RESEARCH NOTE

Treatment of *Solanum torvum* seeds improves germination in a batch-dependent manner[1]

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ABSTRACT

The *Solanum torvum* species can grow in soils with a heavy load of nematodes and pathogenic fungi. It is currently much in demand in intensive agriculture as a rootstock of Solanaceae species, such as eggplant and tomato. This study aimed at comparing treatments, in order to determine the best method to accelerate the germination of *S. torvum* seed batches. Three seed batches were submitted to four treatments to overcome dormancy (water, potassium nitrate, gibberellic acid and pre-imbibition in gibberellic acid). The first germination count, germination percentage, germination speed index, mean germination time and mean germination speed were assessed. Treatments with gibberellic acid, with either pre-imbibition or only moistened substrate, exhibited the best germination speed index, mean germination time and mean germination speed. The final germination percentage showed a significant interaction between treatments and seed batches. Therefore, the treatments affect the final germination in a batch-dependent manner.

KEYWORDS: Solanaceae; dormancy breaking; gibberellic acid; potassium nitrate.

RESUMO

Tratamento de sementes de *Solanum torvum* melhora a germinação de maneira dependente do lote

A espécie *Solanum torvum* consegue desenvolver-se em solos com forte carga de nematoides e fungos patogênicos. Atualmente, é muito demandada na agricultura intensiva como porta-enxerto de espécies de Solanaceae, tais como berinjela e tomate. Objetivou-se comparar tratamentos, buscando o melhor método para acelerar a germinação de lotes de sementes de *S. torvum*. Três lotes de sementes foram submetidos a quatro tratamentos para superação de dormência (água, nitrato de potássio, ácido giberélico e pré-embebição em ácido giberélico). Avaliaram-se a primeira contagem de germinação, porcentagem de germinação, índice de velocidade de germinação, tempo médio de germinação e a velocidade média de germinação. Os tratamentos com ácido giberélico, com pré-embebição ou apenas com o substrato umedecido, apresentaram os melhores índices de velocidade de germinação, tempo médio de germinação e velocidade média de germinação. A porcentagem final de germinação apresentou interação significativa entre tratamentos e lotes de sementes. Portanto, os tratamentos afetam a germinação final de maneira dependente do lote.

PALAVRAS-CHAVE: Solanaceae; quebra de dormência; ácido giberélico, nitrato de potássio.

The *Solanum* genus is a hyperdiverse taxon. There are around two thousand *Solanum* species worldwide, distributed primarily in tropical and subtropical areas, with a small portion in temperate zones (Edmonds & Chweya 1997).

The *Solanum torvum* species is native to Latin America. It is shrubby, reproduces by seeds and is dispersed mainly by birds that feed on its berries. It is widely distributed in Pakistan, India, Malaysia, China, Philippines and tropical America (Zakaria & Mohd 1994). The species is used both in the pharmacological and agronomic areas, but is little studied and has no methodological description rules for seed testing in Brazil (Brasil 2009).

The species is highly vigorous, rustic, wild and known in many equatorial countries as an invader capable of colonizing poor and inhospitable zones. Due to its robust root system, it manages to develop in soils with a heavy load of nematodes and pathogenic fungi, thus recently becoming much in demand in intensive agriculture as a rootstock of Solanaceae species, such as eggplant and tomato (Miceli et al. 2014, Scrimali 2014).

In southern Croatia and part of Montenegro, *S. torvum* is used in around 70 % of protected crops

with positive results, when compared to nongrafted eggplant (*Solanum melongena*), but grown in soils disinfested with methyl bromide. The species provides a good economic and environmental advantage, since the rootstock vigor allows a biannual eggplant cropping, with significantly lower planting costs and a considerable increase in agricultural sustainability (Scrimali 2014).

This species has also been widely exploited for its chemical constituents. Several parts (fruits, leaves and roots) are used to isolate a vast array of compounds. Its aqueous extracts inhibit pathogenic fungi such as *Pyricularia oryzae*, *Alternaria alternata*, *Trichoconiella padwickii*, *Fusarium oxysporum* and *Fusarium solani* (Jaiswal 2012). In pharmacological studies, several Solanaceae species, including *S. torvum*, have shown hypotensive action in the cardiovascular system (Batitucci 2003).

The main limitation for the practical use of *S. torvum* as a rootstock in the commercial production of grafted eggplant, as well as in genetic breeding programs, is the poor and irregular germination caused by seed dormancy (Ginoux & Laterrot 1991, Miura et al. 1993, Gousset et al. 2005, Hayati et al. 2005).

Among the procedures that may increase seed germination is seed imbibition in water or solutions capable of promoting growth, whether by immersion or simply with moistened substrate (Rosseto et al. 2000). The use of potassium nitrate (KNO_3), reported as one of the primary agents to overcome dormancy in numerous species, may cause structural changes in the seeds, decreasing water absorption by the pericarp, thereby increasing germination (Faron et al. 2004). Gibberellins, in turn, play a key role in regulating germination. As endogenous enzyme activators, they are involved in both dormancy breaking and reserve hydrolysis control (Soares et al. 2009).

Methods that make germination more regular and predictable are necessary in production systems that use rootstocks, for synchrony between the production of seedlings to be grafted and that of rootstocks. Thus, this study aimed at comparing treatments that improve the germinative parameters of *S. torvum* seed batches, in order to facilitate and accelerate the rootstock production.

The study was conducted between April and July 2014, at the University of Bologna, in Bologna, Italy.

The experimental design was completely randomized, in a 3 x 4 factorial scheme. Three *S. torvum* seed batches were assessed in storage and submitted to four treatments: substrate moistening with water (H_2O), potassium nitrate (0.2 % KNO_3) and gibberellic acid (0.05 % GA_3) and seed imbibition with gibberellic acid for 24 h and subsequent planting in substrate moistened with GA_3 (imbibition with 0.05 % GA_3).

A total of fifty *S. torvum* seeds were sown per plate (containing 3 ml of the respective treatment), with three replications per treatment. The substrate used was germination-specific filter paper. The seeds were incubated in chambers with a controlled environment, under 16 h of light at 20 ºC and 8 h of dark at 30 ºC. The Petri dishes were randomly disposed inside the chamber and rotated daily.

Germination count occurred daily up to 35 days after sowing (DAS), when the experiment was finalized. Seeds were considered germinated when they exhibited root protrusion of more than 2 mm. The following variables were calculated: first germination count: conducted at 7 DAS by counting the number of seeds with root protrusion; germination (G): calculated by the formula $G = (N/50) \times 100$, where N = number of germinated seeds at the end of the test (Labouriau & Valadares 1976), with results expressed in percentage; germination speed index (GSI): calculated by the formula $GSI = \sum (ni/ti)$, where ni = number of seeds that germinated in time i and ti = time after starting the test, with $i = 1 \rightarrow 35$ days (Maguire 1962), dimensionless; mean germination time: calculated by the formula $MGT = (\sum ni\ ti)/\sum ni$, where ni = number of seeds germinated per day and ti = incubation time, with $i = 1 \rightarrow 35$ days (Labouriau & Valadares 1976), in days; mean germination speed (MGS): calculated by the formula $MGS = 1/t$, where t = mean germination time (Kotowski 1926), in days.

The data were submitted to analysis of variance, using the F-test. Data that did not fit some Anova assumption were transformed to $(x + 1)^{0.5}$. If significant, the averages of the treatments were compared by the Tukey test at 5 %.

The interaction batch x treatment was significant for all the germination parameters analyzed. The analysis for first germination count showed a significant difference among treatments only for batch 3 (Table 1), where seeds pre-imbibed in gibberellic acid for 24 h exhibited the largest number of germinated seeds at 7 DAS. When GA_3 was used

only to moisten the substrate, the response differed statistically from the other treatments, being only lower than the treatment involving pre-imbibition of seeds in gibberellic acid. This response shows the marked effect of gibberellic acid in the germination process, activating hydraulic enzymes that are active in deploying reserve substances.

The effects of GA_3 on germination depend largely on the difference in physiological conditions among seeds caused by their ripening, post-ripening and aging conditions (Suzuki & Takahashi 1968). The positive response of GA_3 observed only in batch 3 is possibly due to its better physiological condition, when compared to the others. This could occur due to the larger amount of reserves accumulated in the seeds. Seed size was not measured in this study.

The final germination percentage at 35 DAS did not differ statistically among treatments for batches 1 and 3 (Table 2). However, there was a difference among treatments for batch 2, which exhibited a larger number of germinated seeds when treated with 0.2 % potassium nitrate, not differing statistically from the treatment with substrate moistened with GA_3. Lower germination percentages at the end of the assessments were obtained for treatments with water and pre-imbibition with GA_3.

In a study with *S. torvum*, Ranil et al. (2015) found that treatments with GA_3 and KNO_3, among others, such as immersion in water for 24 h and light irradiation, have highly positive effects on germination stimulation. Similarly, applications of GA_3 or KNO_3 were also efficient for other *Solanum* species (Hayati et al. 2005, Wei et al. 2010, Gisbert et al. 2011).

The germination speed index was higher for treatments with GA_3, whether only in the substrate or in pre-imbibition for batches 1 and 2 and only in pre-imbibition for batch 3. Similarly, studies with *Genipa americana* L. seeds pre-imbibed in liquid gibberellin (4 % GA_3) for 12 h obtained a higher germination speed index, when compared to pre-imbibition in water (Prado Neto et al. 2007). The germination speed index of potassium nitrate did not differ from the standard treatment with water. In some species, moistening seeds with potassium nitrate do not produce significant effects on dormancy breaking (Martins et al. 2012).

Although KNO_3 is widely used in laboratories to overcome dormancy, its use is recommended mostly in species whose coats are impermeable to gases, since it is believed that the contact with substances in the pericarp decreases resistance and facilitates gas exchanges (Frank & Nabinger 1996). Applying KNO_3 may accelerate water and oxygen capture, as well as improve the nutritional status of seeds (McIntyre et al. 1996).

Hayati et al. (2005) observed that low concentrations of KNO_3 (0.1 %) were efficient in breaking the *S. torvum* dormancy, and that germination percentages declined significantly with an increase in KNO_3. The positive effects of this chemical substance are not always observed, because it decreases the osmotic pressure of the substrate, in relation to the seeds, thereby precluding imbibition (Xia & Kermode 2000).

Table 1. First germination count (%) of three *Solanum torvum* seed batches submitted to different treatments.

Treatment	Batch 1	Batch 2	Batch 3	Mean
Water	0.000 Aa	0.000 Aa	0.000 Ca	0.000
0.2 % KNO_3	0.000 Aa	0.000 Aa	0.000 Ca	0.000
0.05 % GA_3	0.000 Ab	0.000 Ab	1.333 Ba	0.444
Imbibition with GA_3	0.000 Ab	0.000 Ab	12.000 Aa	4.000
Mean	0.000	0.000	3.333	
CV (%)		12.03		

Means followed by the same lower case letter do not differ horizontally, while those followed by the same upper case letter do not differ vertically. Data were transformed by the formula $(x + 1)^{0.5}$.

Table 2. Germination percentage and germination speed index of three *Solanum torvum* seed batches submitted to different treatments.

Treatment	Germination percentage				Germination speed index			
	Batch 1	Batch 2	Batch 3	Mean	Batch 1	Batch 2	Batch 3	Mean
Water	86.000 Ab	79.000 Bc	95.000 Aa	87.000	25.168 BCb	24.996 Bb	55.019 Ca	35.061
0.2 % KNO_3	81.000 Ac	89.000 Ab	97.000 Aa	89.000	22.570 Cc	26.760 Bb	55.844 Ca	35.058
0.05 % GA_3	85.000 Ab	85.000 ABb	96.000 Aa	89.000	26.700 ABc	31.359 Ab	61.318 Ba	39.792
Imbibition with GA_3	80.000 Ab	81.000 Bb	95.000 Aa	85.000	28.739 Ac	32.140 Ab	69.320 Aa	43.400
Mean	83.000	83.000	96.000		25.794	28.813	60.375	
CV (%)		3.99				3.92		

Means followed by the same lower case letter do not differ horizontally, while those followed by the same upper case letter do not differ vertically.

The exposure of *S. torvum* seeds to gibberellic acid decreased the mean germination time for the three batches assessed, that is, fewer days were needed between the first and last germinated seed (Table 3). The treatment with pre-imbibition of seeds in GA$_3$, for two of the batches assessed, required fewer days for germination. Treatments with water and potassium nitrate were not statistically different, but exhibited higher mean germination time values, if compared to treatments with GA$_3$. Studies with *Solanum betaceum* indicated no statistical difference for mean germination time with the use of gibberellic acid, when compared to hydro-priming (Kosera Neto et al. 2015).

Mean germination speed data corroborate those observed for mean germination time. The treatment with pre-imbibition in GA$_3$ displayed higher mean germination speed for two of the batches assessed. However, for the third batch, this treatment did not differ statistically from the use of GA$_3$ only in the substrate. Batch 3 showed greater physiological potential, given that lower mean germination time and higher germination speed index and mean germination speed values were observed, when compared to the other batches.

By comparing batches, it was observed that batch 3 obtained a higher germination percentage, germination speed index and mean germination speed, and lower mean germination time than the other batches (Table 3), irrespective of treatment. The superiority of batch 3 may be associated with a greater physiological vigor. Vigor influences all germinative aspects, particularly characteristics such as speed, uniformity and mass of emerged seedlings (Carvalho & Nakagawa 2000).

The use of gibberellins in the germination phase may improve seed vigor and germination in a number of species, as observed here for *S. torvum*, but they become more important when the seeds are under adverse conditions (Ferreira et al. 2005, Lopes & Sousa 2008). However, gibberellins accelerate the germination and emergence of several species, while for others they promote a slight or no response (Soares et al. 2009). Studies with GA$_3$ in *Coffea arabica* L. seeds *in vitro* showed that this regulator do not contribute to accelerate germination or final seedling development. This may be due to the fact that seeds exhibit an adequate level of endogenous gibberellin, not interfering with performance during germination (Moraes et al. 2012).

Dormancy in *S. torvum* seeds is not attributed to their seed coat, as a physical barrier to water absorption (Hayati et al. 2005). However, the physical resistance of the endosperm may represent a barrier to root protrusion (Nomaguchi et al. 1995, Leubner-Metzger 2002).

In tomato (*Solanum lycopersicum*), the embryo is embedded in a rigid endosperm. The region of the endosperm near the root tip weakens to allow the embryo emergence (Groot & Karrssen 1987). Enzymes such as expansin, β-1,3-glucanase, endo-β-mananase and xyloglucan endotransglucosylase/hydrolase are involved in weakening the endosperm capsule. The levels of mRNA transcription of the genes that codify these enzymes are induced by gibberellic acid (Chen & Bradford 2000, Nonogaki et al. 2000, Wu et al. 2001, Chen et al. 2002). Thus, the gibberellic acid may be involved in weakening the endosperm rigidity, decreasing the resistance to root penetration, estimulating root growth and resulting in accelerated germination. Hayati et al. (2005) concluded that the dormancy mechanisms involved in *S. torvum* may be due to the mechanical resistance of the endosperm, presence of inhibitors in seed coats and physiological status of the embryo.

The treatments with GA$_3$, with pre-imbibition or only moistened substrate, showed the best germination speed index, mean germination time

Table 3. Mean germination time and mean germination speed of three *Solanum torvum* seed batches submitted to different treatments.

Treatment	Mean germination time (days)				Mean germination speed (days)			
	Batch 1	Batch 2	Batch 3	Mean	Batch 1	Batch 2	Batch 3	Mean
Water	20.711 ABa	19.578 Ab	11.807 Ac	17.365	0.0487 Bb	0.0510 BCb	0.085 Ca	0.0614
0.2 % KNO$_3$	21.508 Aa	20.307 Ab	11.757 Ac	17.857	0.0467 Bb	0.0493 Cb	0.085 Ca	0.0603
0.05 % GA$_3$	20.160 Ba	18.072 Bb	10.479 Bc	16.237	0.0497 Bc	0.0553 ABb	0.095 Ba	0.0668
Imbibition with GA$_3$	18.383 Ca	17.050 Bb	08.813 Cc	14.749	0.0547 Ac	0.0590 Ab	0.114 Aa	0.0758
Mean	20.190	18.752	10.714		0.050	0.054	0.095	
CV (%)	3.30				3.07			

Means followed by the same lower case letter do not differ horizontally, while those followed by the same upper case letter do not differ vertically.

and mean germination speed. The response for final germination percentage did not differ among treatments for batches 1 and 3, while, for batch 2, the best treatment was KNO_3.

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