

Germination Performance in Seeds and Initial Development of Forage Seedlings Under Different Concentrations of Salicylic Acid

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Abstract

Introduction: The use of *Urochloa* genre pastures in the forage industry is expanding, increasingly demanding high quality seeds. However, forage seed species commonly present dormancy and to overcome it an efficient and economically viable method is to soak seeds in acid, and among these, the salicylic acid may be a viable option. **Objective:** Thus, this study aimed to evaluate dormancy breaking in seed and early development of forage seedlings under different doses of salicylic acid. **Material and Methods:** The experiment was conducted in Dracena Campus of São Paulo State University - UNESP. The experimental design was completely randomized with 15 treatments and 4 repetitions of 20 seeds. Seeds of *Urochloa brizantha* cv. Marandu, *U. brizantha* cv. Xaraés and *U. ruziziensis* were immersed for 30 minutes in the salicylic acid levels: 0; 0.0025; 0.005; 0.01 and 0.02 mol/L and placed in gerbox boxes. Fifteen days after sowing, the following variables were evaluated: GSI - germination speed index; SG - Speed of germination; %G - Percentage of germination; LAP - Length of the air part; RL - Root length; DMAP - Dry mass of the air part and DMR - Dry mass of root. **Conclusion:** The use of salicylic acid at doses of 0.0025 and 0.02 mol/L increased the germination of both cultivars of *Urochloa brizantha* Xaraés and *Urochloa ruziziensis*. The germination of *Urochloa brizantha* cv. Marandu was not influenced by the application of salicylic acid. The 0.005 mol/L dose of salicylic acid resulted in higher initial growth and higher dry matter deposition in all studied species.

Key words: *Urochloa brizantha*, *Urochloa ruziziensis*, Marandu, Xaraés.

INTRODUCTION

High-quality seeds, with high germination power, are fundamental to the pasture establishment, however, in several species of tropical grasses is remarkable the presence of dormancy in the freshly harvested seeds, which is associated with the physiological causes (Usberti and Martins, 2007; Costa, 2011).

Dormancy represents one of the main strategies used by plant species in order to achieve high survival rates and the establishment of young plants. In plants of the genus *Urochloa*, short term dormancies are related to the embryo, while the long terms ones are imposed by wraps, usually preventing high germination percentage (Sallam and Ibrahim, 2015; Heidari *et al.* 2015). This physiological phenomenon hampers the establishment of uniform populations and at the same time, promotes the emergence of invasive plants on pasture (Malik and Silva, 2003).

According to Carneiro (1994), in several species of tropical grasses the presence of numbness in the freshly harvested seeds is remarkable, and, as described by some authors, the treatment with hormones can influence the reducing of the dormancy intensity and, therefore, the methods for overcoming this mechanism have been continuously studied, in order to facilitate its implementation quickly and efficiently (Oliveira and Mastrocola, 1983; Kabori *et al.*, 2013; Ozpinar *et al.* 2017).

Salicylic acid (SA) can be used as a regulator to perform dormancy breaking or even as germination promoter in grasses, its endogenous use may influence different process in plants, including seeds germination and dormancies related to embryo (Heidari *et al.* 2015). According to Sallam and Ibrahim (2015), this compounding also acts on oxidative stress at germination process of seedlings, in which concentrations above 1,2 g/L promote synthesis of catalase (CAT), chlorophyll A and B and peroxidase (POD), while SA doses below 0,6 g/L promote an increase in yield of carotenoids, by acting as defense mechanism of the plant against pathogenic agents Yang *et al.* (2016).

SA acts in physiology of oxidative stress and, with α -amylase enzyme, on the degradation of the starch present in the cotyledons endosperm, initiating the seed germination process (Maia *et al.*, 2000, Silva *et al.*, 2014). Besides, by acting with others hormones, SA may collaborate with cell expansion in roots, which allows a more efficient substrate fixation (Taiz and Zeiger, 2013).

In this sense, this study aimed to evaluate dormancy breaking in seed and early development of forage seedlings under different doses of salicylic acid

MATERIAL AND METHODS

Installing the experiment:

The experiment was carried out in July, 2015, in Laboratory of Vegetal Morphophysiology and Forages of College of Agricultural and Technological Sciences, São Paulo State University (Unesp), Dracena, Brazil.

The experimental design was completely randomized, in a factorial scheme of 3x5, in which the first factor was composed by *Urochloa* seeds: *Urochloa brizantha* cv. Marandu, *U. brizantha* cv. Xaraés and *U. ruziziensis*, obtained from a company in the Dracena region, and the second factor was the SA doses: 0; 0.0025; 0.005; 0.01 and 0.02 mol / L, with 4 replicates. Fifty viable seeds of forages were selected to compose each replicate. The seeds were stored in gerbox boxes, containing a germitex paper moistened with 2.5 times its mass with distilled water, following the model of Vieira *et al.* (1998).

Then, the boxes were stored in a BOD, with 12 hrs photoperiod (darkness/light) under a constant 25°C temperature.

Destructive and non-destructive assessments:

Non-destructive:

After 15 days of sowing were evaluated: germination rate (TG), being considered germinated caryopses that presented radicle protrusion (Mott *et al.* 1976), not being considered its length (mm); speed of germination (SG), set from mathematical equation proposed by Edmond and Drapala (1958):

$$SG = \frac{(N1G1)+(N2G2)+\dots+(NnGn)}{(G1+G2+\dots+Gn)}$$

In which: N1 is the number of days for the first counting, G1 is the number of emerged seedlings at the first counting, N2 is the number of days at the second counting, G2 is the number of emerged seedlings at the second counting; Nn is the number of days at the last counting, Gn is the number of emerged seedlings at the last counting; index of speed of germination (ISG), set from the mathematical equation proposed by Maguire (1962):

$$ISG = \frac{G1}{N1} + \frac{G2}{N2} + \dots + \frac{Gn}{Nn}$$

In which: G1, G2, Gn are the numbers of germinated seedlings in each counting; N1, N2, Nn are the numbers of days from sowing at each counting.

Destructive:

Length of the air part (LAP) and root length (RL) were set by using a millimeter ruler; dry matter of aerial part (DMAP) and dry matter of root (DMR) were obtained by grams, through the drying of the material

Statistical analyzes:

All variables were submitted to the F test ($p < 0.05$) and the regression analysis was applied to the SA doses, in which their models were tested: linear, quadratic and cubic (Banzatto and Kronka, 2013), by using Assisat 7.7 static software (Silva and Azevedo, 2016).

RESULTS AND DISCUSSIONS

Analysis of variance:

Chart 1 displays values of variance analysis of the evaluated variables submitted to different SA concentrations.

Chart 1: Analysis of variance of 7 production characteristics evaluated in the culture *Urochloa* submitted to concentrations of salicylic acid.

Forage	FV	Middle Square							
		GL	ISG	SG	%G	LAP	RL	DMAP	DMR
Marandu	Concentration	4	1.775	0.589	121.87	0.125	0.369	0.00001	0.00001
	Residue	15	0.902	0.456	79.166	0.260	0.170	0.00001	0.00001
	Regression	1	Ns	Ns	Ns	Ns	Ns	Ns	Ns
	CV (%)	-	21.15	16.37	12.27	11.18	10.54	12.93	16.85
Xaraés	Concentration	4	0.489	2.633	64.375	1.490	3.290	0.00008	0.00002
	Residue	15	0.236	0.497	77.089	0.266	0.239	0.00001	0.00000
	Regression	1	Ns	Q**	Ns	L**	L**	Q**	L**
	CV (%)	-	16.51	12.18	13.15	11.48	9.67	13.39	19.04
Ruziziensis	Concentration	4	0.462	0.031	83.125	0.317	1.405	0.00002	0.00001
	Residue	15	0.425	0.052	37.500	0.210	0.342	0.00001	0.00000
	Regression	1	Ns	Ns	L*	Ns	L**	L**	L**
	CV (%)	-	8.29	9.31	6.84	8.41	9.64	9.90	10.22

Ns- $p > 0.05$; * $0.01 < p < 0.05$; ** $p < 0.01$. L – polynomial of 1st degree. Q – polynomial of 2nd degree. CV – Coefficient of variation. GSI – germination speed index; SG – Speed of germination; %G – Percentage of germination; LAP – Length of the air part; RL – Root length; DMAP – Dry mass of the air part; DMR – Dry mass of root. Source: Research data, 2016.

Non-destructive assessments:

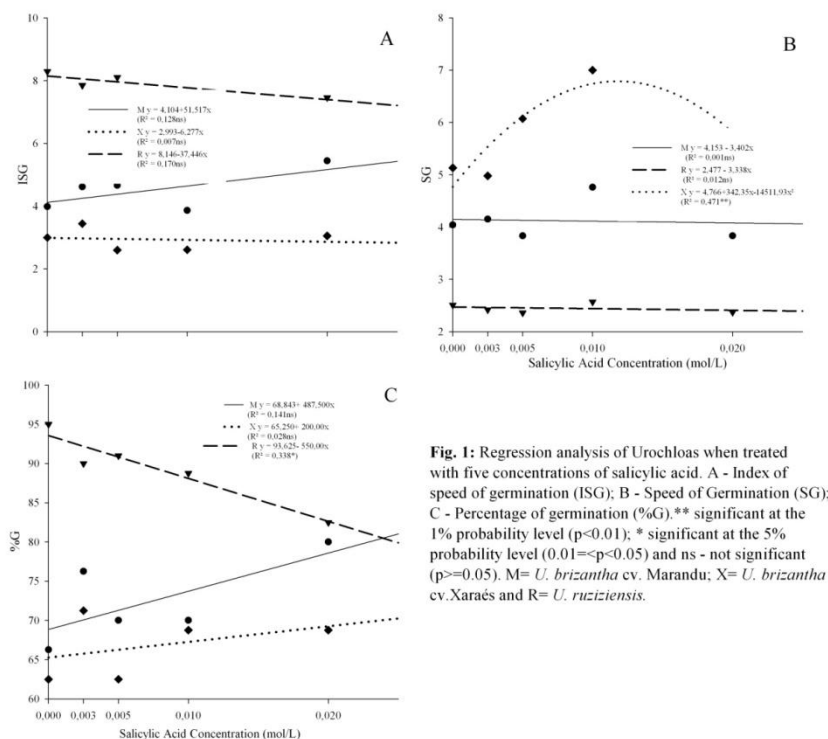
Figure 1A shows germination speed index in the three different species of *Urochloa* treated with SA. *Urochloa brizantha* cv. Marandu and cv. Xaraés presented a decrease in the germination speed from 0,005 mol/L dose, repairing its growth in values till reach the final 0,02 mol/L dose. In general, greater SA doses resulted in an increase on germination speed index. To *Urochloa ruziziensis*, the trend was inversely proportional between the used dose and analyzed variable, being represented by the lowest values at 0,01 and 0,02 mol/L doses.

Speed of germination (Figure 1B) of species *U. brizantha* cv. Marandu and *U. brizantha* cv. Xaraés displayed increasing trend at 0,00mol/L doses and decay to the highest dose, 0,02 mol/L, being the lowest doses, 0; 0,0025 e 0,005 mol/L, the ones that displayed intermediate values. The speed of germination to *U. ruziziensis* was steady at all studied doses. All species showed, at some dose, values higher than the control.

Percentage values to germination rate are available in Figure 1C. *U. ruziziensis* presented the highest rate among the studied species in all doses of SA, with directly proportional decreasing values, resulting in greater rates at smaller doses. In *U. brizantha* cv Xaraés, it was observed directly proportional increasing values, with the highest values at the greater doses, as Marandu cultivar obtained steady and less elevated values than the other ones.

Zanet (2011) found out, by studying the use of SA in *Urochloa humidicola*, that youngest the lot of seeds lead to greater germination rate, the author also report that seeds stored for a longer-term may suffer a increasing in germination rate as submitted to SA.

Marandu displayed an unlike behavior as compared to *U. ruziziensis* and obtained the highest trend in germination rate at 0,02mol/L dose, which oppositely occurred in Xaraés, the forage with the lowest germination rate, with its development curve reach its peak at 0,0025 mol/L dose. Lacerda *et al.* (2010) also found out values higher than 70% of germination by treating *U. brizantha* cv. Marandu seeds with others dormancy breaking methods.



Destructive assessments:

Regarding to the growth of seedling, 15 days after the sowing, Figure 2 shows values of length of the aerial part.

Urochloa ruziziensis displayed the greater length in all analyzed doses. All studied species presented a bigger growth at 0,005 mol/L as compared to the control, as *U. ruziziensis* e *U. brizantha* cv. Xaraés showed a decreasing trend in shoot length at other tested doses, Marandu presented an increase on seedlings growth at the highest dose, 0,02mol/L.

As well as the shoot length data (Figure 2A), root length ones presented a greater value at 0,005mol/L doses as compared to the control. Regarding to root length, all the species presented decreasing trends as the doses were increased. *U. ruziziensis* presented a greater growth in its roots as compared to others studied species.

Dry mass of the aerial part (Figure 3A) follows the same trend on shoot length (Figure 4), in which 0,005mol/L doses presented values greater than control.

However, at 0,02mol/L doses it was detected an inversion in species dormancy, and, differently of the shoot length results, *U. ruziziensis* presented lowest values of mass as compared to the others, indicating a higher tall with no relation with mass deposition. It may occurred due to the greater *U. ruziziensis*'s growth rate as compared to the others studied species, corresponding to a lower initial deposition of dry mass. Pacheco *et al.* (2010) reached similar results by studying different species of tropical forages and found taller plants to *U. ruziziensis* than *U. brizantha*, being correspondent to this study.

Likewise, by comparing the two variables, the species *U. ruziziensis* and *U. brizantha* cv. Marandu maintained their decreasing trend lines with an increase in the last dose. A *U. brizantha* cv. Xaraés was the only species that presented greater mass deposition in the aerial part at the 0,02mol/L dose, as compared to the growth in centimeters.

Unlike the observed in aerial part, data analyses regarding the dry mass of root (Figure 3B) on *U. ruziziensis* suggest growth in size matching dry mass deposition, since both variables in this specie present higher values as compared to others species. Pacheco *et al.* (2010) and Menezes and Leandro (2004) also found more prominent radicle growth in *U. ruziziensis*.

When Heidari *et al.* (2015) studied the effect of nitrogen along with the AS action on pinto beans, which verified the opposite of this work, since the crop showed an increase in its dry mass. They reported that the association between these factors favored the chlorophyll indexes and even the grains production. Najjari *et al.* (2015) reported that SA provided an increase between 7.0 and 10.0% in forage mass production in corn as cultivated under nitrogen fertilization.

Both *Urochloa* species showed greater values of mass deposition in roots at 0,005 mol/L as compared to control. In general, obtained data matches the expected, based on charts presentation of others analyzed data. Studies carried out by Ozpinar *et al.* (2017) corroborate the results, since the authors reported that a decrease occurred in the root development as the SA concentration increased.

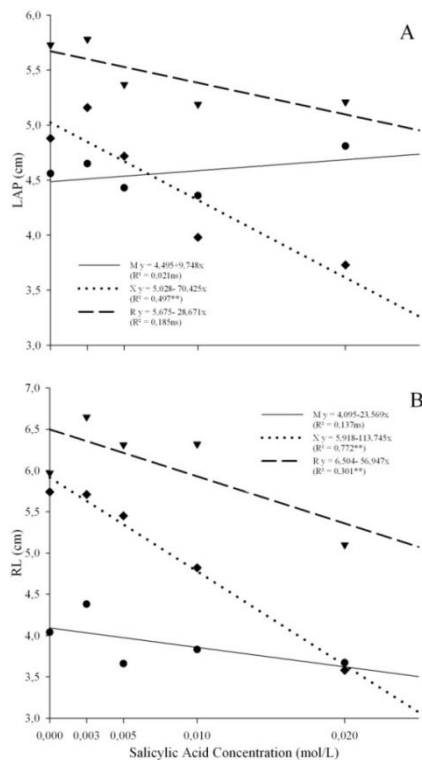


Fig. 2: Regression analysis of Urochloas when treated with five concentrations of salicylic acid. A - Length of the air part (LAP); B - Root length (RL). ** significant at the 1% probability level ($p < 0.01$); *significant at the 5% probability level ($0.01 < p < 0.05$) and ns – not significant ($p > 0.05$). M= *U. brizantha* cv.Marandu; X= *U. brizantha* cv.Xaraés and R= *U. ruziziensis*.

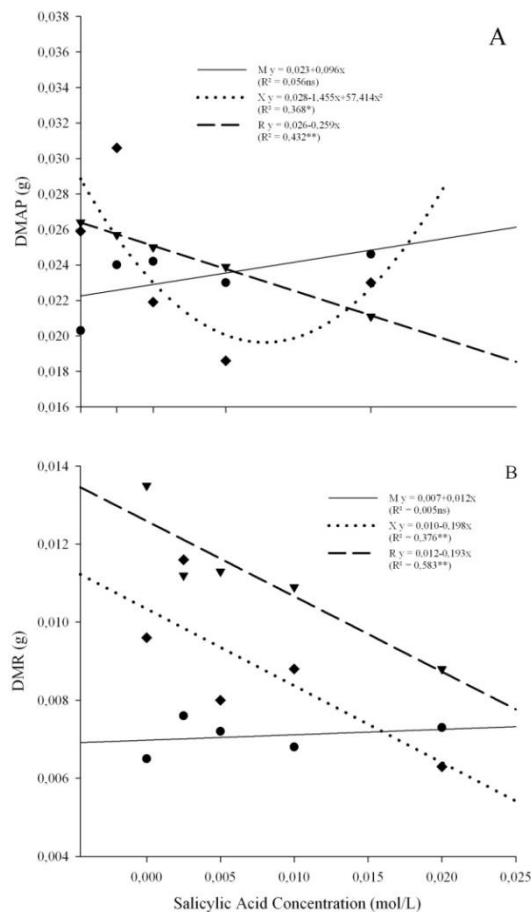


Fig. 3: Regression analysis of Urochloas treated with five concentrations of salicylic acid. A - Dry mass of the aerial part (DMAP); B - Dry mass of root (DMR). **significant at the 1% probability level ($p < 0.01$); *significant at the 5% probability level ($0.01 < p < 0.05$) and ns – not significant ($p > 0.05$). M= *U. brizantha* cv.Marandu; X= *U. brizantha* cv.Xaraés and R= *U. ruziziensis*.

Conclusion:

The use of salicylic acid at doses of 0.0025 and 0.02 mol/L increased the germination of both cultivars of *Urochloa brizantha* Xaraés and *Urochloa ruziziensis*.

The germination of *Urochloa brizantha* cv. Marandu was not influenced by the application of salicylic acid.

The 0.005 mol/L dose of salicylic acid resulted in higher initial growth and higher dry matter deposition in all studied species.

REFERENCES

- Banzatto, D.A., S.N. Kronka, 2013. Experimentação Agrícola. 4.Ed. Funep, 237.
- Carneiro, J.W.P., 1994. Influência da temperatura na porcentagem de germinação de sementes de *Urochloa brizantha* Stapf, cv. Marandu. *Revista Brasileira de Sementes*, 16(2): 183-186. (in Portuguese) <https://doi.org/10.17801/0101-3122/rbs.v16n2p183-186>.
- Costa, C.J., R.U. Araújo, H.D.C. Villas Bôas, 2011. Treatments for seed dormancy release in *Brachiaria humidicola* (Rendle) Schweick. *Pesquisa Agropecuária Tropical*, 41(4): 519-524. (in Portuguese) <http://dx.doi.org/10.5216/pat.v41i4.15100>.
- Edmond, J.U., W.J. Drapala, 1958. The effects of temperature, sand and soil, and acetone on germination of okra seed. *Proceedings of the American Society Horticultural Science*, 71: 428-434.
- Heidari, A., F. Shekari, J. Saba, G. Shahidi, 2015. The effect of seed priming with salicylic acid on growth and grain yield of pinto bean under nitrogen levels. *International Journal of Biosciences*, 6(1): 298-307. <http://dx.doi.org/10.12692/ijb/6.1.298-307>.
- Kobori, N.N., G.M. Mascarin, S.M. Cicero, 2013. Métodos não sulfúricos para superação de dormência de sementes de mucuna-preta (*Mucuna aterrima*). *Informativo Abrates*, 23(1): 25-32. (in Portuguese)
- Lacerda, M.J.R., J.S.R. Cabral, J.F. Sales, K.R. Freitas, A.J. Fontes, 2010. Seed dormancy-breaking of *Brachiaria brizantha* cv. Marandu. *Semina: Ciências Agrárias*, 31(4): 823-828. (in Portuguese) <http://dx.doi.org/10.5433/1679-0359.2010v31n4p823>.
- Maguire, J.D., 1962. Speed of germination - Aid in selection and evaluation for seedling emergence vigor. *Crop Science*, 2(1): 176-177. <http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x>
- Maia, F.C., D.M. Moraes, R.C.P. Moraes, 2000. Ácido salicílico: efeito na qualidade de sementes de soja. *Revista Brasileira de Sementes*, 22: 264-270.
- Martins, L., W.R. Silva, 2003. Immediate and latent effects of thermal and chemical treatments on seeds of *Brachiaria brizantha* cv. Marandu. *Bragantia*, 62(1): 81-88. (in Portuguese) <http://dx.doi.org/10.1590/S0006-87052003000100011>.
- Menezes, L.A.S., W.M. Leandro, 2004. Biomass from different ground cover species with potential for use in a no-tillage system. *Pesquisa Agropecuária Tropical*, 34(3): 173-180. (in Portuguese)
- Mott, J.J., G.M. Mckeon, C.J. Moore, 1976. Effects of seed bed conditions on the germination of four *Stylosanthes* species in the Northern Territory. *Australian Journal Agriculture Research*, 27: 811-823. <https://doi.org/10.1071/AR9760811>.
- Najjari, S., Seghatoleslami, M., Mousavi, G., 2015. The effect of drought stress, salicylic acid and nitrogen on yield and yield components of forage maize. *International Journal of Life Sciences*, 9(6): 72-74. <https://doi.org/10.3126/ijls.v9i6.12741>.
- Oliveira, P.R.P., M.A. Mastrocola, 1983. *Brachiaria Humidicola* (Rendle) Schwickerdt: Observations about its seed viability. *Boletim de Indústria Animal*, 40(1): 49-53. (in Portuguese) disponível em: <http://www.iz.sp.gov.br/pdfs/bia/1380632384.pdf>.
- Ozpinar, H., S. Dag, E. Yigit, 2017. Alleopathic effects of benzoic acid, salicylic acid and leaf extract of *Persica vulgaris* Mill. (Rosaceae). *South African Journal of Botany*, 108: 102-109. <http://dx.doi.org/10.1016/j.sajb.2016.10.009>.
- Pacheco, L.P., F.R. Pires, F.P. Monteiro, S.O. Procópio, R.L. Assis, F.A. Petter, 2010. Depth of sowing and initial growth of forage species used for soil coverage. *Ciência e Agrotecnologia*, 34(5): 1211-1218. (in Portuguese) <http://dx.doi.org/10.1590/S1413-70542010000500019>.
- Sallam, A.M., H.I.M. Ibrahim, 2015. Effect of grain priming with salicylic acid on germination speed, seedling characters, anti-oxidant enzyme activity and forage yield of teosinte. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 15(5): 744-753. <http://dx.doi.org/10.5829/idosi.aejas.2015.15.5.12616>
- Silva, T.C.F.S., R.C.B. Silva, J.E.S.B. Silva, R.S. Santos, C.A. Aragão, B.F. Dantas, 2014. Regulators and protectors vegetable seed germination of watermelon. *Scientia Plena*, 10(3): 1-15. (in Portuguese)
- Silva, F.S., C.A.V. Azevedo, 2016. The Assisat Software Version 7.7 and its use in the analysis of experimental data. *African Journal Agriculture Resarch*, 11(39): 3733-3740. <http://dx.doi.org/10.5897/AJAR2016.11522>
- Taiz, L., E. Zeiger, 2013. *Fisiologia vegetal*. 5. ed. Porto Alegre: Artmed, 918.
- Usberti, R., L. Martins, 2007. Sulphuric acid scarifi cation effects on *Urochloa brizantha*, *U. humidicola* and *Panicum maximum* seed dormancy release. *Revista Brasileira de Sementes*, 29(2): 143-147. <http://dx.doi.org/10.1590/S0101-31222007000200020>.
- Vieira, H.D., R.F. Silva, R.S. Barros, 1992. Superação da dormência de sementes de *Urochloa brizantha* (Hochst.ex A.Rich) Stapf cv. Marandu submetidas ao nitrato de potássio, hipoclorito de sódio, tiouréia e etanol. *Revista Brasileira de Sementes*, 20(2): 44-47.
- Yang, C., L.Y. Hu, B. Ali, F. Islam, Q.J. Bai, X.P. Yun, K. Yoneyama, W.J. Zhou, 2016. Seed treatment with salicylic acid invokes defence mechanism of *Helianthus annuus* against *Orobanche Cumana*. *Annals of Applied Biology*, 169: 408-422. <https://doi.org/10.1111/aab.12311>.
- Zanet, C., 2011. Ácido salicílico em sementes de *Urochloa humidicola* submetidas a estresse hídrico. 29f. Dissertação (Mestrado em Agronomia) – Universidade do Oeste Paulista, UNOESTE: Presidente Prudente.