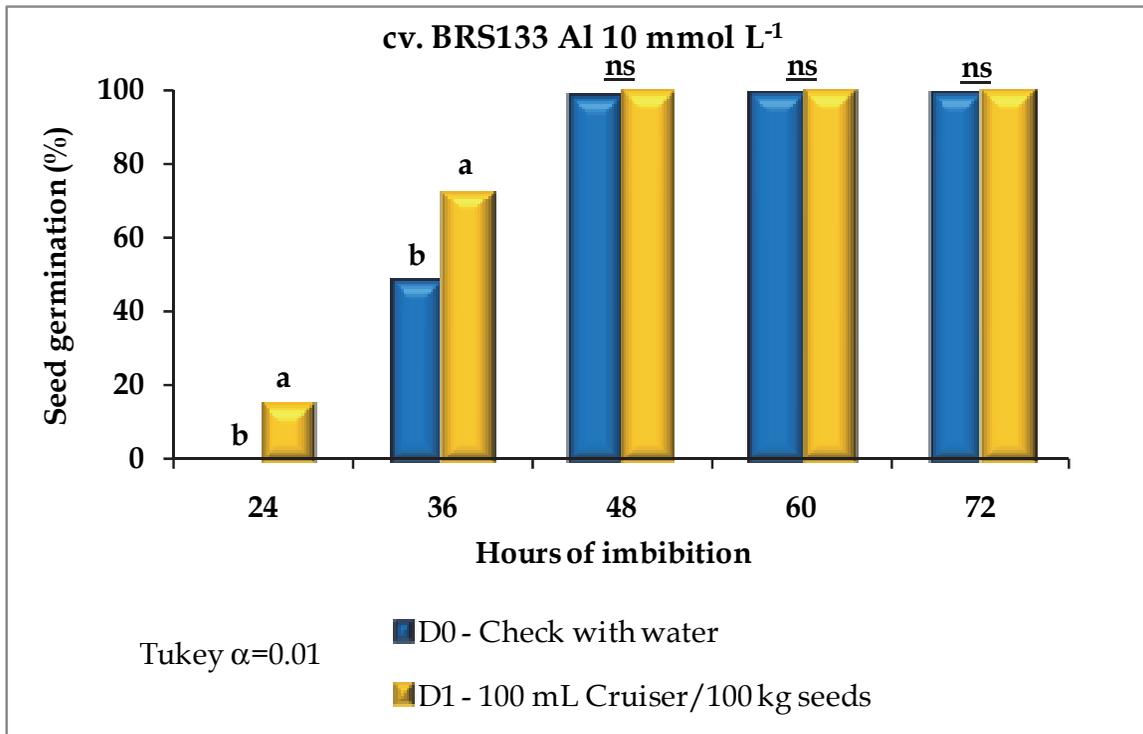


Fig. 4. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under aluminum sulfate 10 mmol L⁻¹. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

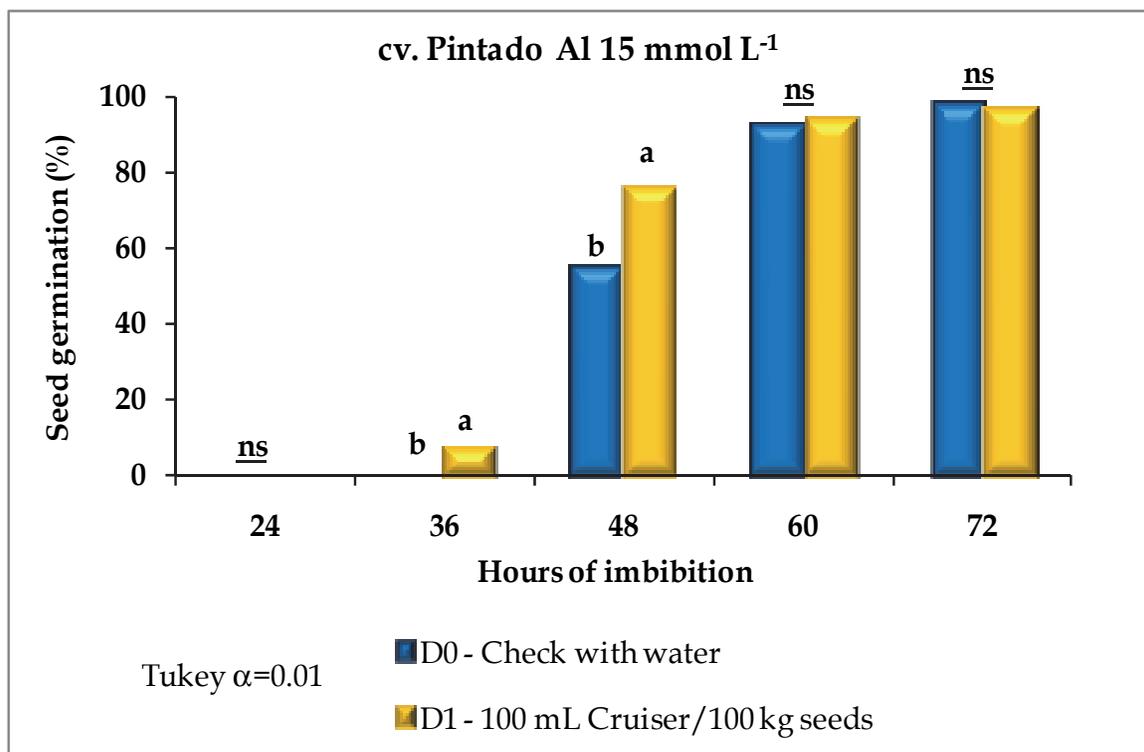
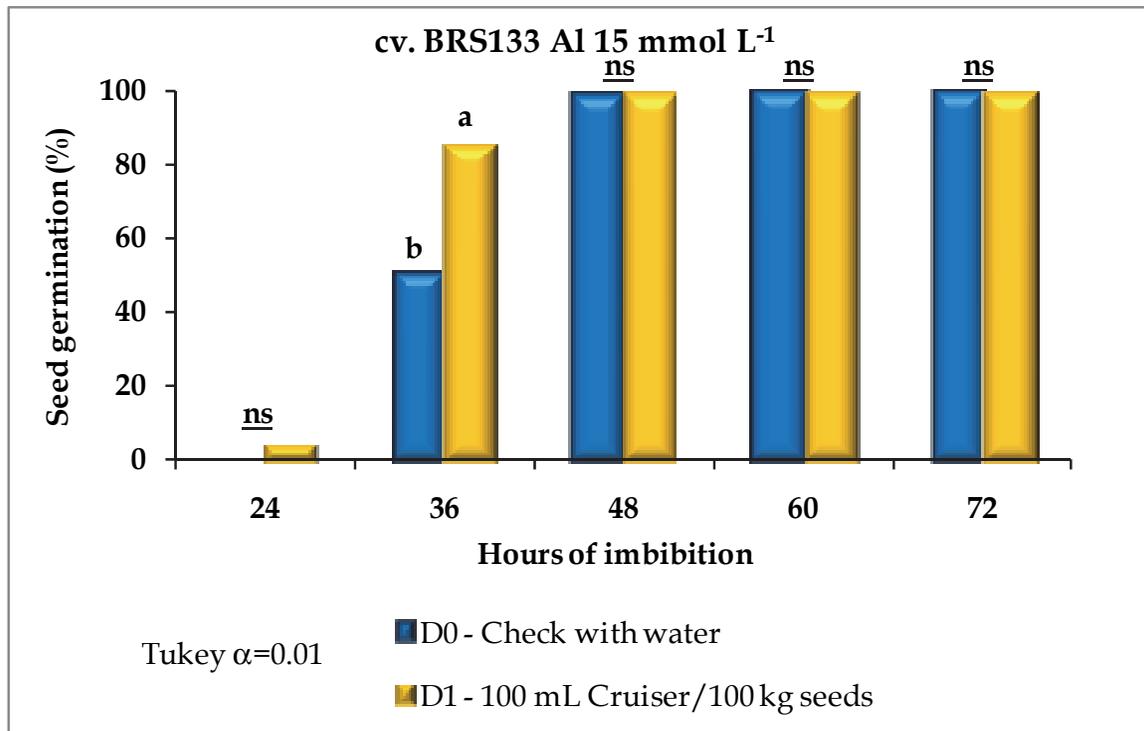


Fig. 5. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under aluminum sulfate 15 mmol L⁻¹. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

Under salinity conditions in the presence of NaCl (Figures 8 to 11), the treatment of soybean seeds of cultivar BRS 133 with Cruiser caused acceleration of germination in the first periods of imbibition evaluated. It was observed that higher the concentration of NaCl, the effect mentioned was observed in the later periods of imbibition, reaching up to 48 h in NaCl concentration of 150 mmol L⁻¹ (Figure 11). It was observed that Cruiser had no effect on germination of cultivar Pintado at concentrations of NaCl 25 (Figure 8) and 100 mmol L⁻¹ (Figure 10). In NaCl concentration of 50 mmol L⁻¹ (Figure 9) Cruiser decreased germination, but in the concentration of 150 mmol L⁻¹ (Figure 11) it increased germination at 48 h of imbibition.

Comparing the results of Cruiser effect on germination of cultivar BRS 133 in the different concentrations of NaCl in the imbibition periods of 24, 36 and 48 h (Figure 12), can be made several considerations: a) NaCl causes decrease in germination, the effect being more pronounced greater the salinity stress; b) at 24 h of imbibition, Cruiser had effect until the NaCl concentration of 100 mmol.L⁻¹; c) at 36 h of imbibition, Cruiser eliminated the effect of salt stress up to the salt concentration of 50 mmol.L⁻¹ and in higher salinity stress, greater was the effect of Cruiser; d) at 48 h of imbibition, Cruiser eliminated any effect of salt stress.

Analyzing the comparisons of the results in Figure 13 can be considered that in cultivar Pintado Cruiser had no effect on germination under salt stress, during imbibition of 24, 36 and 48 h.

The effect of Cruiser on germination of cultivar BRS 133 under water deficit induced by PEG solutions of different water potentials are shown in Figures 14 to 16. At the water potentials of -0.1 and -0.2 MPa, Cruiser had no effect on germination, but at the water potential of -0.3 MPa, Cruiser has caused a significant increase in germination at 72 h of imbibition. In respect of germination of cultivar Pintado under water deficit conditions induced by PEG solutions of different water potentials, it was observed that in the water potential of -0.1 MPa, Cruiser caused increase on germination at 48 and 60 h of imbibition. In water potential of -0.2 MPa the increase on germination by Cruiser effect were observed from 60 to 84 h of imbibition and in the potential of -0.3 MPa only at 72 and 84 h of imbibition.

Comparing the results of the effect of Cruiser on germination of cultivar BRS 133 at different imbibition periods (Figure 17) in the different water potentials, can be made some considerations: a) the decrease of water potential delays germination; b) there is consistency of Cruiser effect in increasing the germination for the three water potentials; c) at 72 h of imbibition, the largest increase in germination under Cruiser effect occurred where the water deficit was higher.

Comparing the effects of Cruiser on germination of cultivar Pintado, at the water potentials used (Figure 18), can be made some considerations: a) water deficiency causes delayed germination; b) Cruiser has effect in combating water stress for all the three tested water potentials; c) at 72 and 84 h of imbibition Cruiser has a greater effect on germination in the largest water deficit.

In Figure 19 is represented, the effect of Cruiser on the weights of embryo axis of soybean cultivars BRS 133 and Pintado under conditions of aluminum presence. Can be inferred that in all concentrations of aluminum used Cruiser has caused increased growth of the embryo axis but, this increase was significantly higher in the absence of aluminum, in the concentration of 10 mmol L⁻¹ for BRS 133 and in the absence of aluminum (0 mmol L⁻¹) to cultivar Pintado. The effect of Cruiser on development of embryo axis occurred in the absence of aluminum in both cultivars. The weight of the embryo axis tended to be equal between the treated and untreated seeds with Cruiser, with the increase of aluminum stress (Figures 57 and 58).

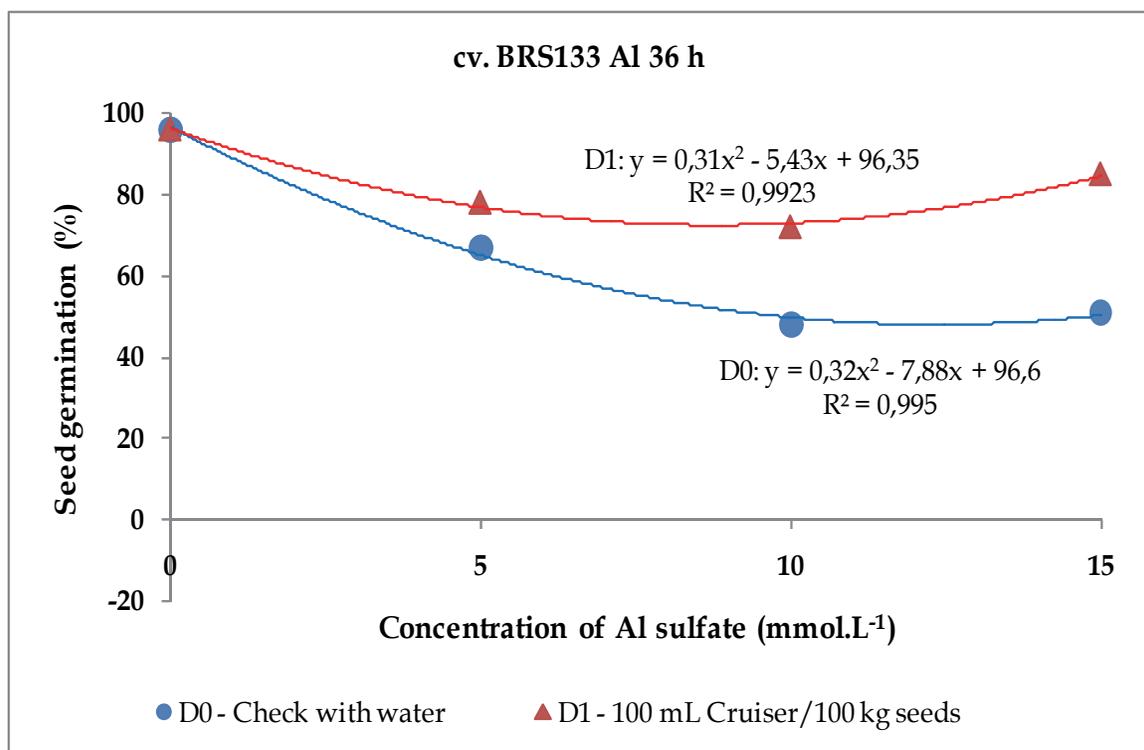
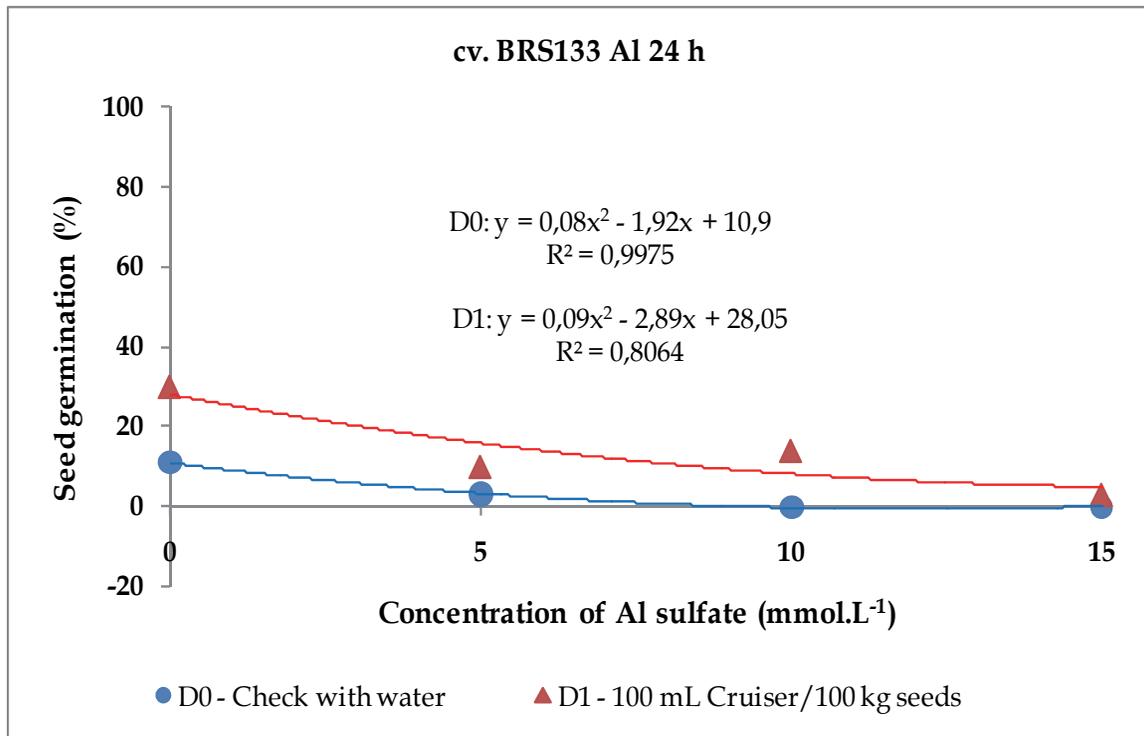


Fig. 6. Comparison of soybean germination percentage cv. BRS 133 treated at recommended dose of Cruiser (D1) and check (D0), under different aluminum sulfate concentrations (5, 10 and 15 mmol L⁻¹) at 24 and 36 h of imbibition.

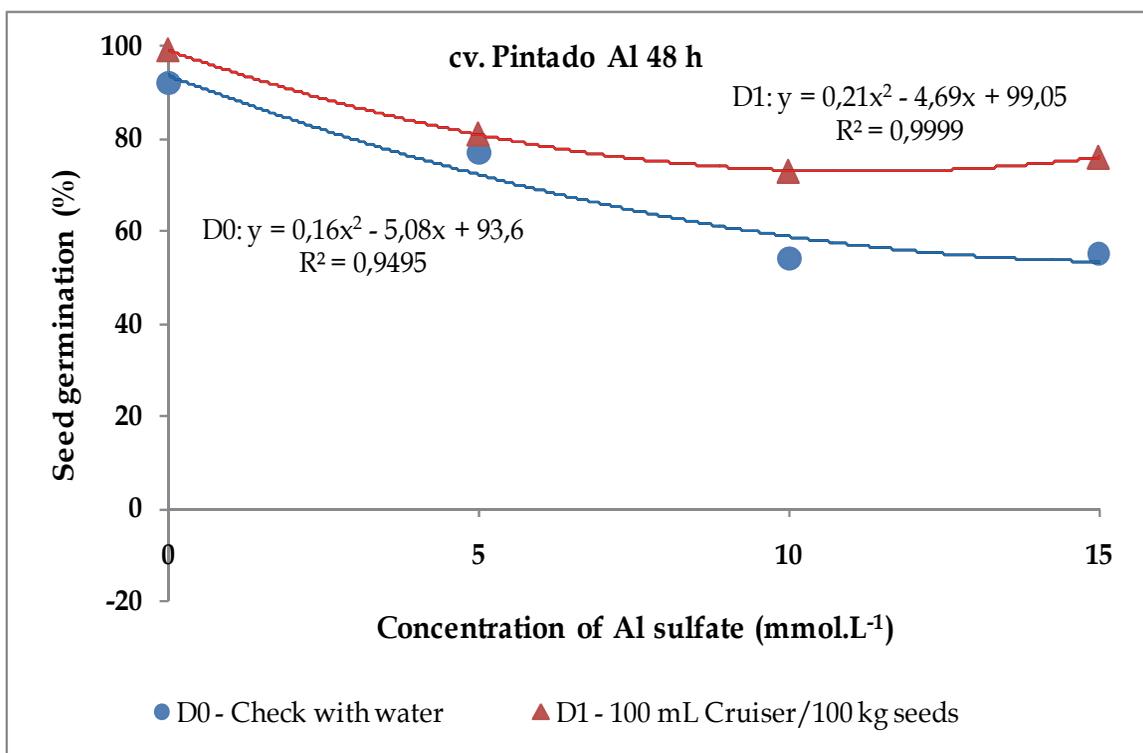
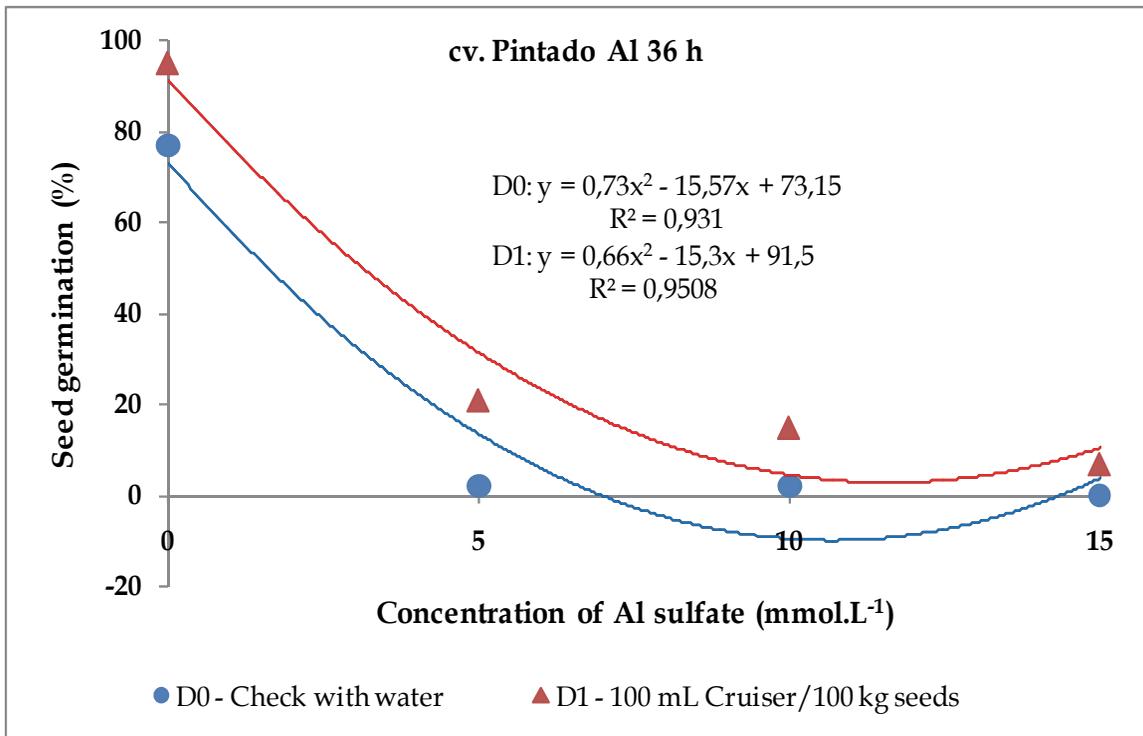


Fig. 7. Comparison of soybean germination percentage cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under different aluminum sulfate concentrations (5, 10 and 15 mmol L⁻¹) at 24 and 36 h of imbibition.

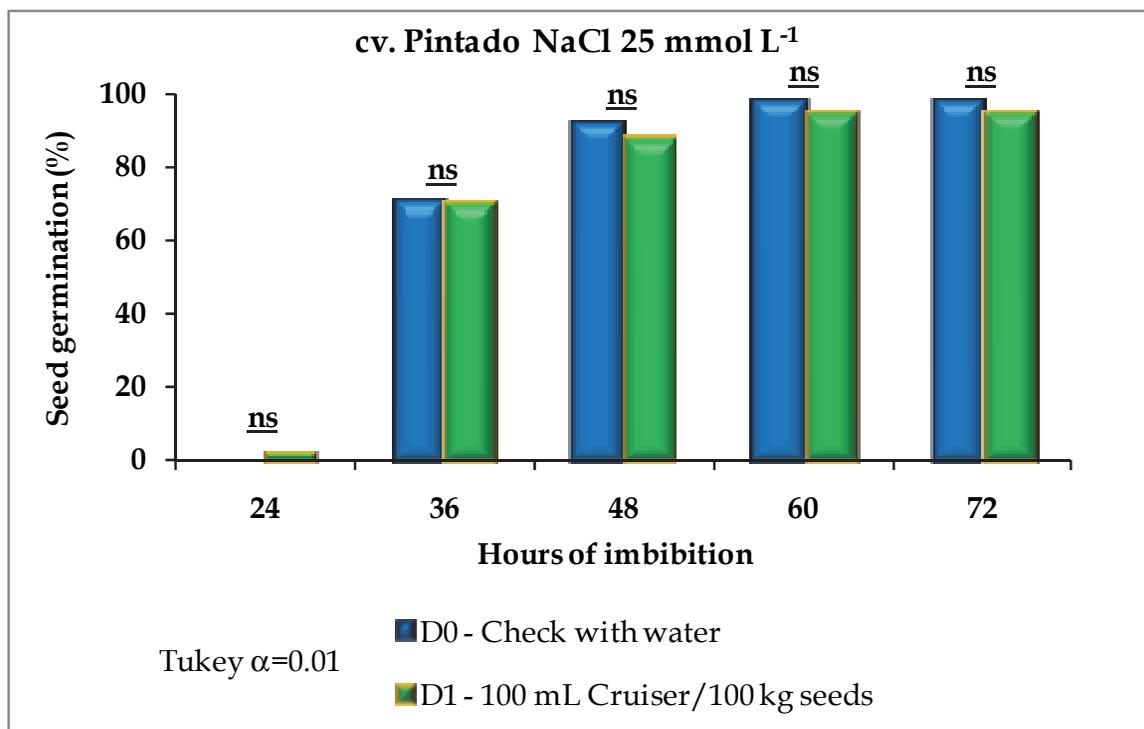
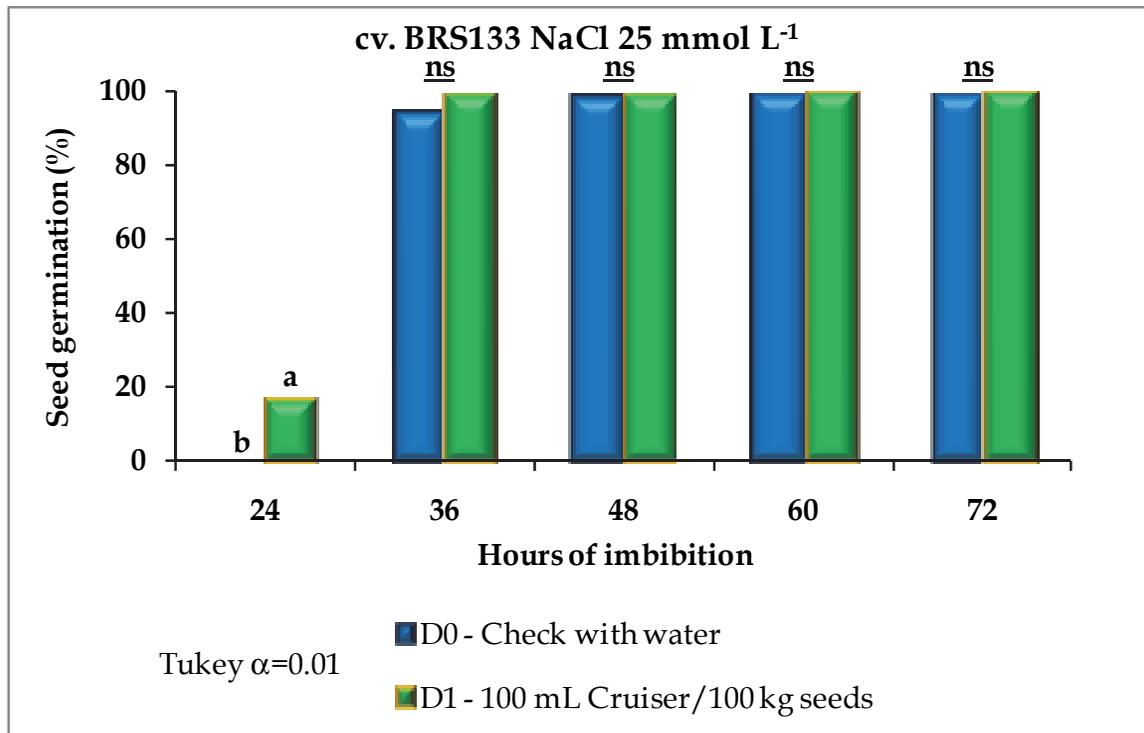


Fig. 8. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under NaCl 25 mmol L⁻¹. Average followed by the same letter did not differ significantly for each imbibition period. ns: not differ significantly for each imbibition period.

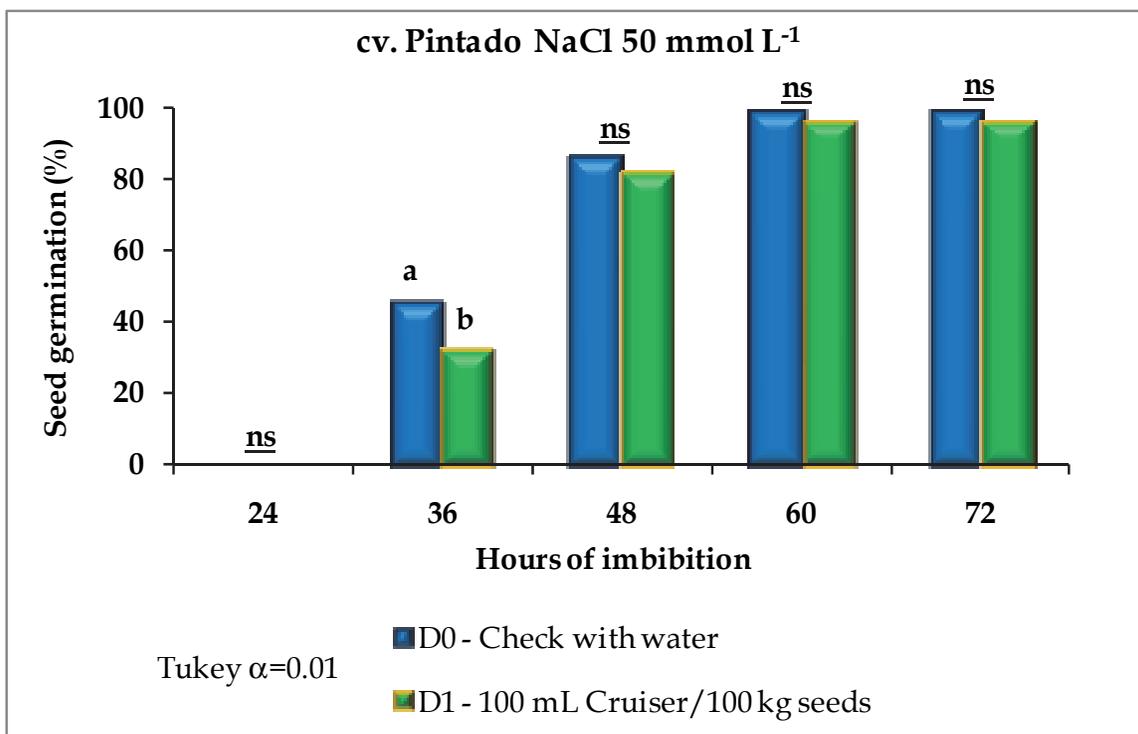
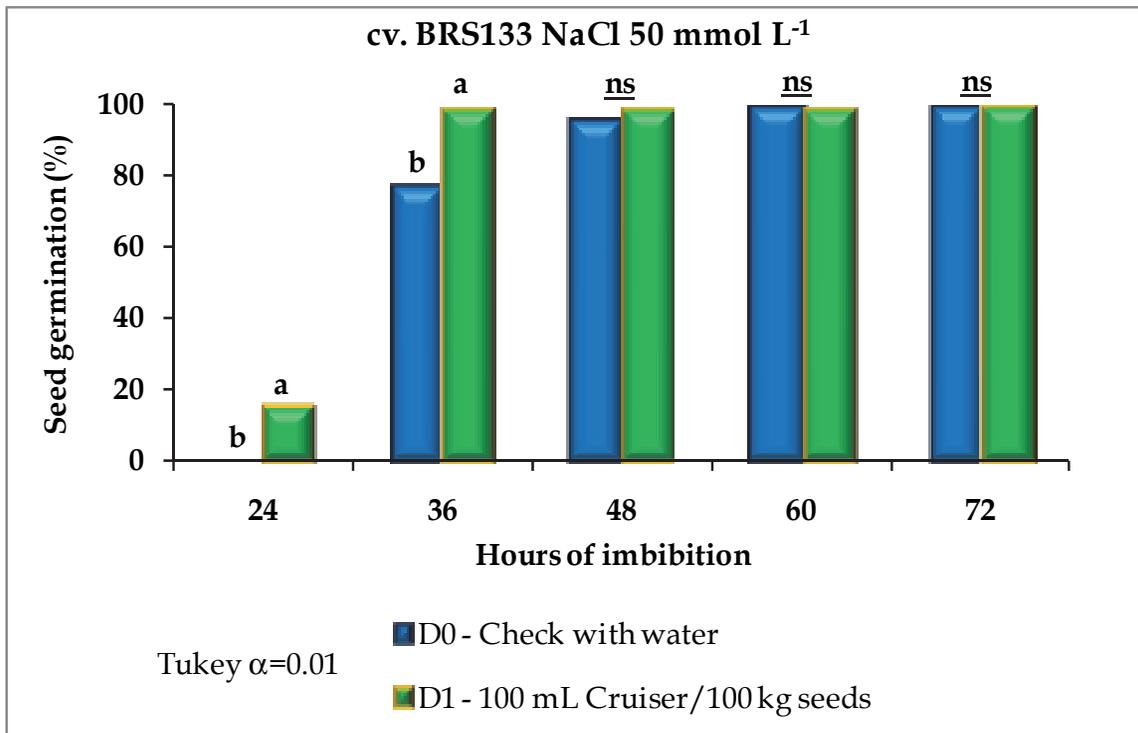


Fig. 9. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under NaCl 50 mmol L⁻¹. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

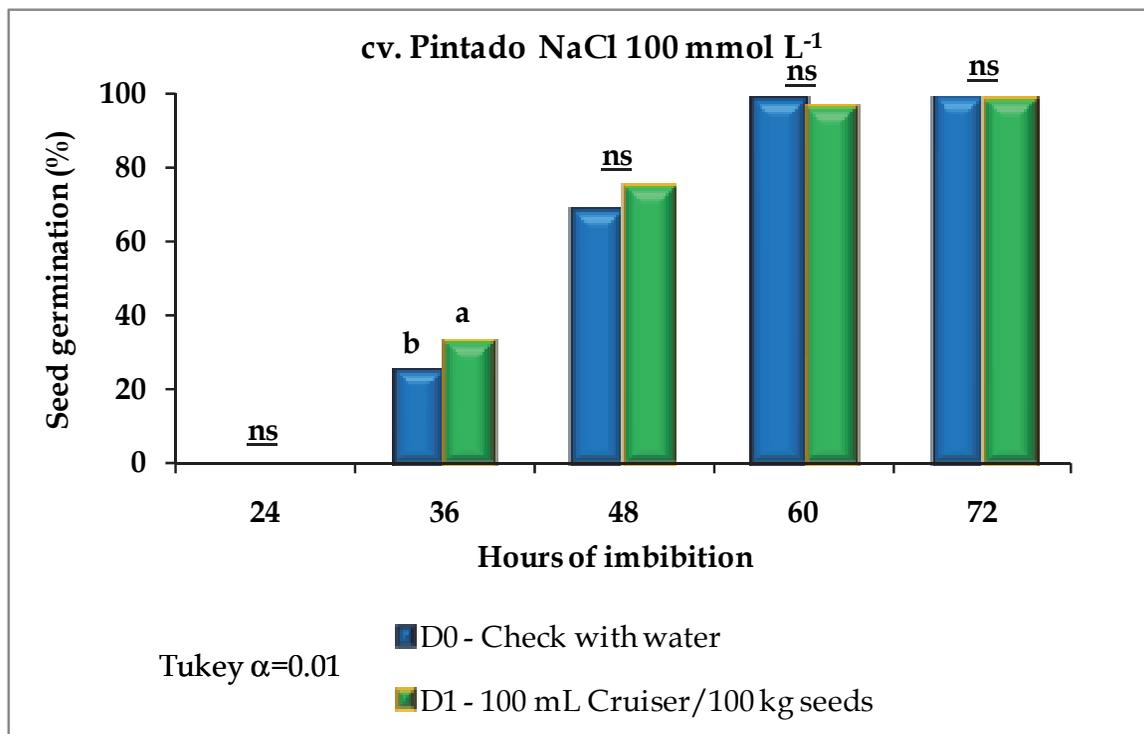
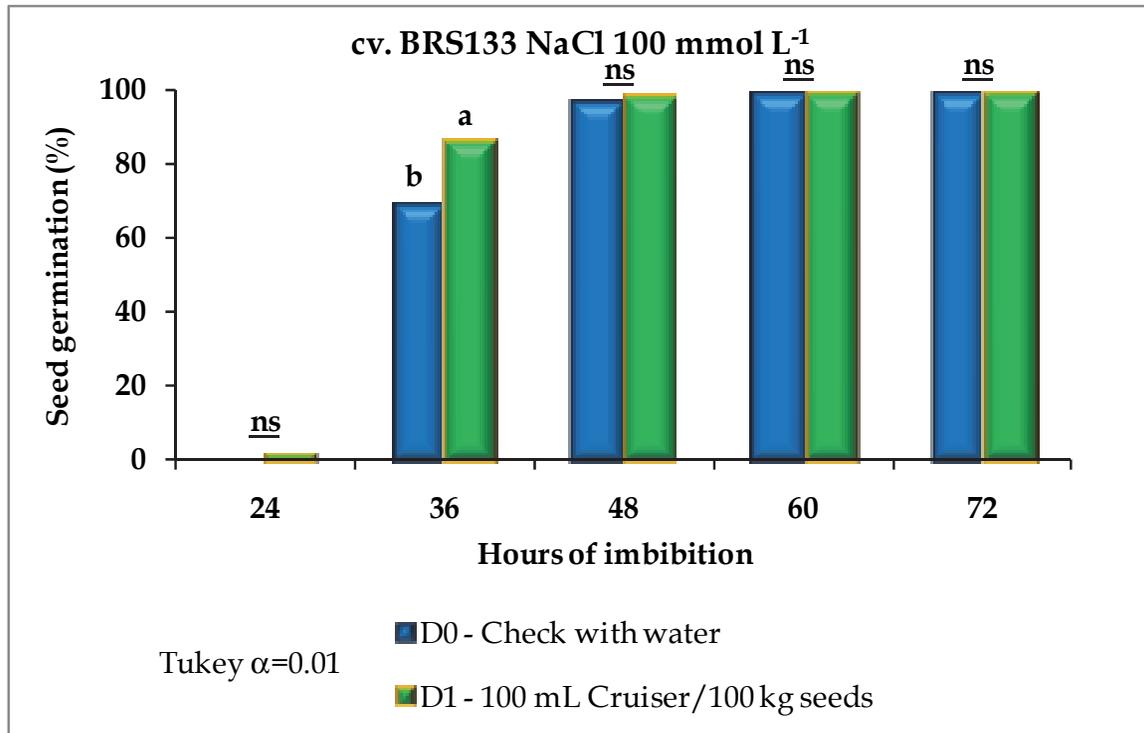


Fig. 10. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under NaCl 100 mmol L⁻¹. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

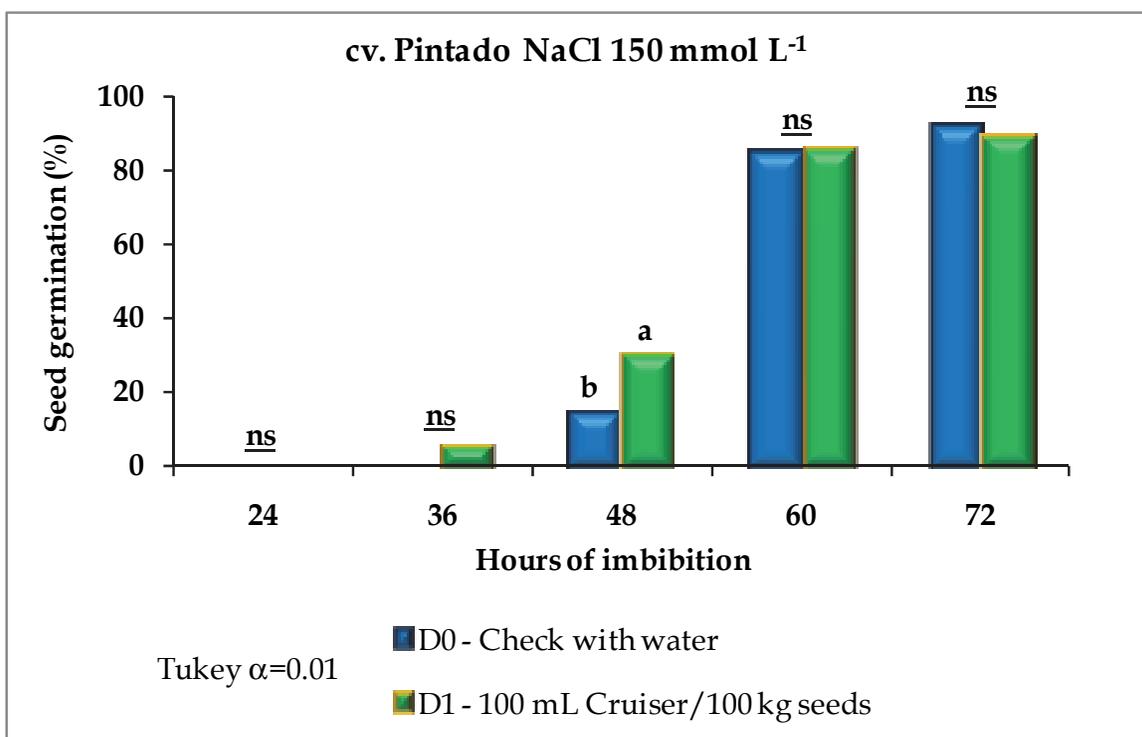
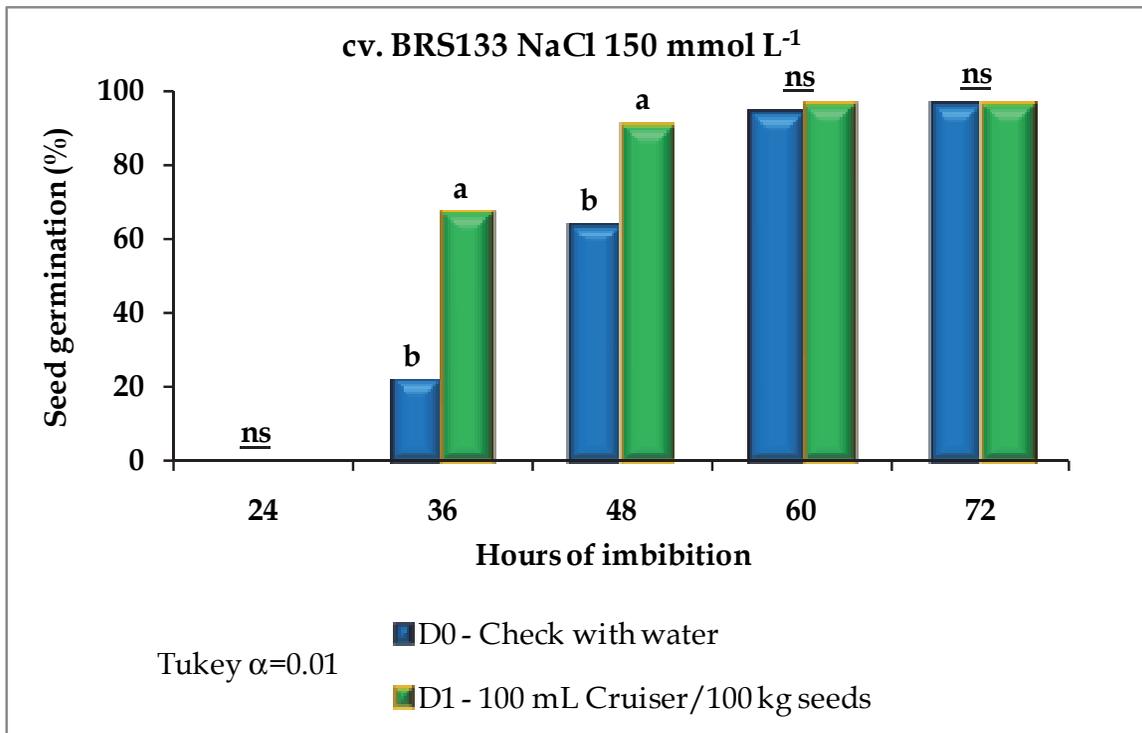
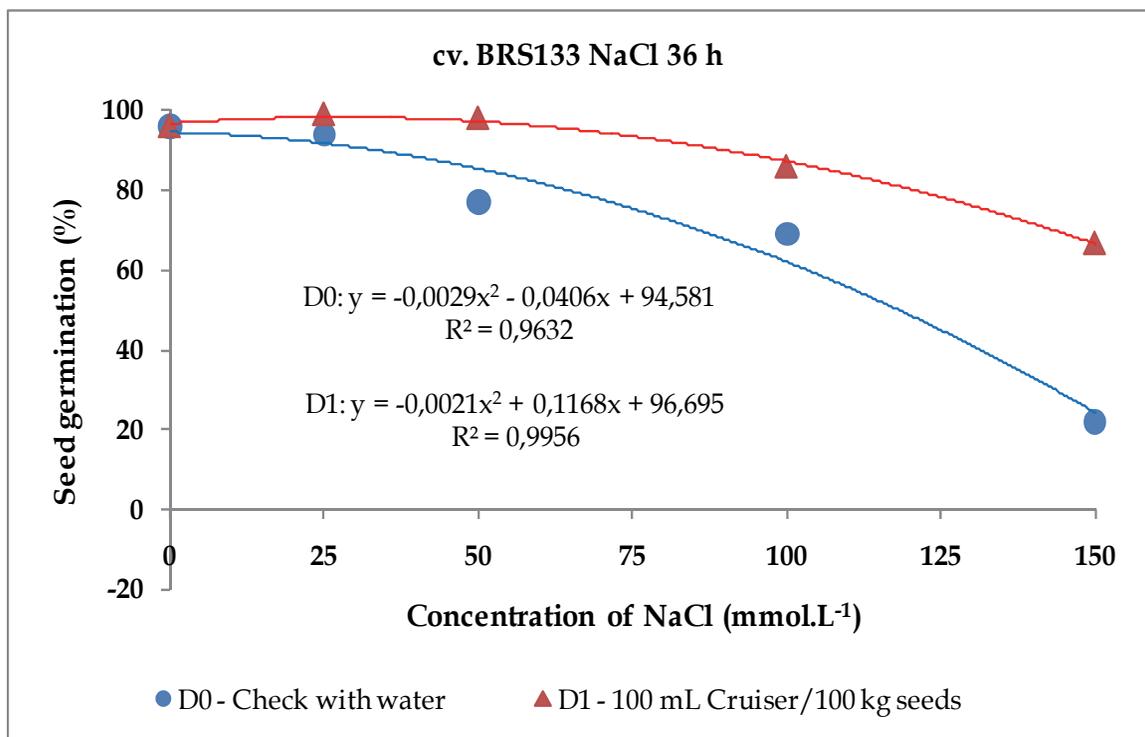
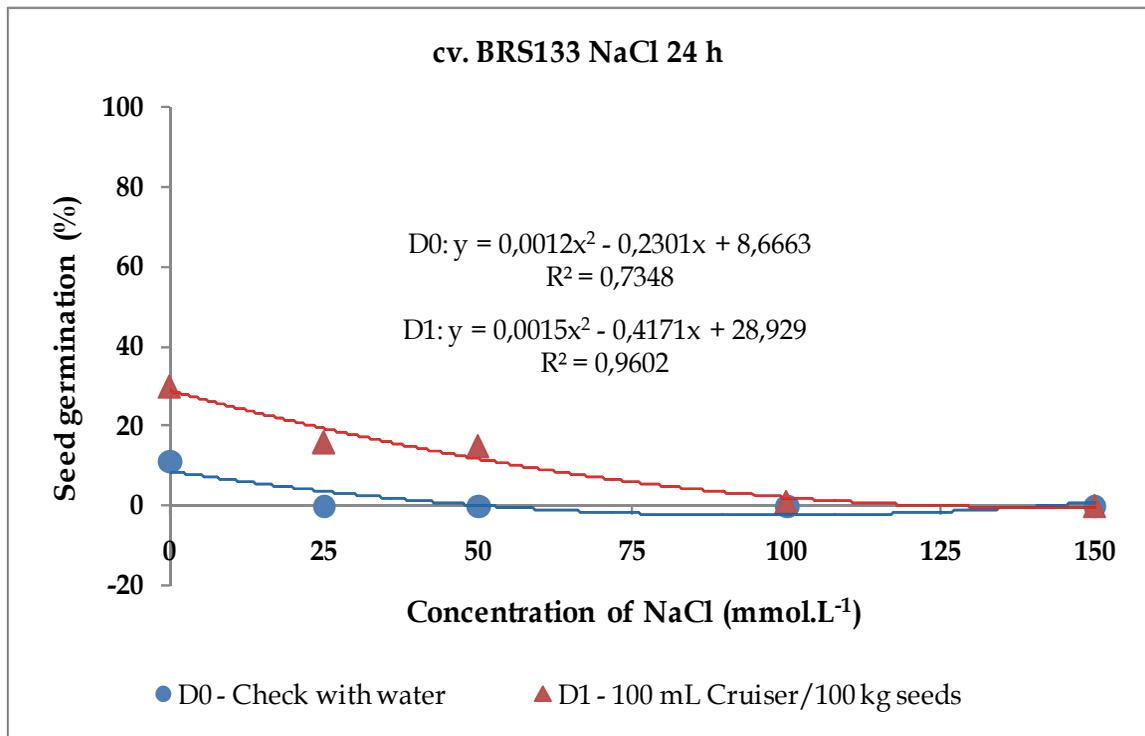


Fig. 11. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under NaCl 150 mmol L⁻¹. Average followed by the same letter did not differ significantly for each imbibition period. ns: not differ significantly for each imbibition period.



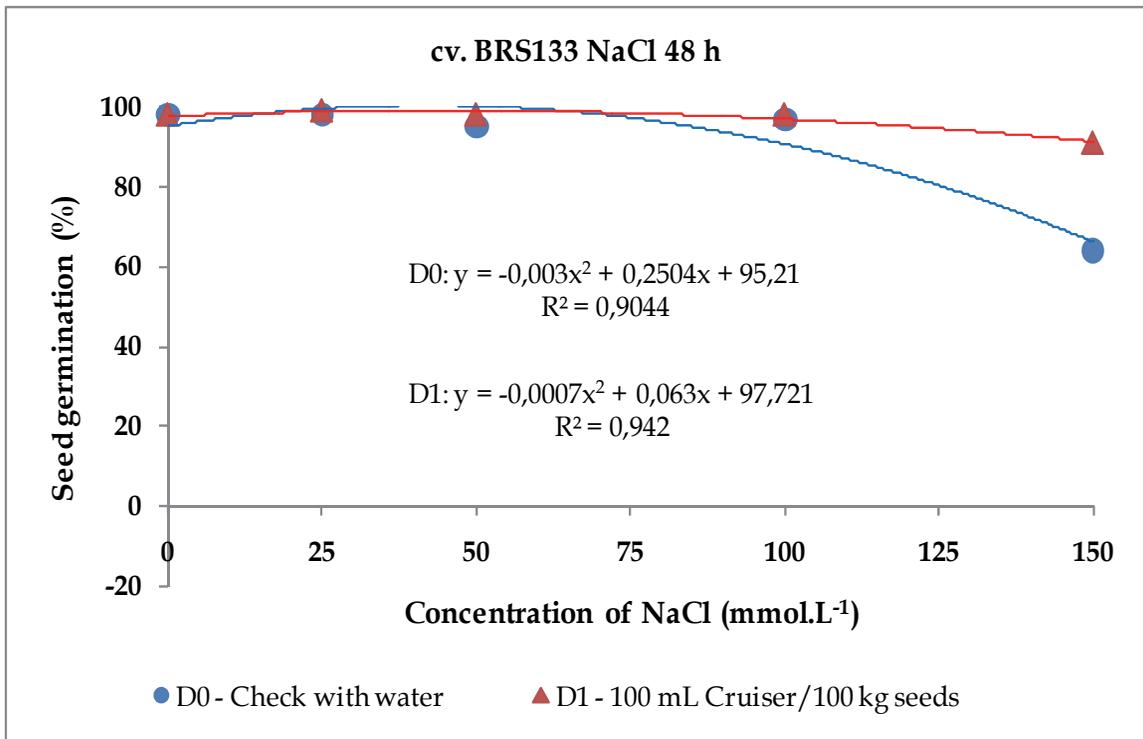
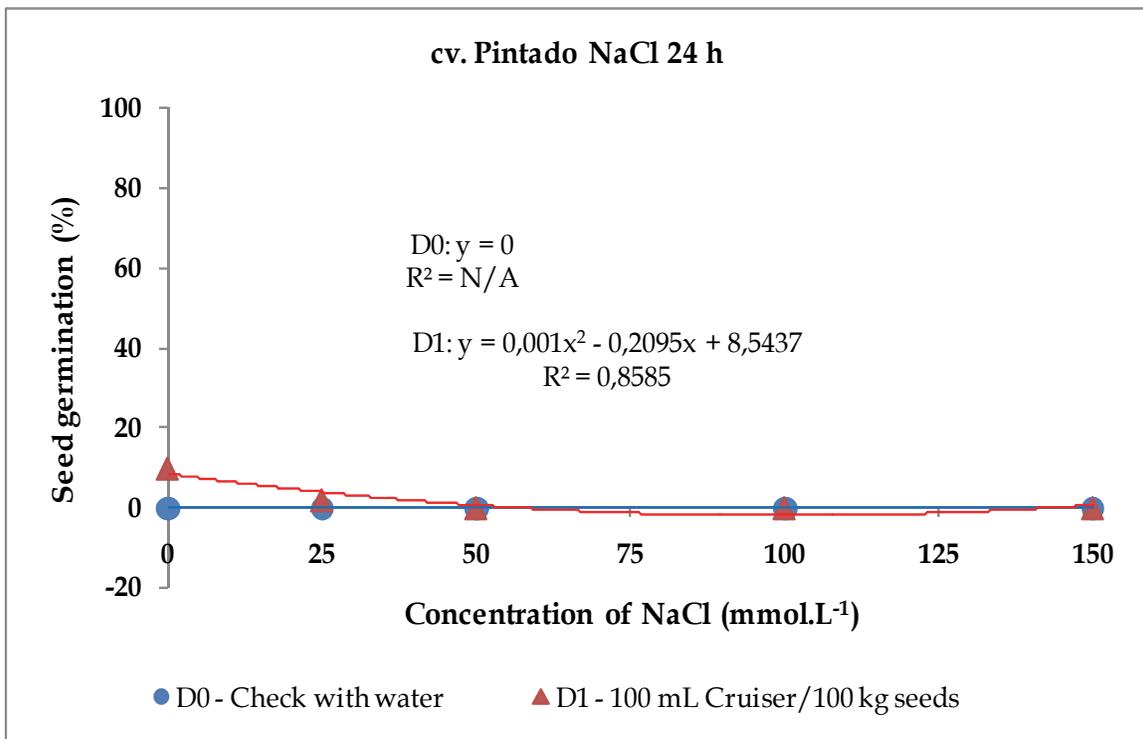


Fig. 12. Comparison of soybean germination percentage cv. BRS133 treated at recommended dose of Cruiser (D1) and check (D0), under different NaCl concentrations (25, 50, 100 and 150 mmol L⁻¹) at 24, 36 and 48 h of imbibition.



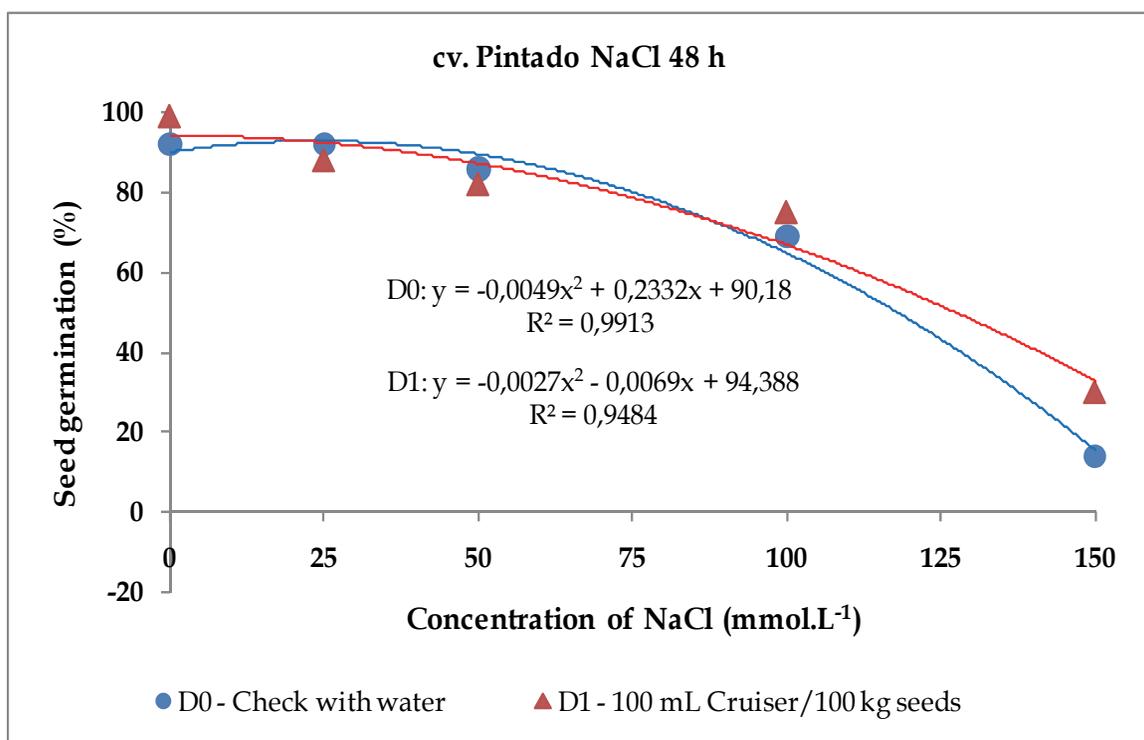
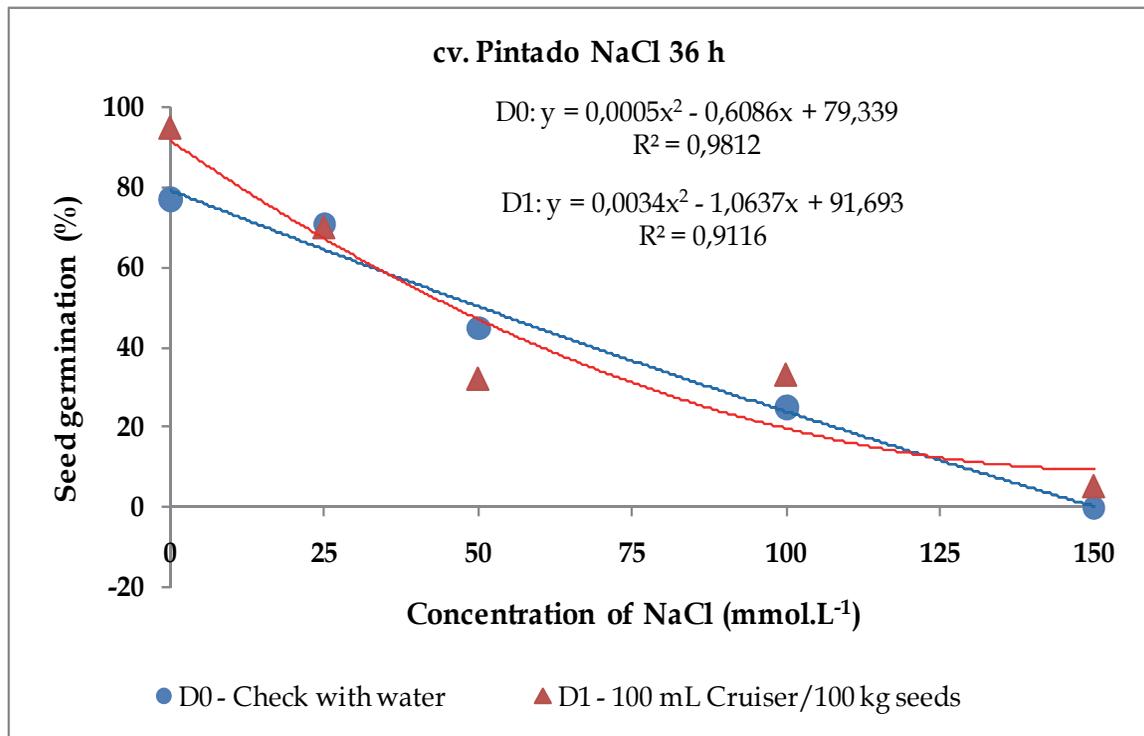


Fig. 13. Comparison of soybean germination percentage cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under different NaCl concentrations (25, 50, 100 and 150 mmol L⁻¹) at 24, 36 and 48 h of imbibition.

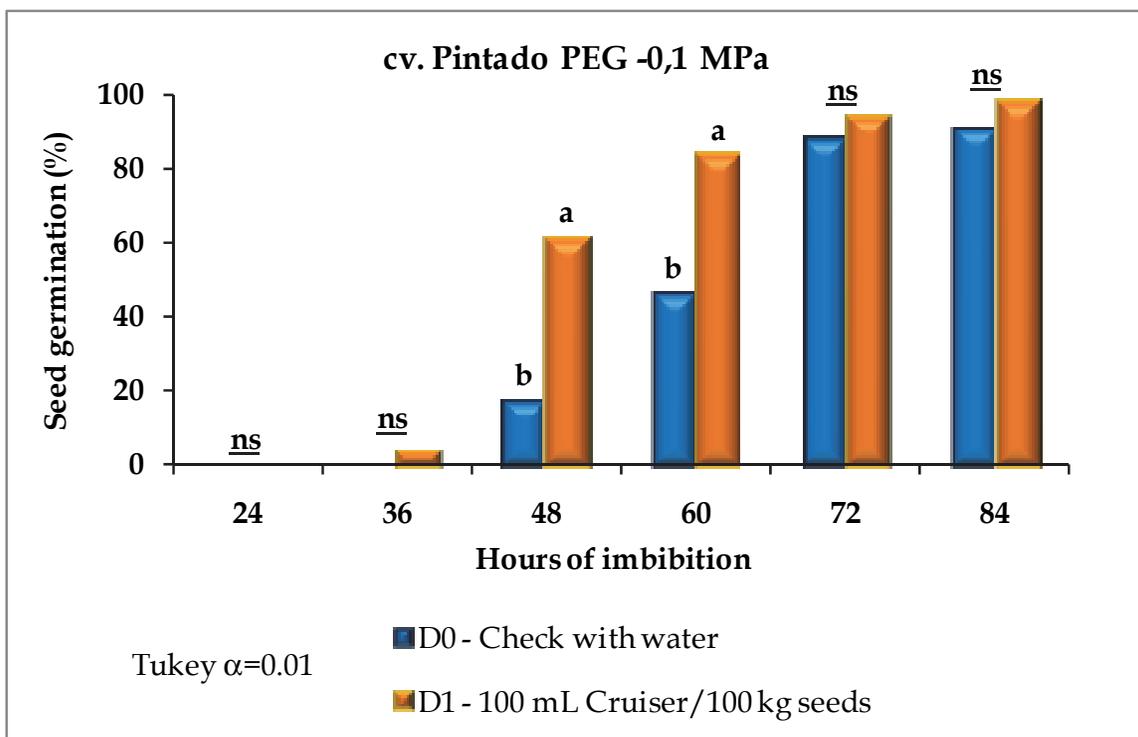
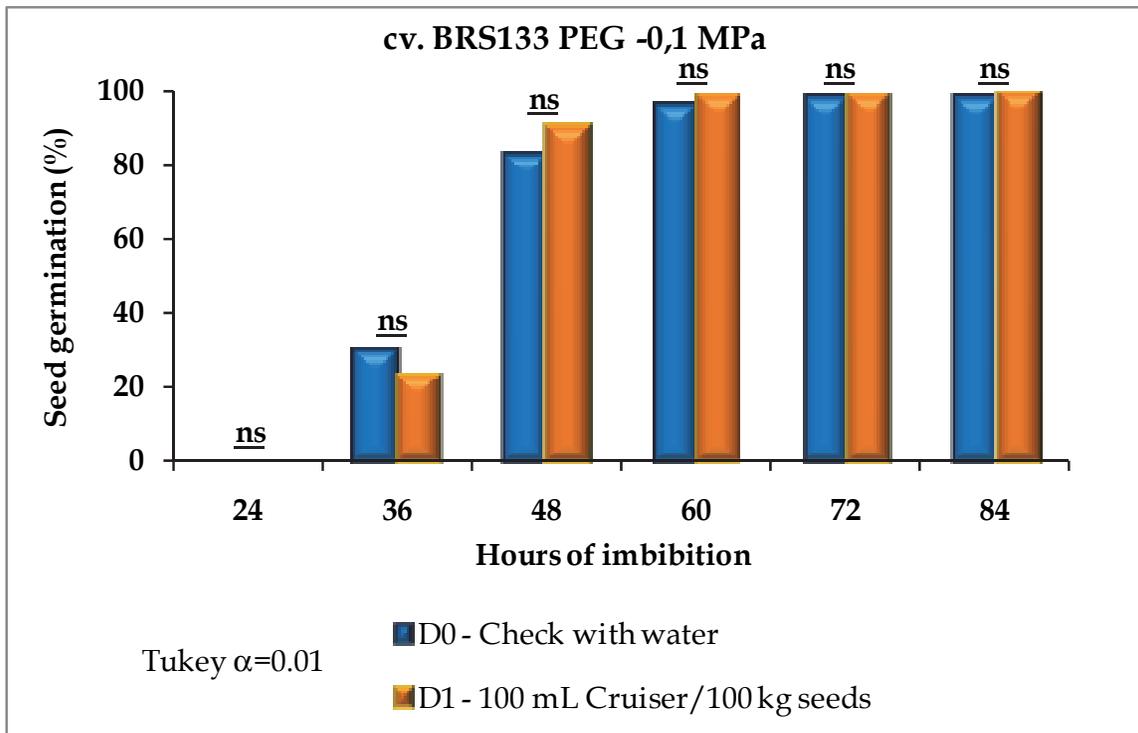


Fig. 14. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under PEG potential -0,1 MPa. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

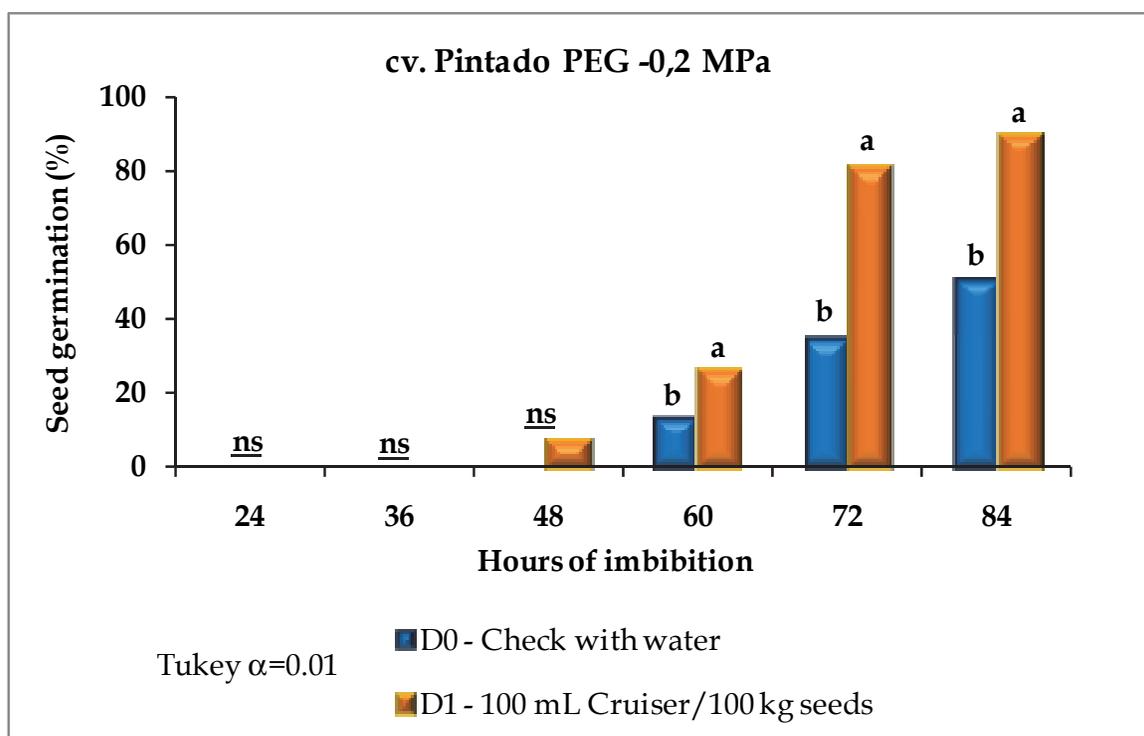
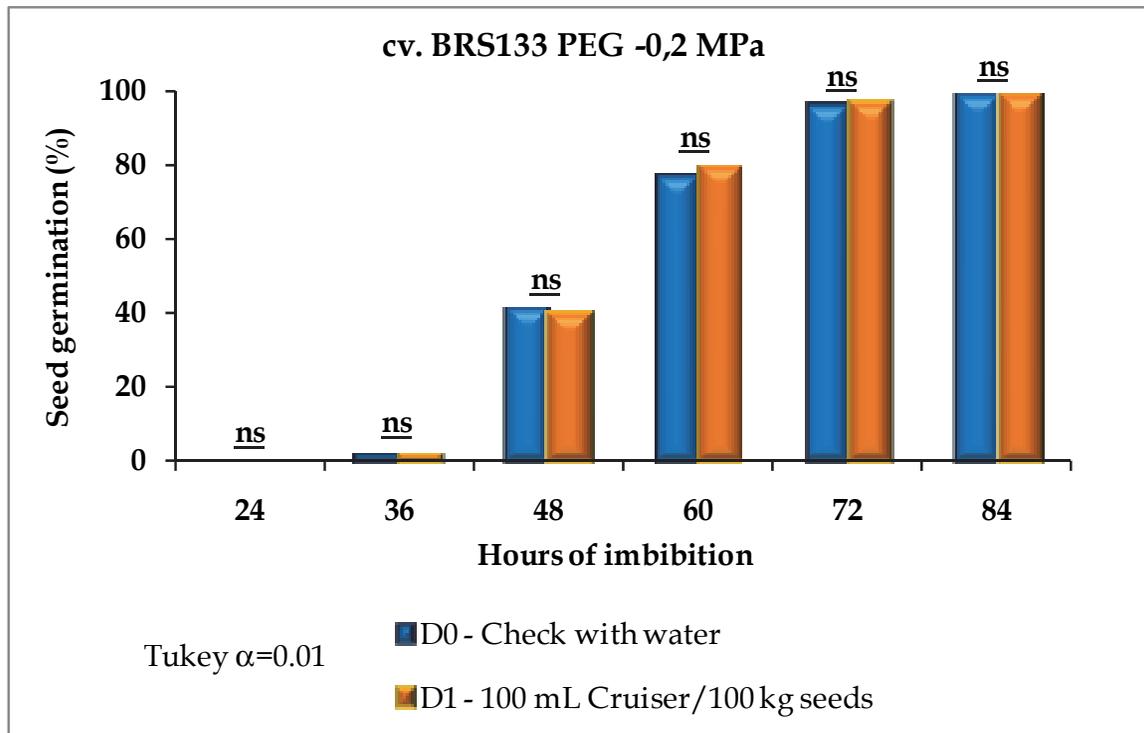


Fig. 15. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under PEG potential -0,2 MPa. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

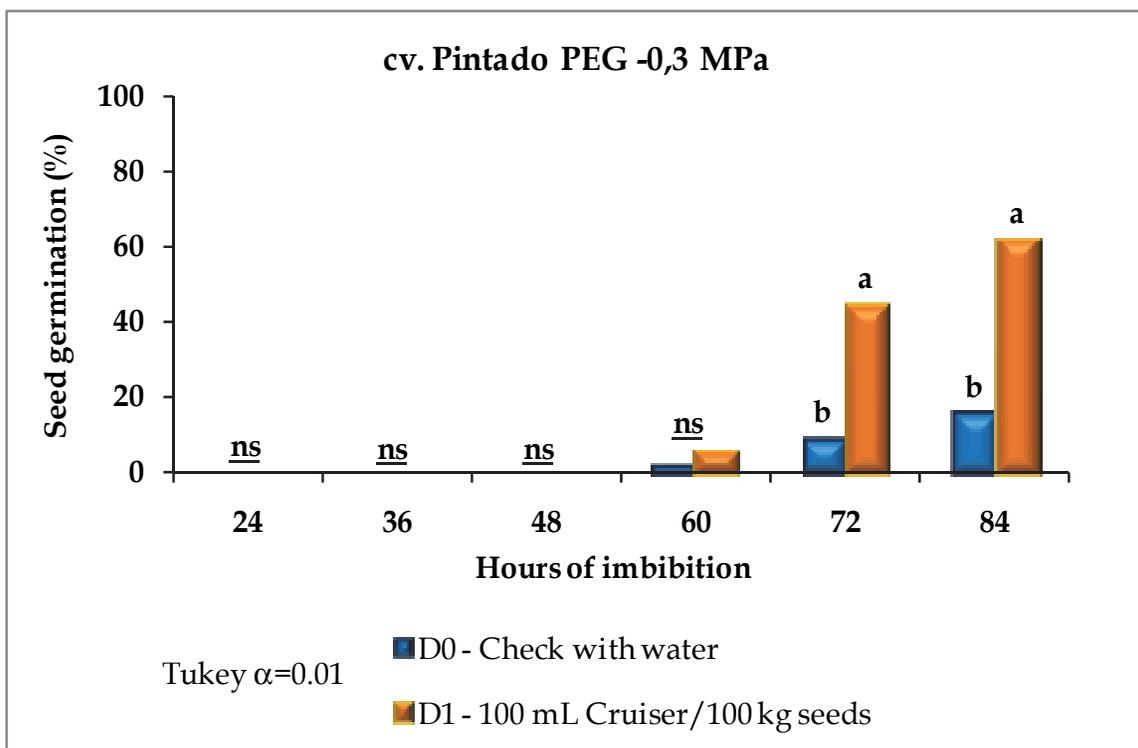
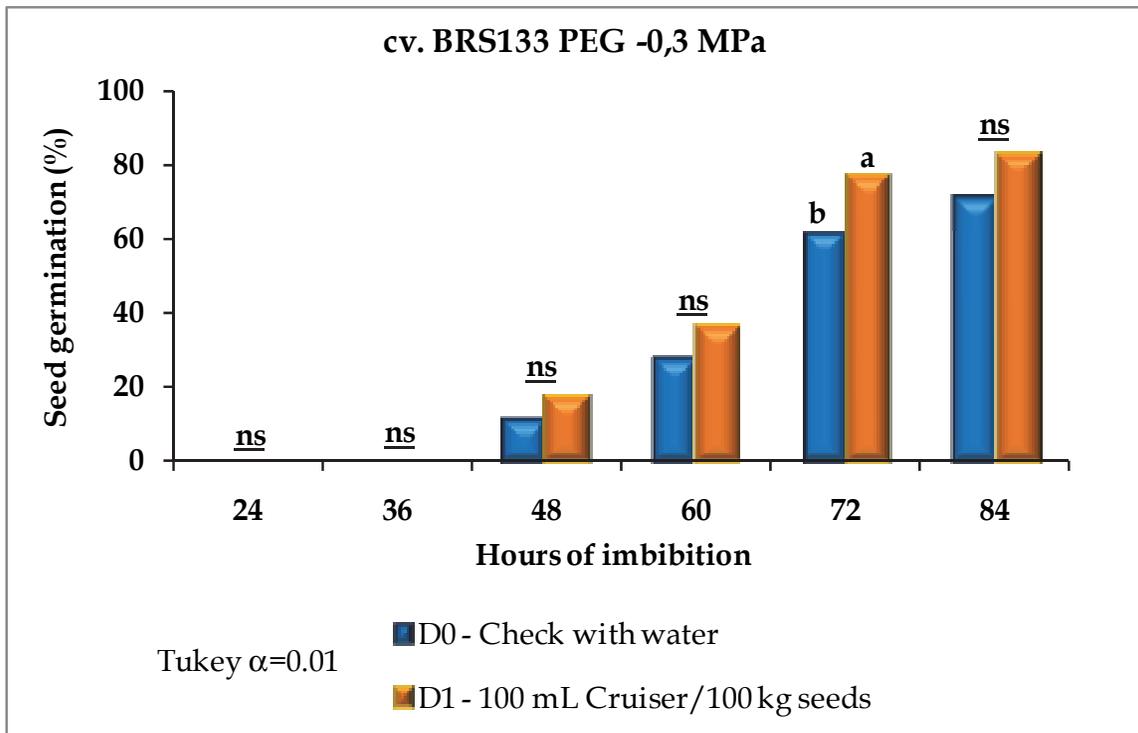
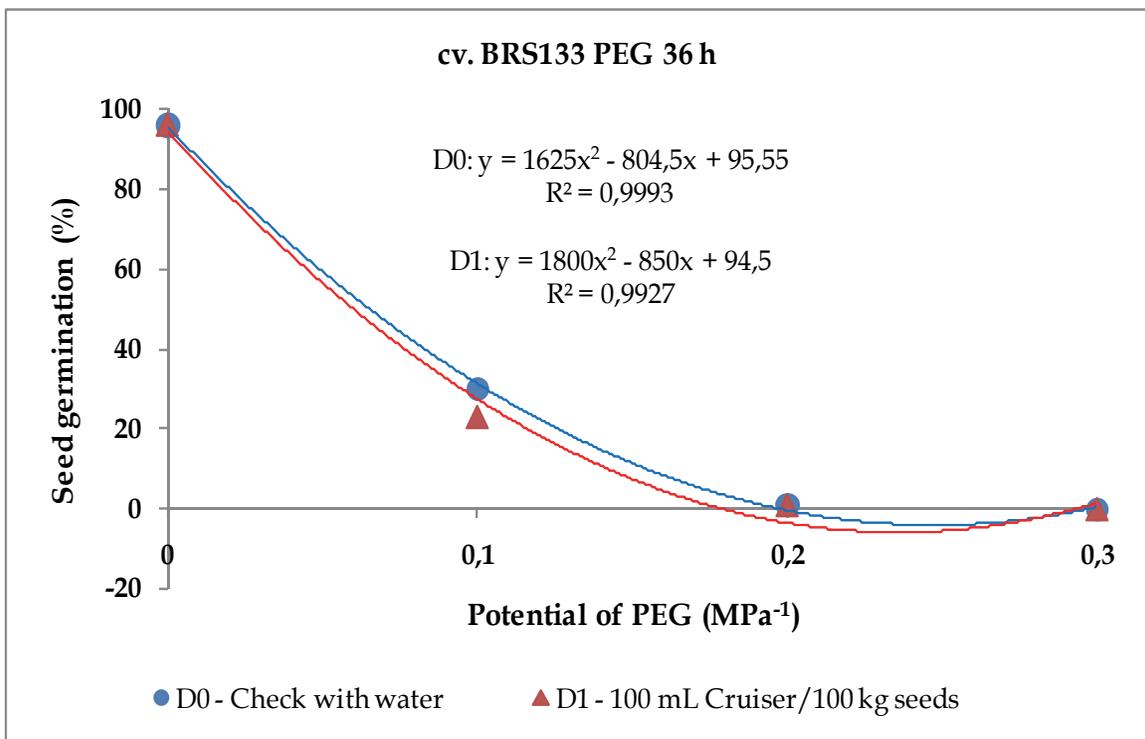
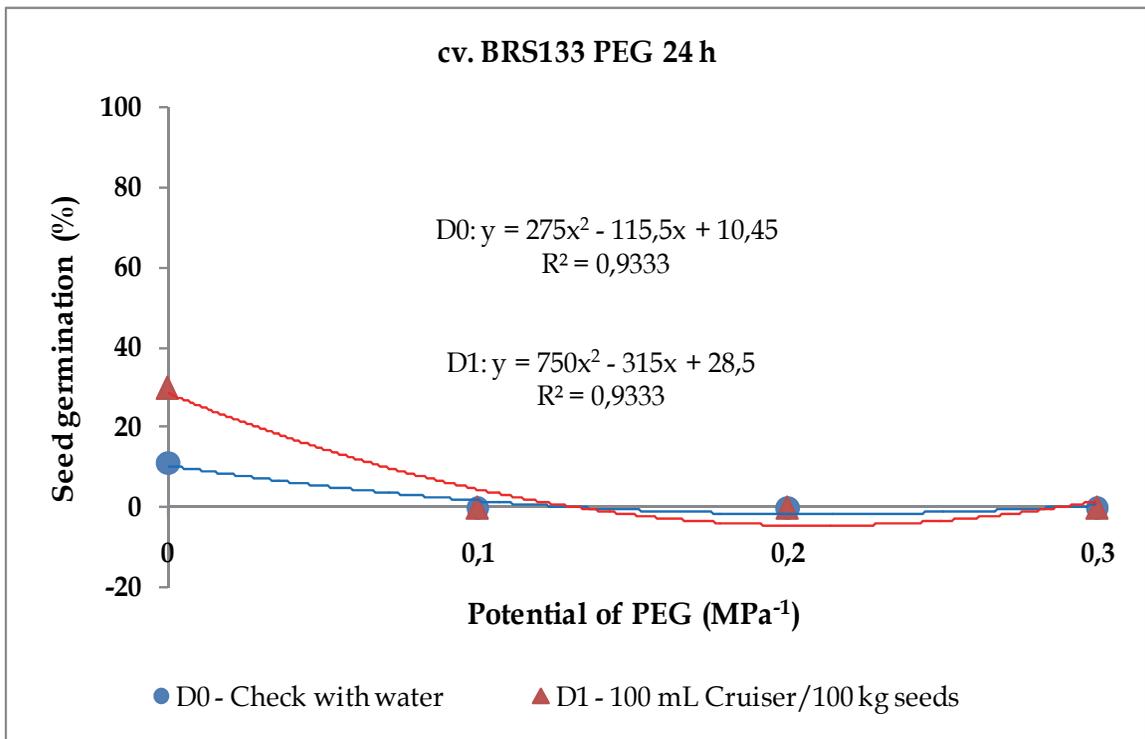
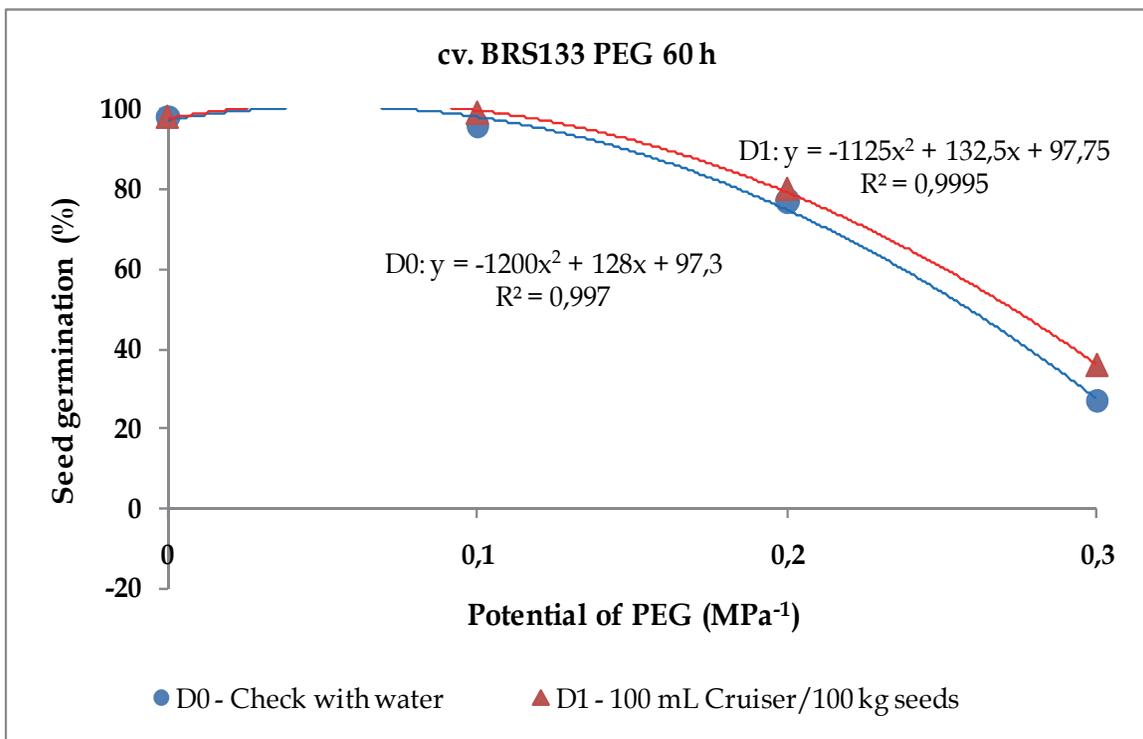
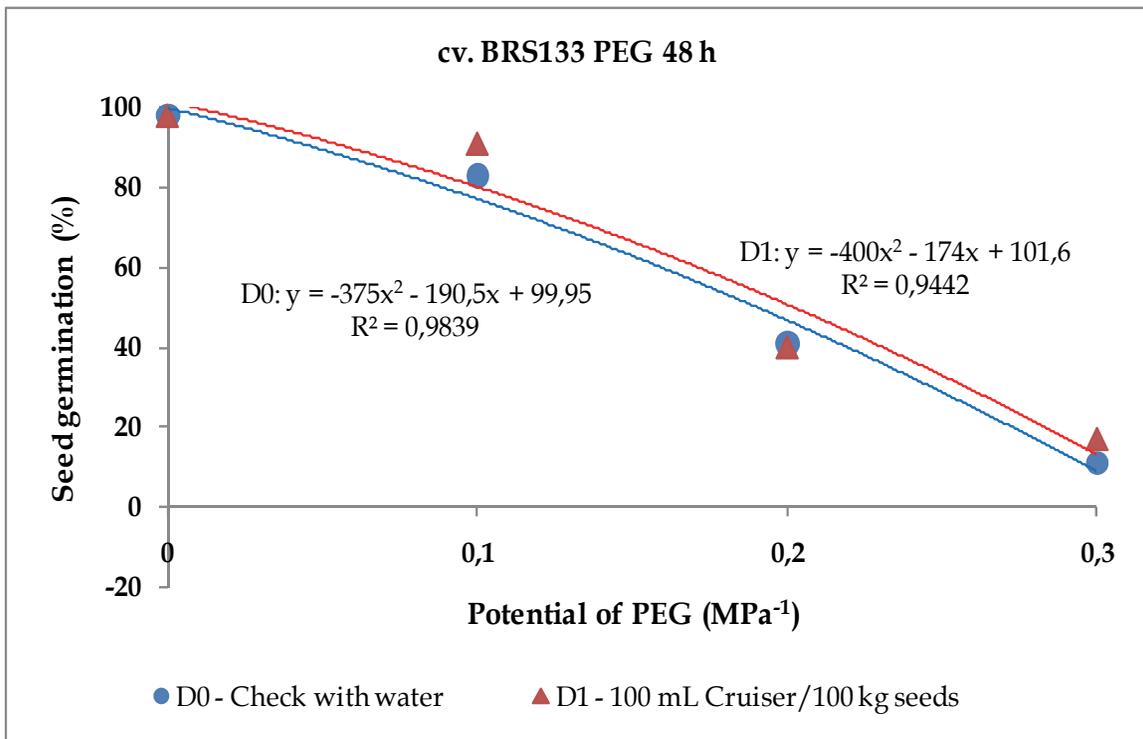


Fig. 16. Soybean germination percentage cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under PEG potential -0.3 MPa. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.





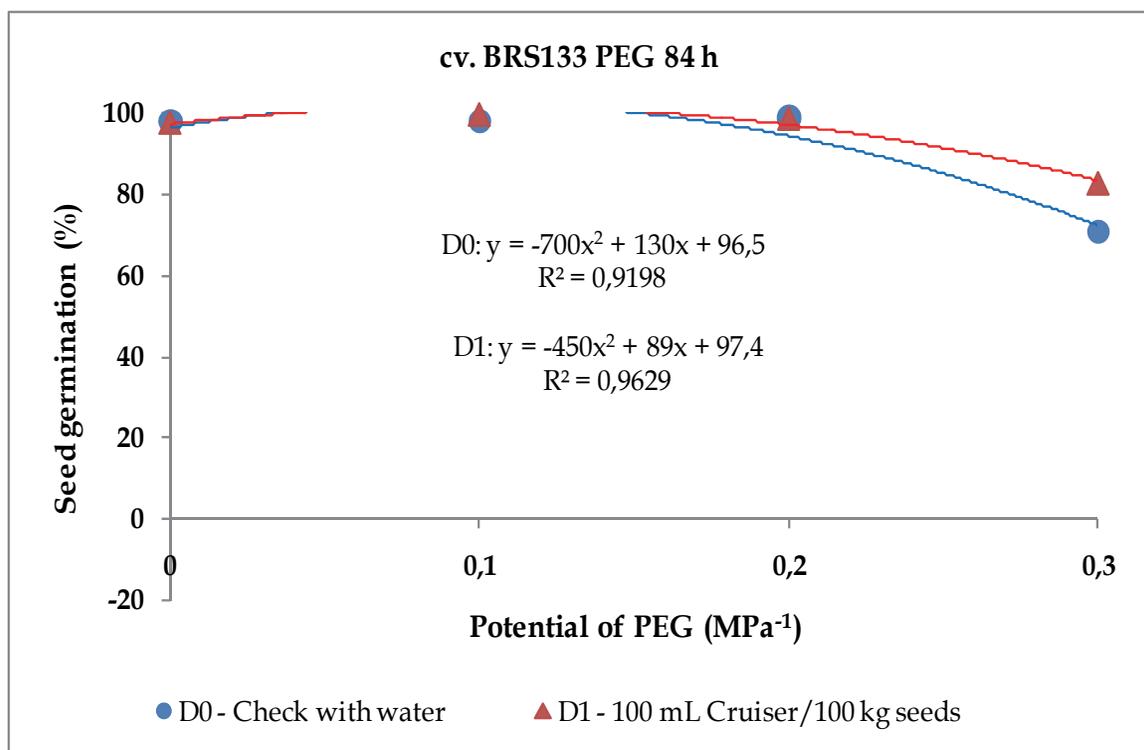
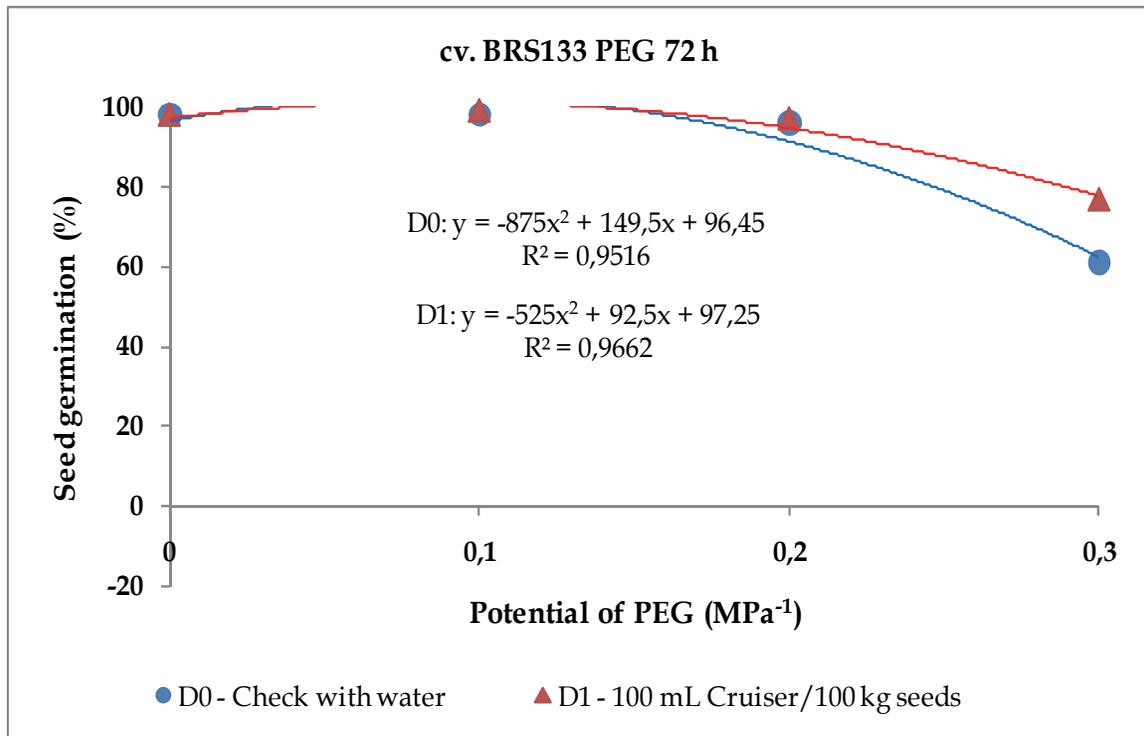
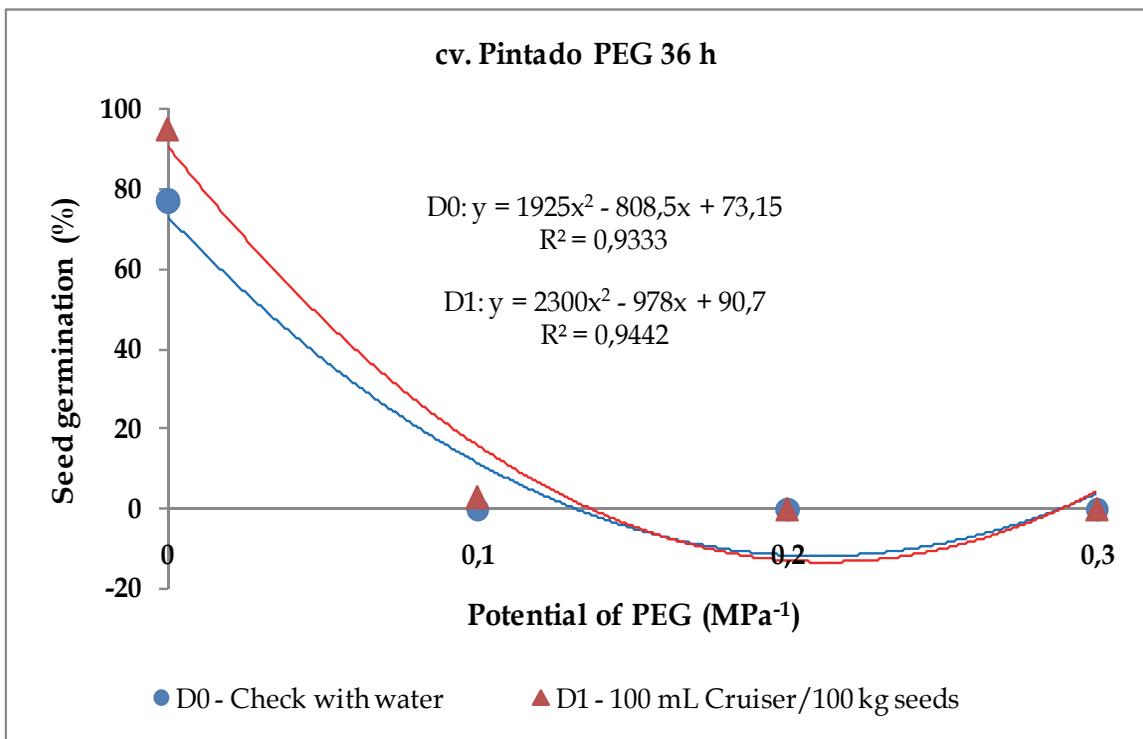
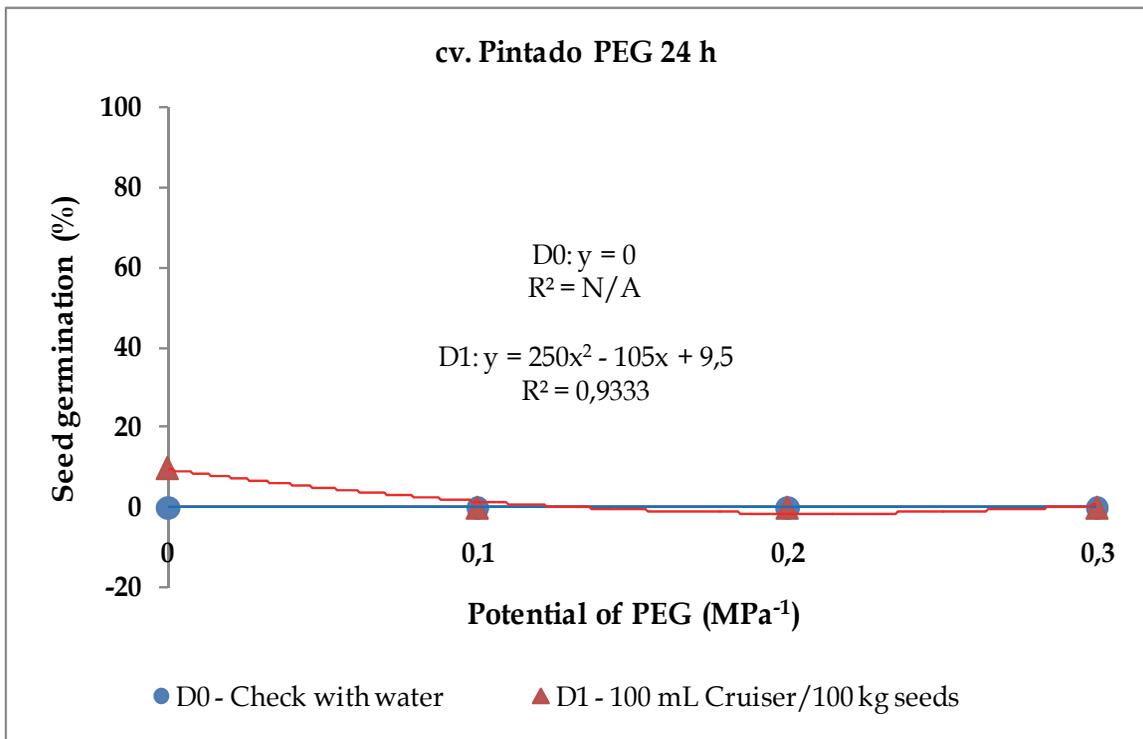
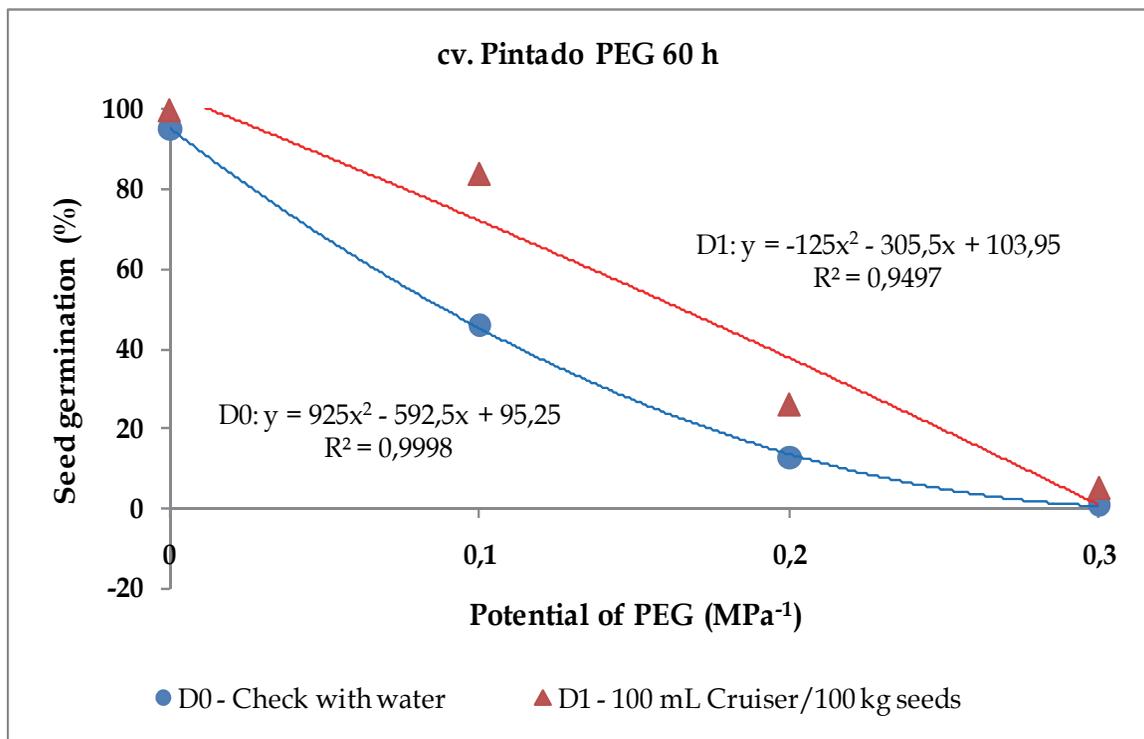
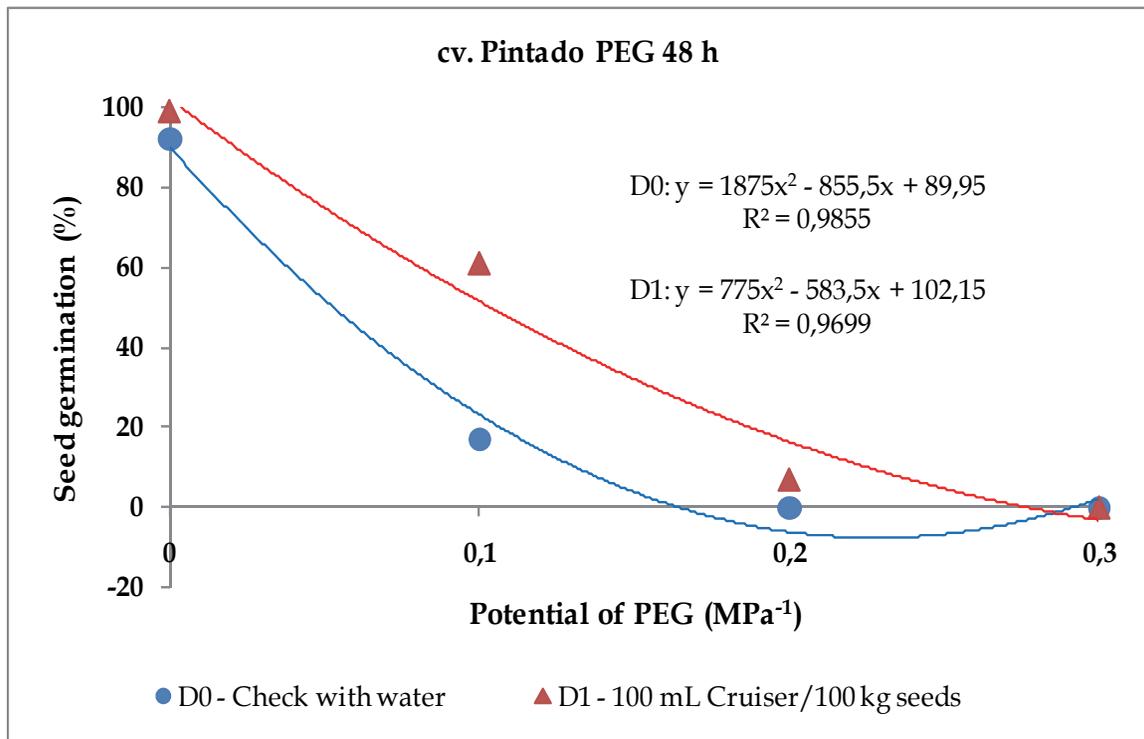


Fig. 17. Comparison of soybean germination percentage cv. BRS133 treated at recommended dose of Cruiser (D1) and check (D0), under different PEG potentials (-0,1; -0,2 and -0,3 MPa) at 24, 36, 48, 60, 72 and 84 h of imbibition.





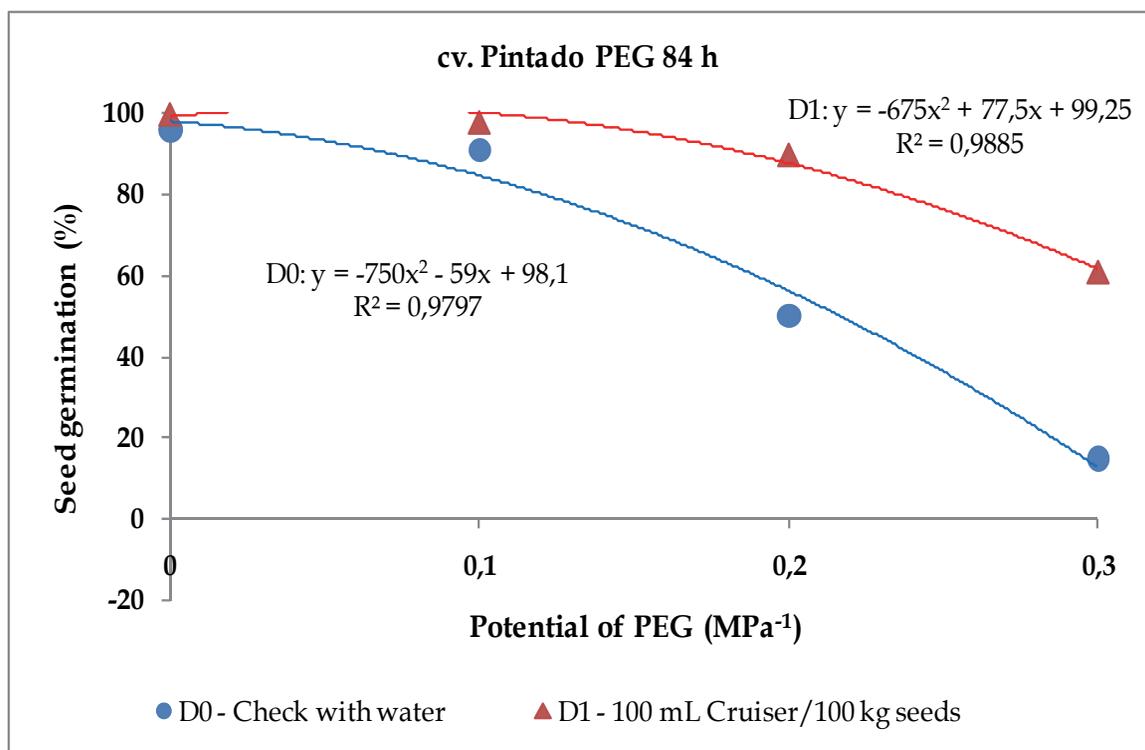
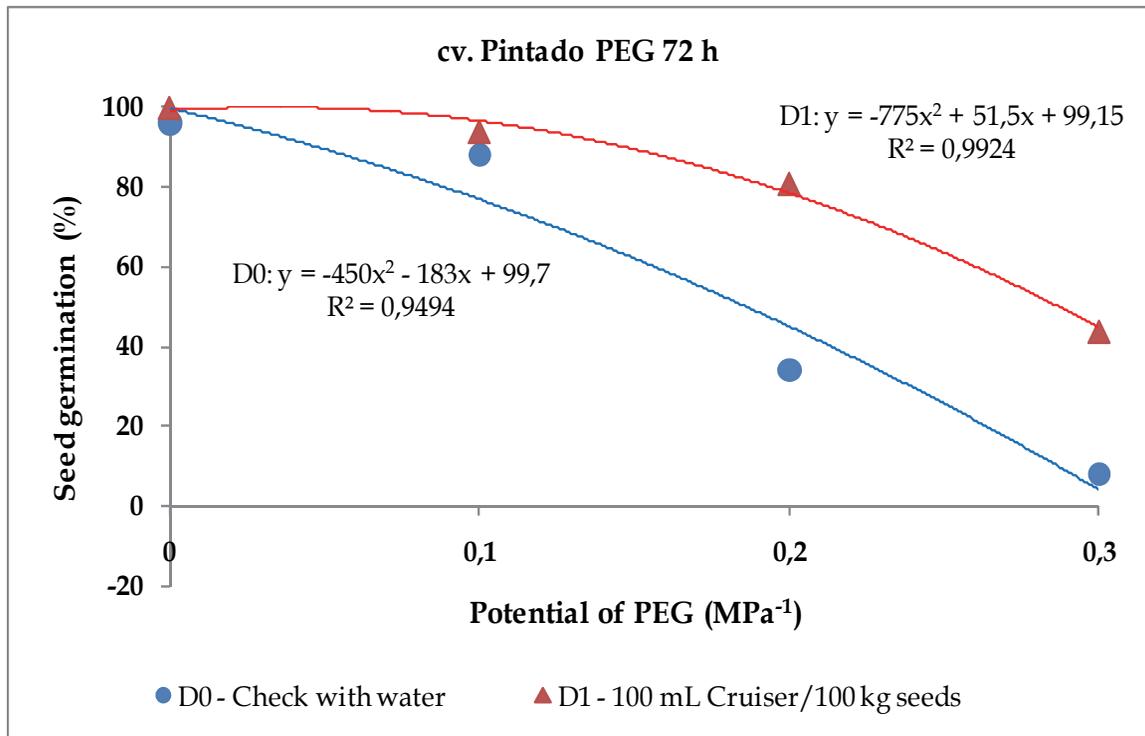


Fig. 18. Comparison of soybean germination percentage cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under different PEG potentials (-0,1; -0,2 and -0,3 MPa) at 24, 36, 48, 60, 72 and 84 h of imbibition.

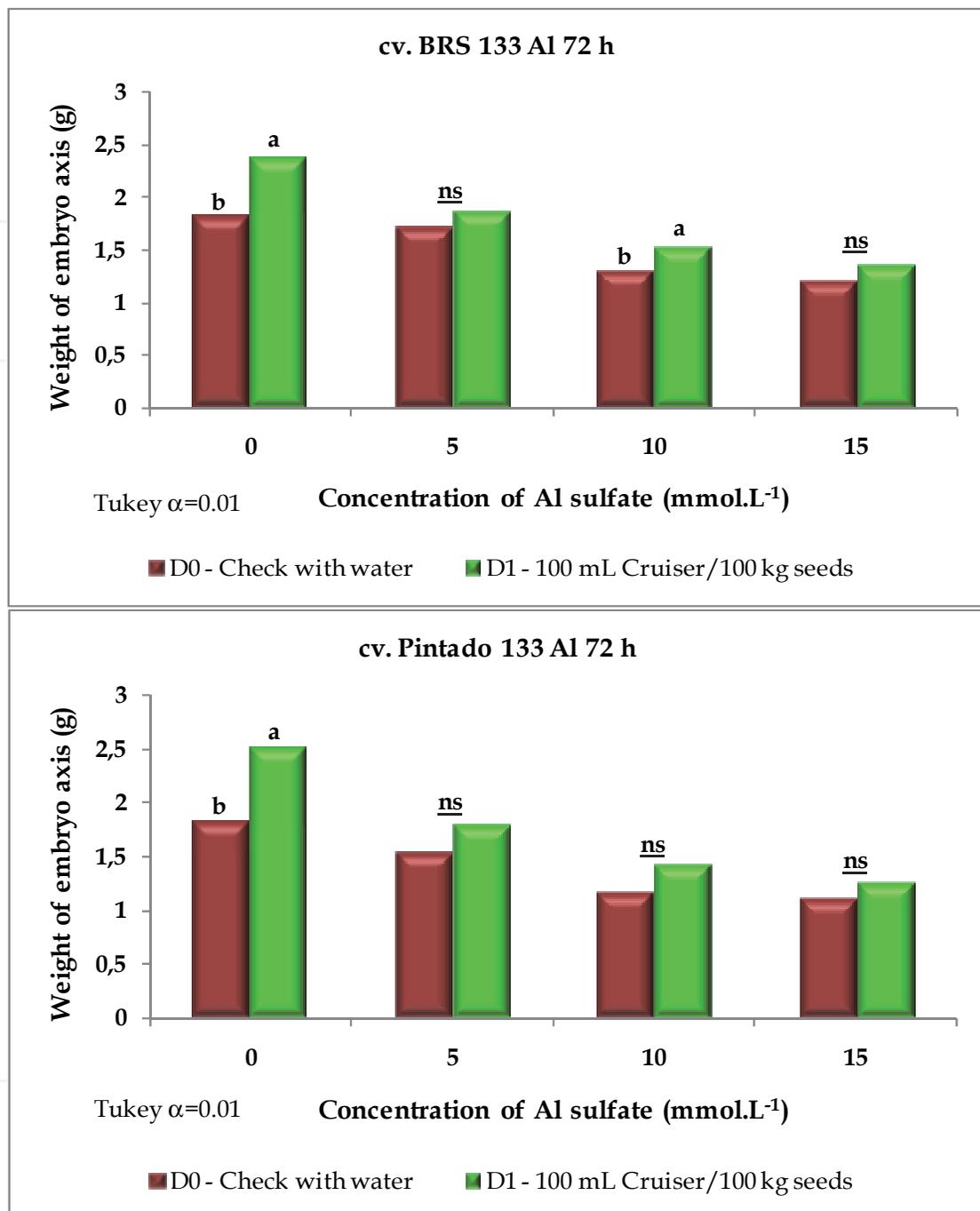


Fig. 19. Weight (g) of embryo axis of soybean seeds cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under different Al concentrations at 72 h of imbibition. Average followed by the same letter did not differ significantly for each concentration or each potential. **ns**: not differ significantly for each imbibition period.

The weights of embryo axis of soybean cultivars BRS 133 and Pintado under salinity are shown, respectively, in Figure 20. In the cultivar BRS 133 Cruiser, generally, caused an increase in the weight of embryo axis at all concentrations of NaCl used except at a concentration of 100 mmol L⁻¹ where there was no significant difference between seeds treated and untreated. In cultivar Pintado was observed a significant increase in the weight of embryo axis in the absence of NaCl and at concentration of 25 mmol L⁻¹; however, at the

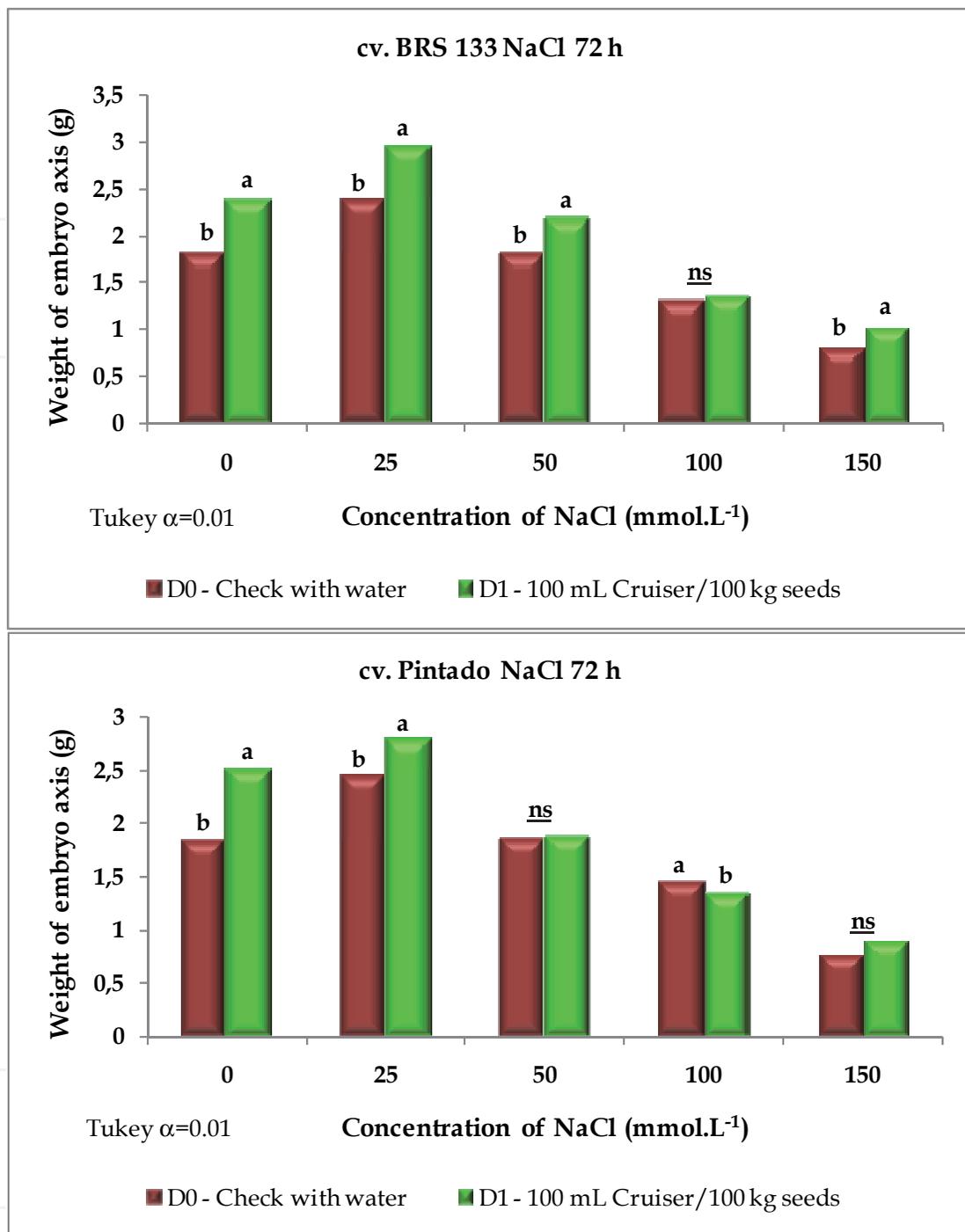


Fig. 20. Weight (g) of embryo axis of soybean seeds cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under different and NaCl concentrations at 72 h of imbibition. Average followed by the same letter did not differ significantly for each concentration or each potential. **ns**: not differ significantly for each imbibition period.

concentration of 100 mmol L⁻¹ Cruiser caused a decrease in axis weight. In saline conditions, Cruiser's effect on the development of the axis in cultivar BRS 133 is smaller with the increase of salt stress and in cultivar Pintado Cruiser has no effect under these conditions. The effect of Cruiser on weight of the embryo axis of cultivars BRS 133 and Pintado under water stress conditions are represented in Figure 21. It was observed that in both cultivars,

Cruiser increased the development of the embryo axis in the water potentials of 0 and -0.1 MPa. In situations of greater water deficit (-0.2 and -0.3 MPa) there was no significant difference between treated and untreated seeds. The effect of Cruiser on the development of embryo axis in conditions of water stress is smaller with increasing of water deficit.

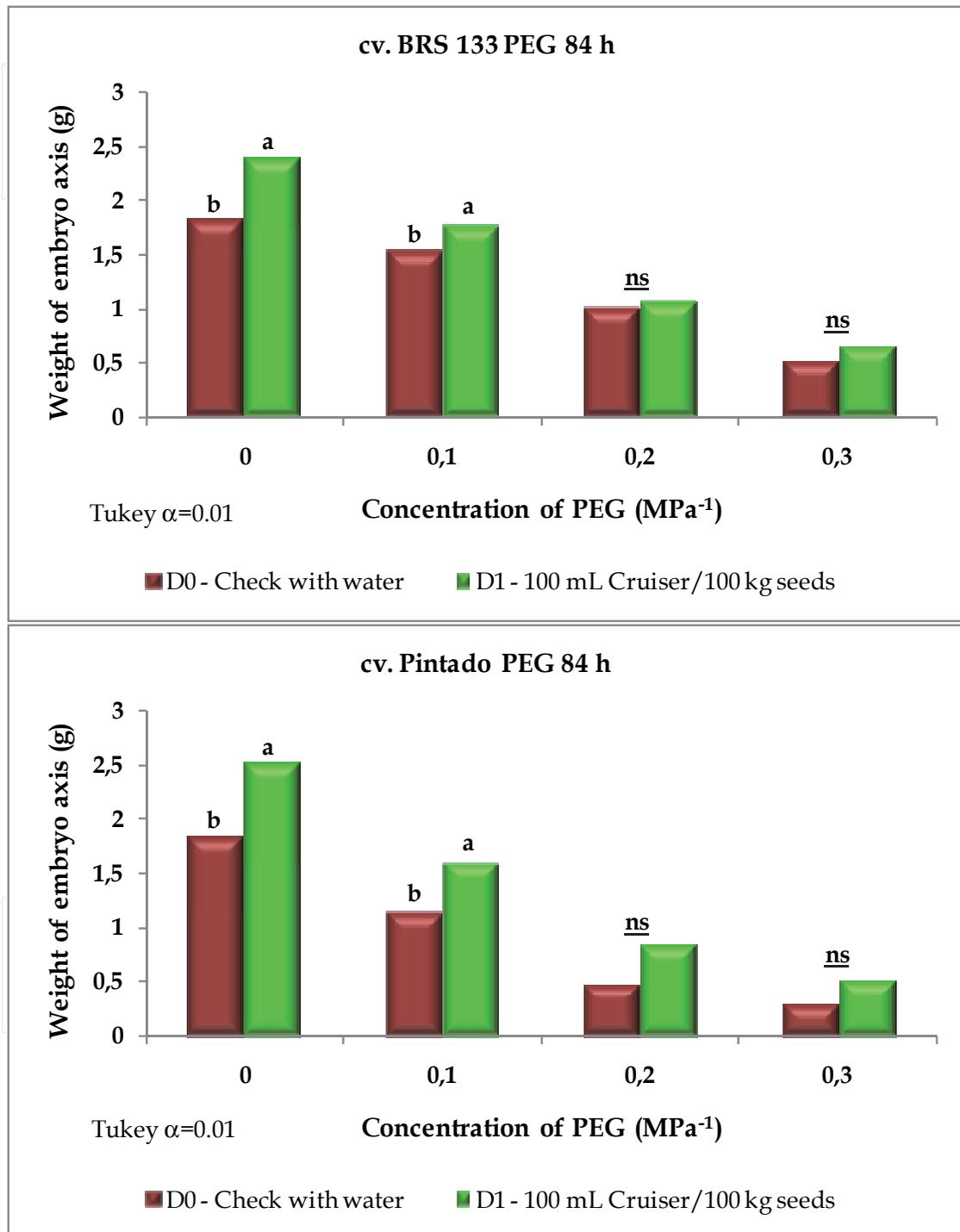


Fig. 21. Weight (g) of embryo axis of soybean seeds cv. BRS 133 and cv. Pintado treated at recommended dose of Cruiser (D1) and check (D0), under different PEG potentials at 84 h of imbibition. Average followed by the same letter did not differ significantly for each concentration or each potential. **ns**: not differ significantly for each imbibition period.

3.3 Third experiment: Cruiser’s action on the enzymes involved in the response to oxidative stress induced by aluminum presence, salinity and water deficit

Cruiser has caused significant increase in peroxidase activity (POD) in BRS 133 and Pintado cultivars at 24 and 36 h of imbibition (Figure 22) when seeds were placed to germinate in distilled water (control).

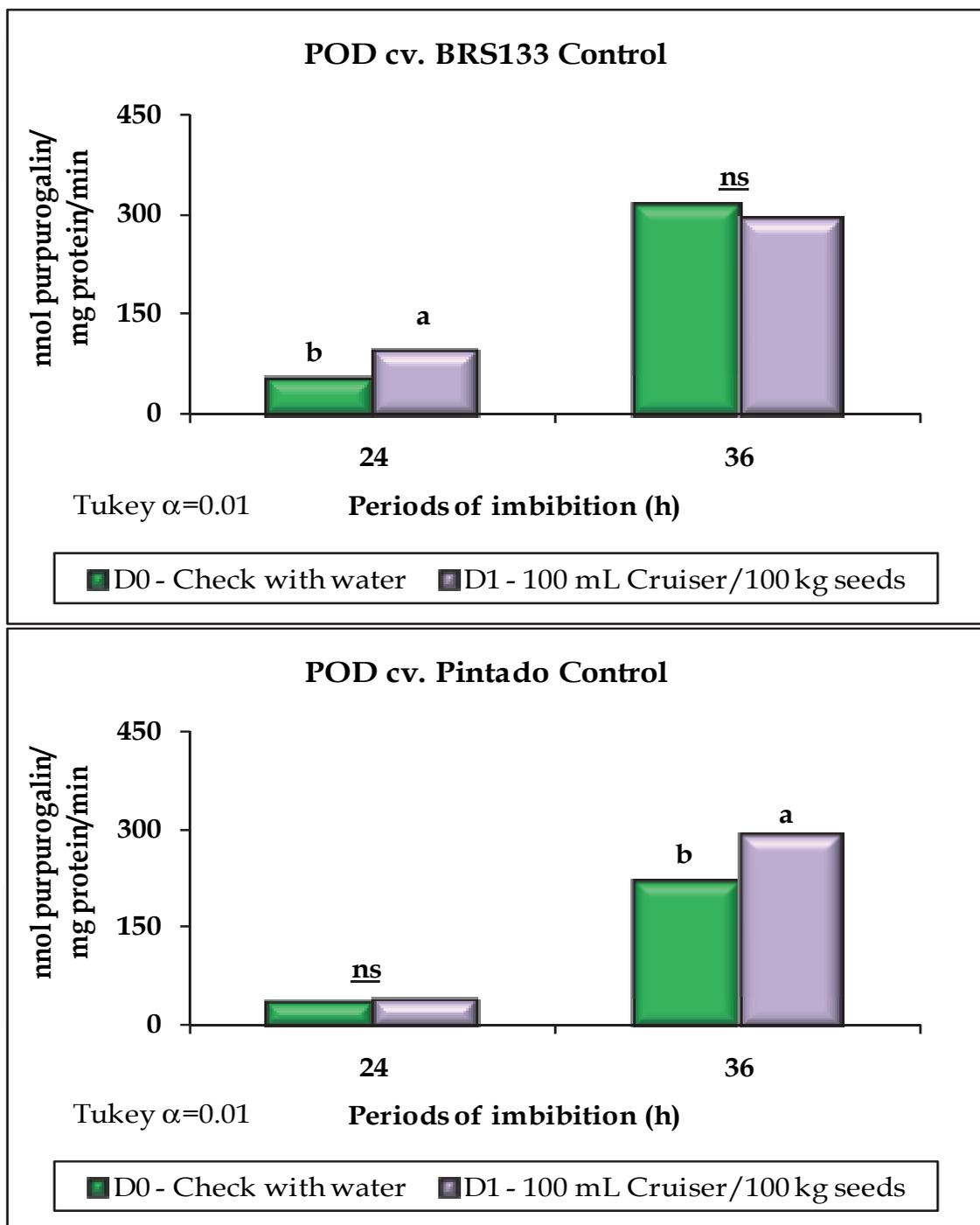


Fig. 22. Peroxidase activity (nmol purpurogalin mg protein⁻¹ min⁻¹) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under distilled water (control). Average followed by the same letter did not differ significantly for each imbibition period. ns: not differ significantly for each imbibition period.

In the cultivar BRS 133 Cruiser has caused increase in POD activity at the aluminum concentration of 10 mmol L⁻¹ (Figure 23) in the two imbibition periods analyzed, at 24 and 36 h. In the cultivar Pintado, Cruiser caused a decrease in POD activity at 36 h of imbibition and increased at 48 h.

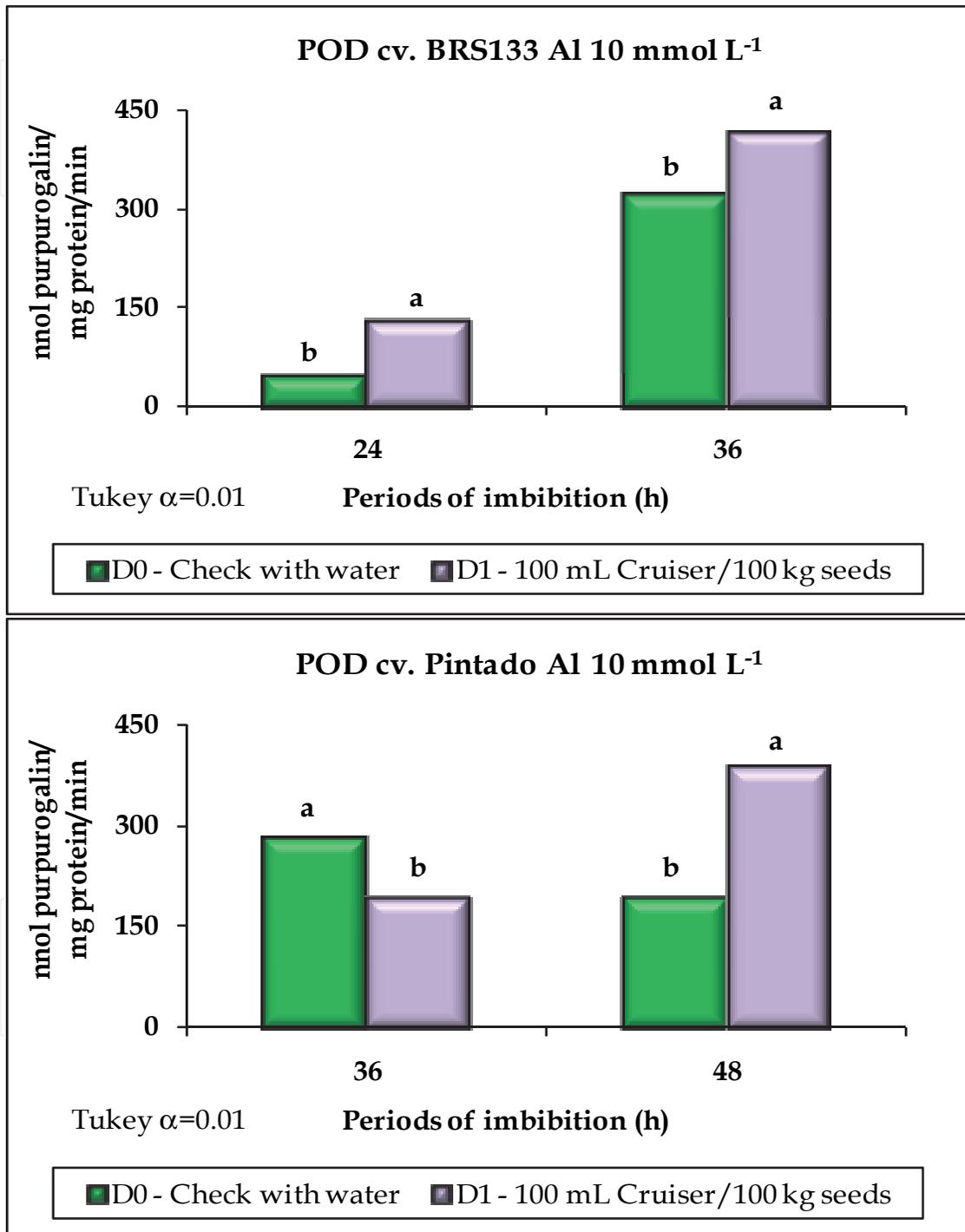


Fig. 23. Peroxidase activity (nmol purpurogalin mg protein⁻¹ min⁻¹) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under aluminum. Average followed by the same letter did not differ significantly for each imbibition period.

In NaCl concentration of 50 mmol L⁻¹, Cruiser caused increase of POD at 36 hours of imbibition in the cultivar BRS 133 (Figure 24). Cruiser used under conditions of NaCl concentration of 100 mmol L⁻¹ in the cultivar Pintado caused a increase in POD activity at 36 h of imbibition and decreased enzyme activity at 48 h of imbibition.

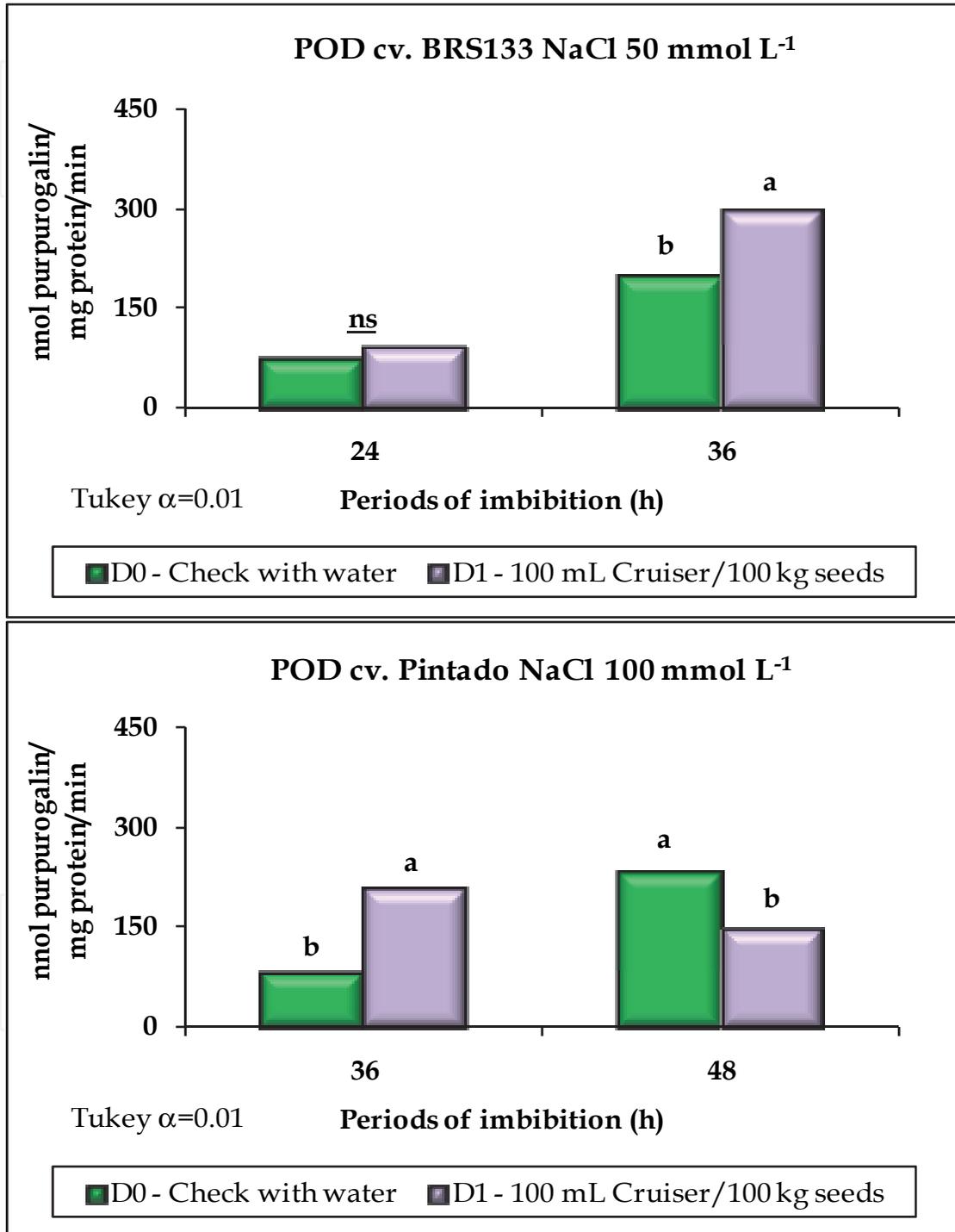


Fig. 24. Peroxidase activity (nmol purpurogalin mg protein⁻¹ min⁻¹) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under NaCl. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

Under water deficit conditions of -0.3 MPa, Cruiser increased activity of POD at 60 and 72 h of imbibition in cultivar BRS 133 (Figure 25), however, in the cultivar Pintado decreased it at 72 h of imbibition and did not alter the enzyme activity at 84 h of imbibition.

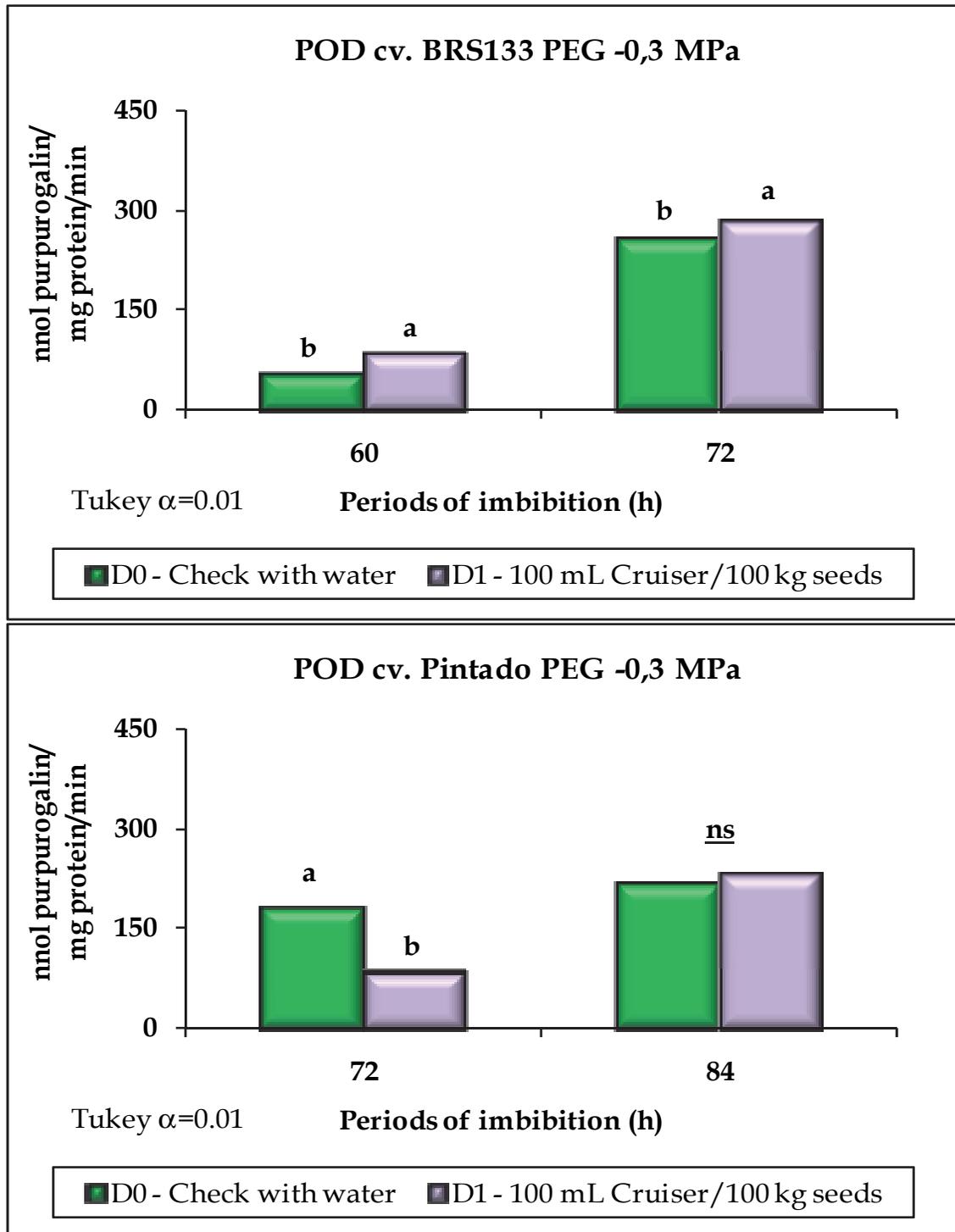


Fig. 25. Peroxidase activity (nmol purpurogalin mg protein⁻¹ min⁻¹) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under PEG. Average followed by the same letter did not differ significantly for each imbibition period. **ns**: not differ significantly for each imbibition period.

Regarding the activity of superoxide dismutase (SOD) (Figures 26 to 29), this did not change as effect of Cruiser when the soybean seeds of both cultivars were germinated under the same conditions of stress, the same imbibition periods analyzed to determine the POD.

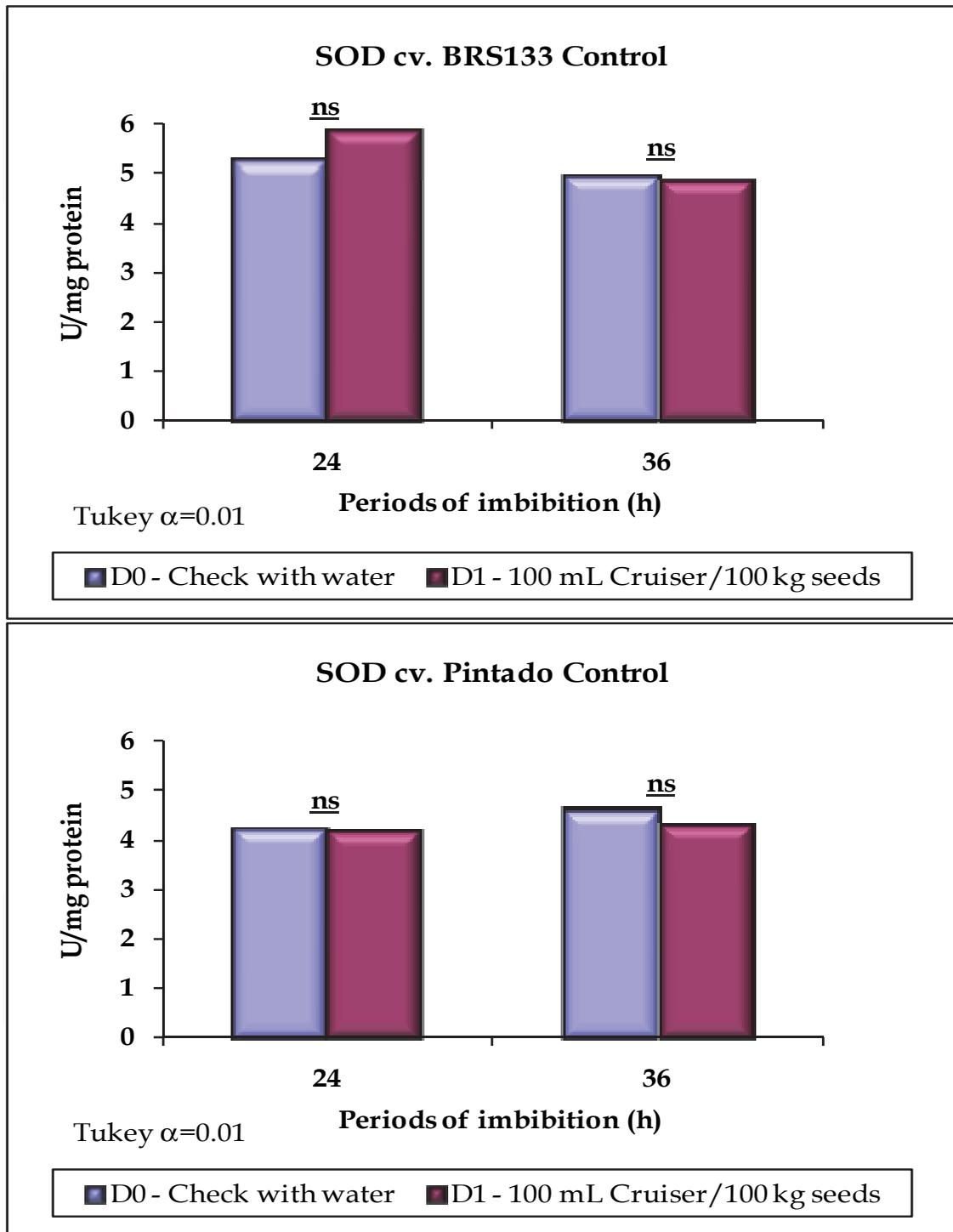


Fig. 26. Superoxide dismutase activity (U mg proteína⁻¹) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under distilled water (control). ns: not differ significantly for each imbibition period.

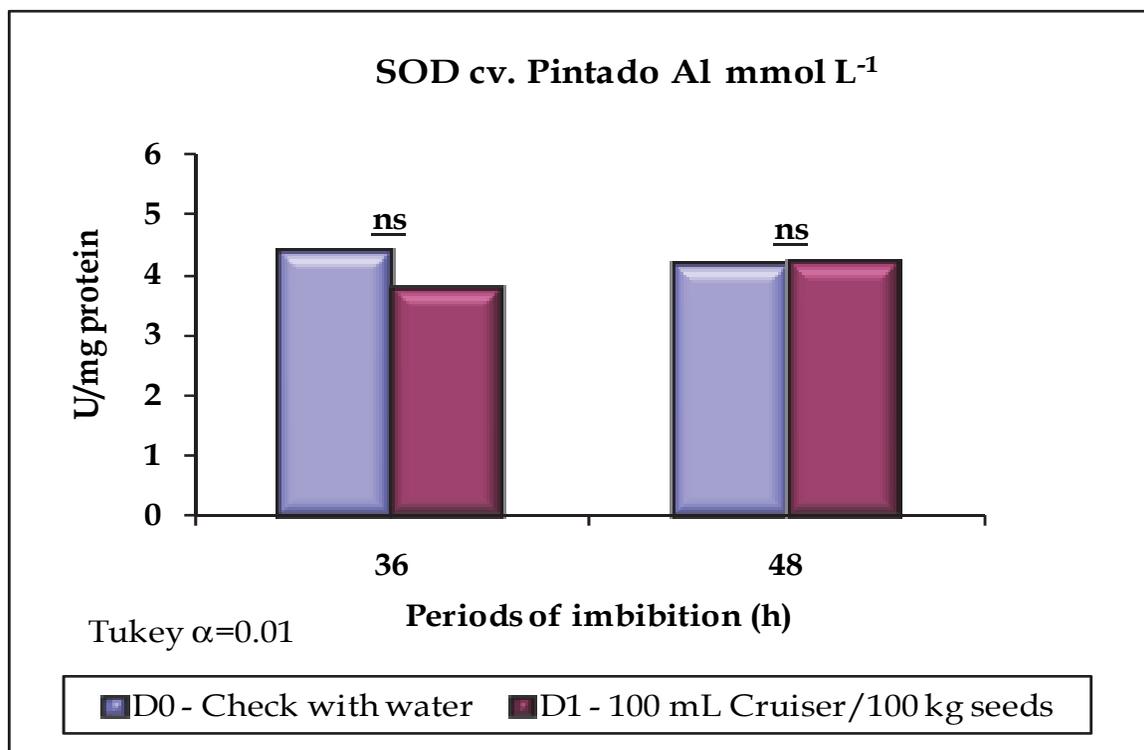
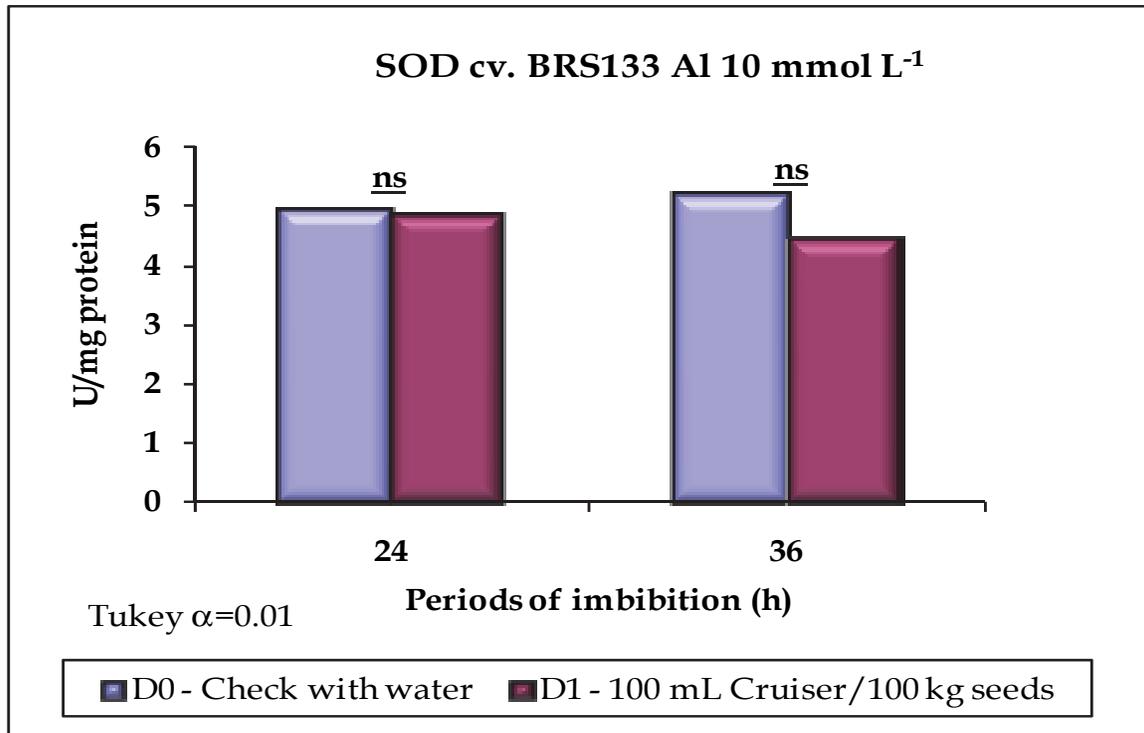


Fig. 27. Superoxide dismutase activity (U mg proteína⁻¹) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under aluminum. **ns**: not differ significantly for each imbibition period.

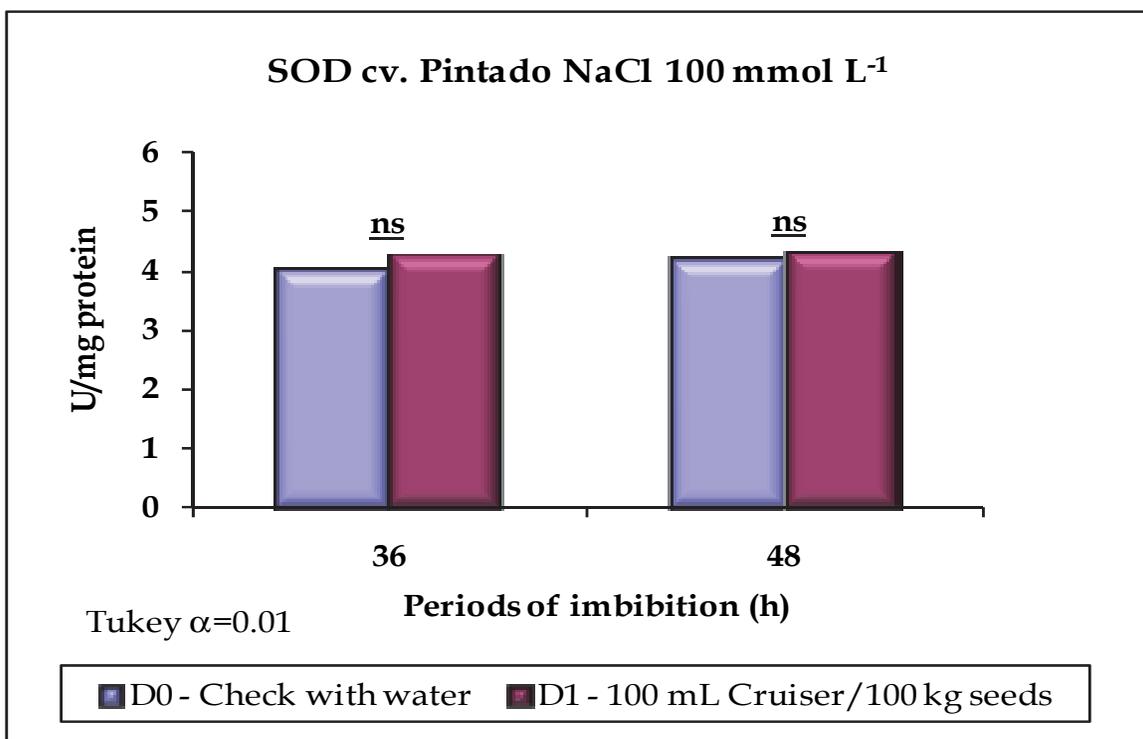
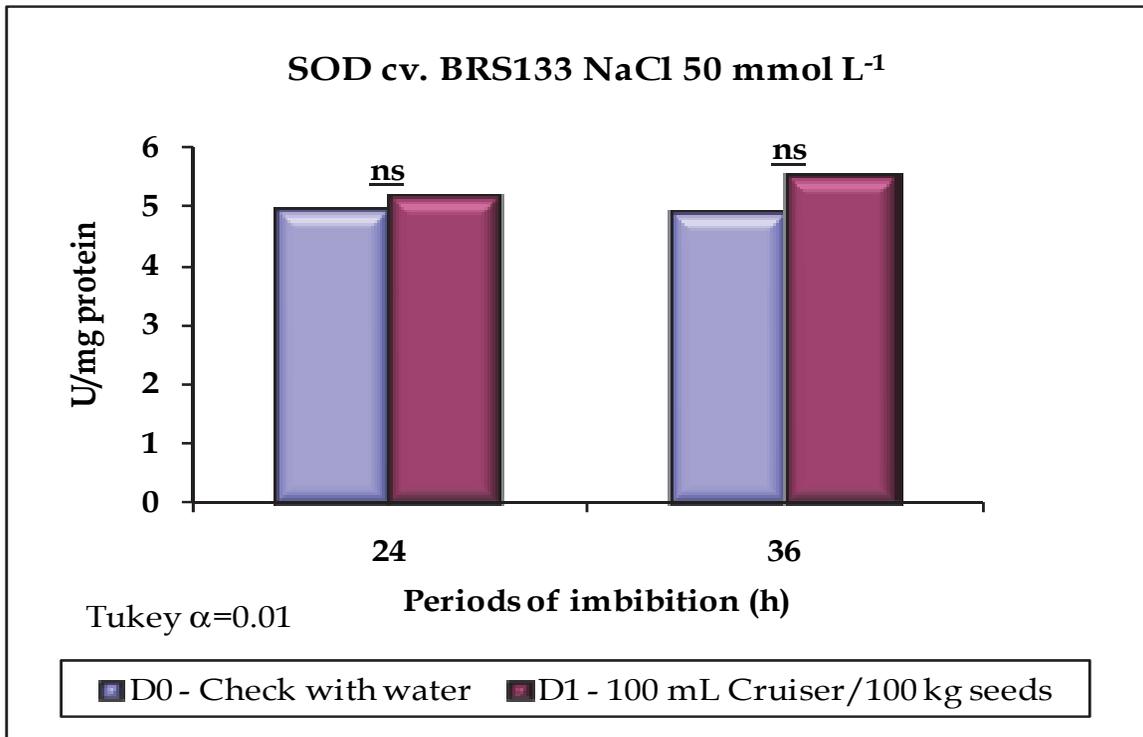


Fig. 28. Superoxide dismutase activity (U mg proteína⁻¹) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under NaCl. **ns**: not differ significantly for each imbibition period.

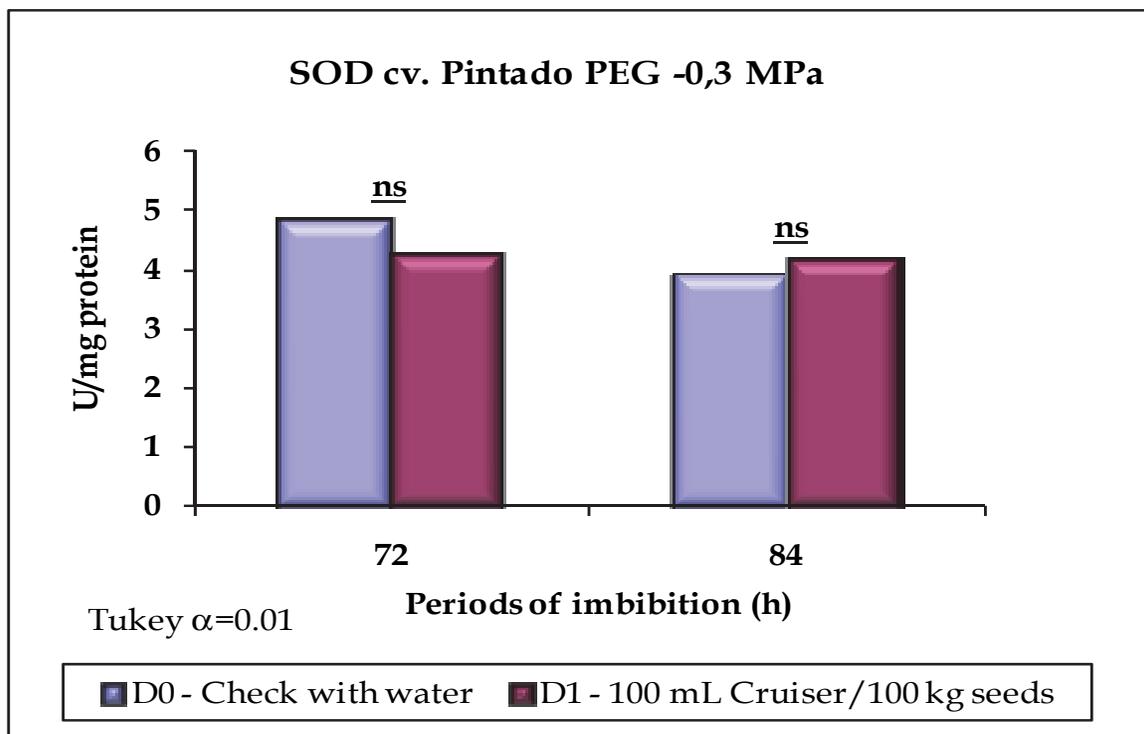
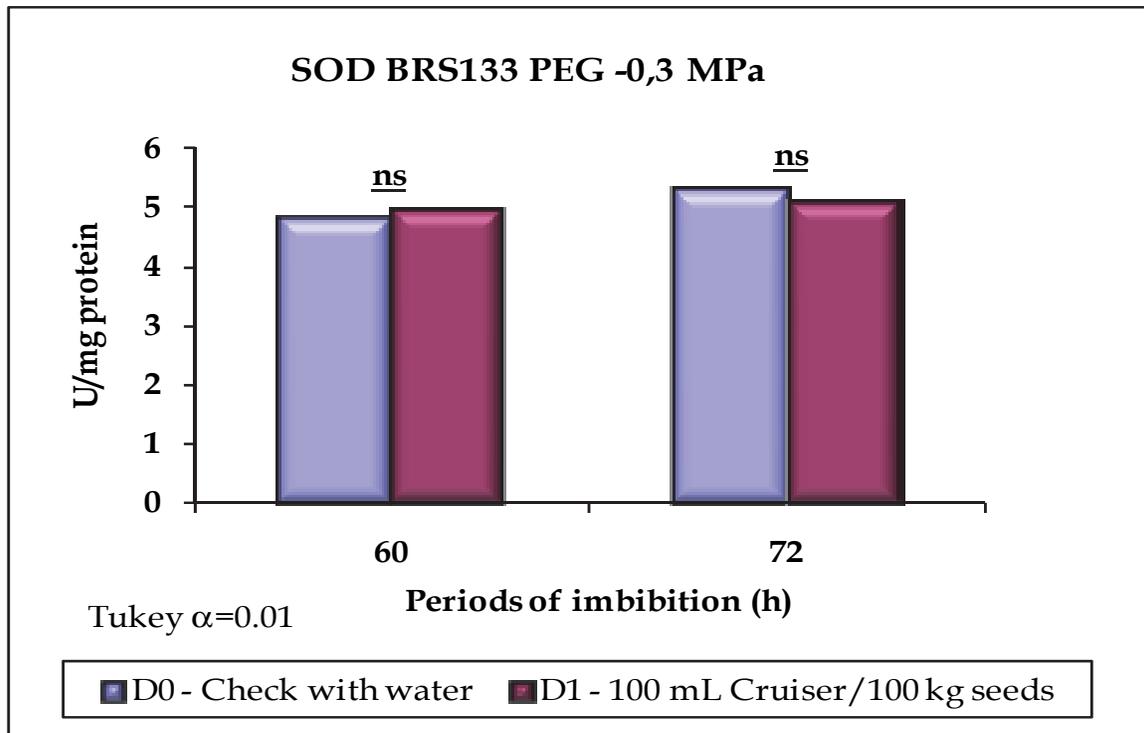


Fig. 29. Superoxide dismutase activity ($U\ mg\ prote\acute{a}na^{-1}$) in soybean seeds cv. BRS133 and Pintado treated at recommended dose of Cruiser (D1) and check (D0), under PEG. **ns**: not differ significantly for each imbibition period.

4. Discussion

Cruiser used as treatment for soybean seeds cultivars BRS 133 and Pintado, accelerated germination, the effect being more pronounced at twice the recommended level. Therefore, the Cruiser's action on the germination reduces the time for crop establishment in the field, reducing the negative effects of competition with weeds or essential nutrients in the soil.

Have been reported that seed germination and seedling development are delayed by high concentrations of aluminum (Matsumoto, 2000; Echart & Cavalli-Molina, 2001, Rout et al., 2001), salinity (Ashraf & McNeily, 1988; Hampson & Simpson, 1990; Ramoliya & Pandey, 2003, Soltani et al., 2004, Luo et al., 2005) and drought (Davidson & Chevalier, 1987; Passioura, 1988, Soltani et al., 2004).

According to Kochian (1995), Matsumoto (2000) and Rout et al. (2001) high aluminum concentrations inhibit root elongation, being proposed that the effect is due to inhibition of cell division, disjunction of cell wall, inhibition of ions flow, loss of membrane integrity and increased production of reactive oxygen species (ROS).

Aluminum causes a delay in germination of the two soybean cultivars in the control treatment and least in treatment with Cruiser, being more pronounced at higher concentrations of this heavy metal.

Salinity causes growth inhibition, being related to a decrease in extensibility of cell walls in the regions of root expansion (Neumann et al. 1994; Chazen et al., 1995), decreases the hydration of the seed (Allen et al. 1986), affects the physiological activities of the embryo due the toxicity of the absorbed ions (Khan et al., 1989), change the metabolism of carbohydrates (Corchete & Guerra, 1986), proteins (Ramagopal, 1990; Dell'Áquila & Spada, 1993) and nucleic acids (Gomes Filho et al., 1983). These changes make difficult to mobilize seed reserves, delaying the emergence of embryonic tissues, or even become non-viable seed (Rogers et al. 1995; Khan & Ungar, 1997).

NaCl causes a delay in germination but Cruiser reduces the negative effect of salinity on germination of soybean cultivar BRS 133, being more evident higher is the concentration of NaCl. To cultivar Pintado no answer was observed.

Cruiser has no effect on germination of soybean cultivar BRS 133 in conditions of drought, but in the cultivar Pintado, Cruiser accelerates germination being the effect more clear in situations of severe water stress.

The reduction on percentage of seeds germination in water stress conditions is attributed to lower diffusion of water through the integument. Water stress causes a prolongation of the stationary phase of the imbibition due to reduced enzyme activity and, consequently, a smaller meristematic development and delay on radicle protrusion (Falleri, 1994).

Seed germination and seedling development of various cultures decrease, influenced by conditions of low water availability, as reported by Owen (1972); Kiem & Krostad (1981), Davidson & Chevalier (1987); Passioura (1988); Soltani et al. (2004).

According to Soltani & Galeshi (2002) the decrease in germination and seedling development, as effect of environmental adversities, with consequent deficiency on crop establishment can cause: a) decreasing the competitiveness of the crop with weeds; b) less protection of soil surface and subsequently greater loss of soil water through evaporation and therefore, less available water for crop; c) lower light interception and yield potential.

It can also be considered that the loss in germination in situations of water stress might result in lower seedling development in the morning period, when the vapor pressure deficit is low and as result decreases CO₂ fixation (Tanner & Sinclair, 1983; Condon et al., 1993).

It was detected in the two soybean cultivars used on this study that Cruiser induced more development of the embryonic axis in presence of aluminum, salinity and water deficit, the effect being less evident with increasing of stress intensity.

The present results suggest it can be considered that Cruiser reduces the negative effects of stressful situations studied on germination of soybean seeds.

ROS generation during germination and root growth is generally accepted as an active physiological process, controlled in plant development (Chen & Schopfer, 1999; Schopfer et al., 2001), whose basal production is increased during conditions of biotic and abiotic stresses.

POD activity results indicate that Cruiser promotes this enzyme activity under stressful conditions, but has no effect on SOD activity during soybean germination under the same conditions.

According to Passardi et al. (2004), the peroxidases can be considered as bifunctional enzymes that can oxidize many substrates in H_2O_2 presence, but also produce ROS. They can promote cell elongation by ROS generation, or are involved in regulating H_2O_2 concentration, whose reactions cause restriction of growth.

Lin & Kao (2001) suggested that elevated production of H_2O_2 in rice roots during osmotic stress is probably involved in cell wall stiffening catalyzed by peroxidase, as explanation for the reduction of root growth. It was also suggested that the increase of peroxidase activity in situations of salinity and water stress induced inhibition of growth (Bacon et al. 1997; Lin & Kao, 2001).

The peroxidases can also participate in the lignification of new xylem elements in the embryo, hypocotyl, radicle and the hydroxyl radical ($\bullet OH$) produced by its action could help on the break of seed tegument and subsequent cell elongation (Passardi et al., 2004). Amaya et al. (1999), related that the increase on expression of peroxidase associated with cell wall caused higher rates of germination on tobacco seeds, for providing water retention under conditions of osmotic stress induced by NaCl.

Looking at the results of Cruiser's action on the induction of POD activity and compare it with the results of germination determined in the same periods of imbibition and stressful situations, can be generally considered that the increases in germination are related to increased activity of POD, which had one of two consequences:

- a) consumption of ROS originated in stressful situations, thereby preventing the damage caused by these molecules on the cell components and their metabolism or
- b) increased production of ROS, arising in situations of stress and for Cruiser's action, which would cause the stimulation of cell elongation, promoting greater radicle development.

As Cruiser had no effect on SOD activity, future work should be focused on investigating the action of the insecticide on other enzymes such as catalase, ascorbate peroxidase, glutathione peroxidase and lipoxygenase, participants of the enzymatic complex involved in protection against the oxidative stress triggered by the presence of aluminum, salinity and water deficit. It would also be of interest to investigate the action of Cruiser on activity of peroxidase associated with the cell wall, whereas in this study was determined only the total peroxidase.

5. Conclusions

Cruiser used in the treatment of soybean seeds cultivars BRS 133 and Pintado:

- accelerates the germination during the process of imbibition, and the effect is more pronounced at twice recommended level.

- induces further development of the embryonic axis, minimizing the negative effects in situations as presence of aluminum, salinity and water deficit.
- accelerates germination during the imbibition process in the presence of aluminum, being more evident in situations of greater concentration of this heavy metal.
- reduces the negative effect of salinity on germination during the imbibition process for cultivar BRS 133 and has no answer for the cultivar Pintado.
- accelerates germination of the cultivar Pintado under water deficit conditions, the effect being more pronounced with increased stress conditions and has no answer for cultivar BRS 133.
- accelerates germination, stimulates the activity of peroxidase, which can act both in consumption of ROS, preventing oxidative stress, as in the production of ROS, stimulating cell elongation.

6. References

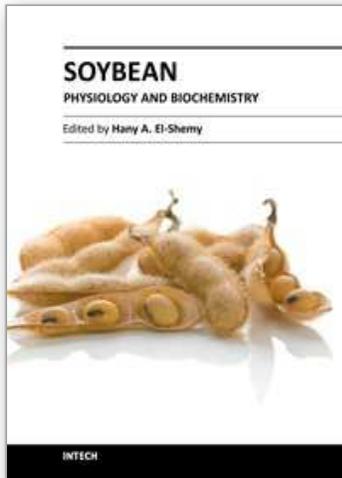
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soyfoods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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