

Influence of germination aids on germination of different species of *Capsicum* sp.

ABSTRACT

Aims: To provide data on the influence of common germination aids for additional pepper species than *Capsicum annuum*, additional species and wild types have been tested with priming media applicable to both hobby and commercial growers.

Place and Duration of Study: Germany, January to September 2017

Methodology: 3x 50 seeds of four different *Capsicum* cultivars "CAP357" (*C. pubescens* L.), "Ancho Poblano" (*C. annuum* L.), "Ulupica from La Paz" (*C. eximium* Hunz.) and "Inca Surprise" (*C. baccatum* var. *baccatum* L.) were primed with either deionized water, 1%(w/v) KNO₃, 1%(w/v) gibberellic acid or 1%(w/v) acetylsalicylic acid. 3x 50 untreated seeds of each cultivar were as control. The seeds were then put to germination on moistened filter paper, maintaining constant temperature (29 ± 1 °C) and air-humidity (85 ± 5%). Seeds were evaluated daily regarding radicle formation for 30 days.

Results: Highest germination rate and germination speed were observed using gibberellic acid as priming agent with overall germination >90 %, followed by potassium nitrate, which improves germination especially for seeds that take longer to germination. Acetylsalicylic acid caused germination rates and speed to decrease. Hydropriming with water showed no significant effect compared to unprimed seeds.

Conclusion: Priming with appropriate media can effectively speed up germination on varieties that take longer to germination such as wild types. On already fast germinating seeds the effect is smaller, however, a more uniform germination can be achieved.

Keywords: capsicum, germination, priming, seeds, potassium nitrate, gibberellic acid

1. INTRODUCTION

Hot (chili) pepper or paprika (*Capsicum* spp.) is one of the most consumed vegetable worldwide. It is not only a large market in various regions of the world, especially India and Pakistan, but the diversity of the *Capsicum* family recently has attracted many hobby growers all over the world. A high germination rate is favorable for every grower but can become substantially relevant for commercial growers, where every plant will eventually generate income.

In order to ensure successful germination, many germination aids or seeds priming agents have been investigated in the past [1]. Potassium nitrate KNO₃ has already been identified as good priming agents by RIVAS and [2] in 1984. More recently various other priming agents such as acetylsalicylic acid [3], 1-hexadecene [4], polyethylene glycol [3,5], NaCl [3,6,7] and again KNO₃ [3,5,8], all reporting beneficial influence on germination speed, seedling uniformity and/or salt tolerance of *Capsicum*. Gibberellic acid (gibberellin) is also likely to speed up the germination process as previous studies on pepper and tomato show [9,10].

The amount of germinated seeds (germination percentage) is one of the first and important parameters in order to evaluate seed health and can also be used to predict future seedling and plant development. High germination percentage and uniform germination time are closely linked to a positive development of the plant. Priming seems to be most advantageous for seed lots with low initial (unprimed) germination percentage [11,12].

However, all major research mainly focuses on *Capsicum annuum* L. cultivars as they are commercially most relevant, while other domesticated species such as *Capsicum pubescens* (Rocoto), *Capsicum baccatum* and wild types are rarely subject of germination studies. There are emergence data of various chili pepper cultivars [13], however, the study investigated cold temperature germination without prior priming.

The present research now provides data for simple and inexpensive seed preparation comparing the germination rates and speeds of a range of *Capsicum* sp. seeds treated with different media prior to germination. The results may not only be relevant for commercial pepper growers but also to hobby

growers who want to improve their seed germination. Media for seed treatment except gibberellic acid can be considered as "readily available" in most countries.

2. EXPERIMENTAL DETAILS

2.1 Germination procedure

50 seeds of each cultivar "CAP357" (*C. pubescens* L.), "Ancho Poblano" (*C. annuum* L.), "Ulupica from La Paz" (*C. eximium* Hunz.) and "Inca Surprise" (*C. baccatum* var. *baccatum* L. wild type), all harvested from self-grown plants in autumn 2016 and stored in the dark at 2-6 °C after dehydration over silica gel, were subjected to each of the following priming agents for 24 h: deionized water, KNO₃ (1%(w/v)), acetylsalicylic acid (1%(w/v)), and gibberellic acid (1%(w/v)). 50 untreated seeds of each lot were used as control group. Seeds were germinated in petri dishes on moistened filter paper under controlled conditions (29 ± 1 °C, 85 ± 5 %rh) for 30 days. Status of germination was checked every 24 hours with radicle formation indicating successful germination [8,14]. Weighing of the seeds has been performed before and after priming in lots of 50 seeds/lot. Every experiment described above has been performed in triplicates.

2.2 Calculations

Overall Germination (G) describes the percentage of germinated seeds in each lot after 30 days. Mean Germination Time (MGT) is a key parameter often used [14,15] to compare the speed of seed germination. It is calculated after Equation 1:

$$MGT = \frac{\sum_{i=1}^n (n_i \cdot d_i)}{\sum n_i} \quad (1)$$

with n_i being the number of germinated seeds on each day d_i . A lower MGT represents a faster germination. Time to 50% germination (G_{50}) as previously described [16] and modified [17] is another index to describe the speed of seed germination.

$$G_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right) \cdot (t_j - t_i)}{n_j - n_i} \quad (2)$$

N denotes the total number of germinated seeds, n_i and n_j represent the cumulative number of germinated seeds adjacent to $N/2$, respectively ($n_i < N/2 < n_j$). t_i and t_j account for the respective days on which n_i and n_j have been counted. For ANOVA with post-hoc Tukey test on significance ($P < .05$) within tested groups statistical evaluation has been performed using Origin Pro 2016.

3. RESULTS AND DISCUSSION

Weighing of dry and primed seeds (Tab. 1) revealed that water uptake is independent of the priming medium but varies for the different *Capsicum* species. Water uptake ranges from 62 % for BAC to 150 % for PUB. A similar water uptake regardless of the priming agent is not unexpected considering all priming media are aqueous solutions (1%). Seeds of smaller size (BAC and EXI) have less surface available for water intrusion, while larger seeds (ANN and PUB, which additionally have a rougher, more uneven surface) are likely able to take up more water.

Table 1. Seed weight dry and after 24 h of priming (50 seeds/measurement).

Means $\pm$ Standard Deviation in g of a 3-fold determination.

priming	weight (dry) [g]				weight (primed) [g]			
	BAC	PUB	EXI	ANN	BAC	PUB	EXI	ANN
SA	0.139 $\pm$ 0.002	0.326 $\pm$ 0.011	0.276 $\pm$ 0.006	0.302 $\pm$ 0.002	0.220 $\pm$ 0.002	0.802 $\pm$ 0.009	0.493 $\pm$ 0.009	0.585 $\pm$ 0.006
	0.141 $\pm$ 0.005	0.323 $\pm$ 0.008	0.262 $\pm$ 0.010	0.306 $\pm$ 0.006	0.227 $\pm$ 0.005	0.810 $\pm$ 0.026	0.495 $\pm$ 0.004	0.598 $\pm$ 0.008
water	0.136 $\pm$ 0.002	0.319 $\pm$ 0.008	0.282 $\pm$ 0.01	0.300 $\pm$ 0.002	0.219 $\pm$ 0.002	0.800 $\pm$ 0.002	0.509 $\pm$ 0.009	0.584 $\pm$ 0.015
	0.135 $\pm$ 0.003	0.320 $\pm$ 0.005	0.271 $\pm$ 0.005	0.301 $\pm$ 0.002	0.226 $\pm$ 0.007	0.798 $\pm$ 0.006	0.487 $\pm$ 0.011	0.637 $\pm$ 0.045
KNO ₃	0.137 $\pm$ 0.002	0.315 $\pm$ 0.011	0.270 $\pm$ 0.002	0.304 $\pm$ 0.002	n.a.	n.a.	n.a.	n.a.
	0.137 $\pm$ 0.002	0.315 $\pm$ 0.011	0.270 $\pm$ 0.002	0.304 $\pm$ 0.002	n.a.	n.a.	n.a.	n.a.
GA-3	0.137 $\pm$ 0.002	0.315 $\pm$ 0.011	0.270 $\pm$ 0.002	0.304 $\pm$ 0.002	n.a.	n.a.	n.a.	n.a.
	0.137 $\pm$ 0.002	0.315 $\pm$ 0.011	0.270 $\pm$ 0.002	0.304 $\pm$ 0.002	n.a.	n.a.	n.a.	n.a.
average	0.137 $\pm$ 0.003	0.321 $\pm$ 0.008	0.272 $\pm$ 0.009	0.303 $\pm$ 0.003	0.223 $\pm$ 0.005	0.803 $\pm$ 0.013	0.496 $\pm$ 0.011	0.601 $\pm$ 0.031
	0.137 $\pm$ 0.003	0.321 $\pm$ 0.008	0.272 $\pm$ 0.009	0.303 $\pm$ 0.003	0.223 $\pm$ 0.005	0.803 $\pm$ 0.013	0.496 $\pm$ 0.011	0.601 $\pm$ 0.031
50 seeds					+62%	+150%	+82%	+99%

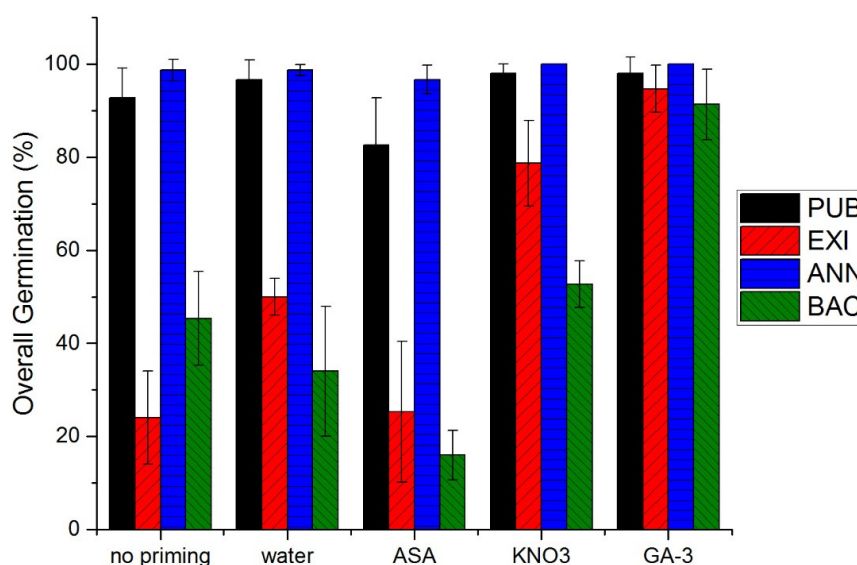


Figure 1. Overall germination for different priming media.

Means $\pm$ Standard Deviation of three experiments.

Overall Germination (Fig.1) after 30 days is first of all strongly dependent on the species. While ANN and PUB display over 90 % germination regardless of the priming method, germination of BAC and EXI is influenced by the choice of the priming agent. In general, no priming and simple deionized water yield almost the same results, and the use of ASA even decreases overall germination for all investigated species. On the other hand, priming with KNO₃ or GA-3 markedly increases germination with GA-3 having the highest effect on seeds. MGT (Fig. 2a) and G₅₀ (Fig. 2b) are both parameters describing the speed of germination. As with Overall Germination, speed is dependent firstly on the species and secondly on the used priming agent. Again, no priming and water priming yield comparable results, ASA prolongs germination of all species, while KNO₃ and GA-3 significantly shorten germination times. In Fig. 3, both overall germination and germination speed are shown in a graph depicting cumulated number of germinated seeds over time.

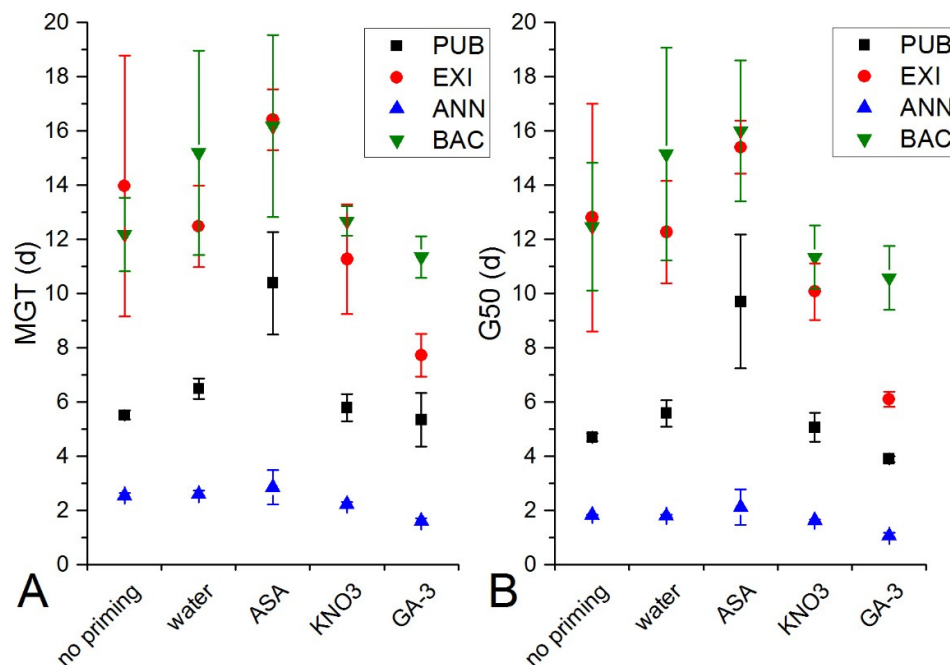


Figure 2. a) Mean Germination Time (MGT) and b) Time to 50% germination (G50) for different priming media.

Means $\pm$ Standard Deviation of three experiments.

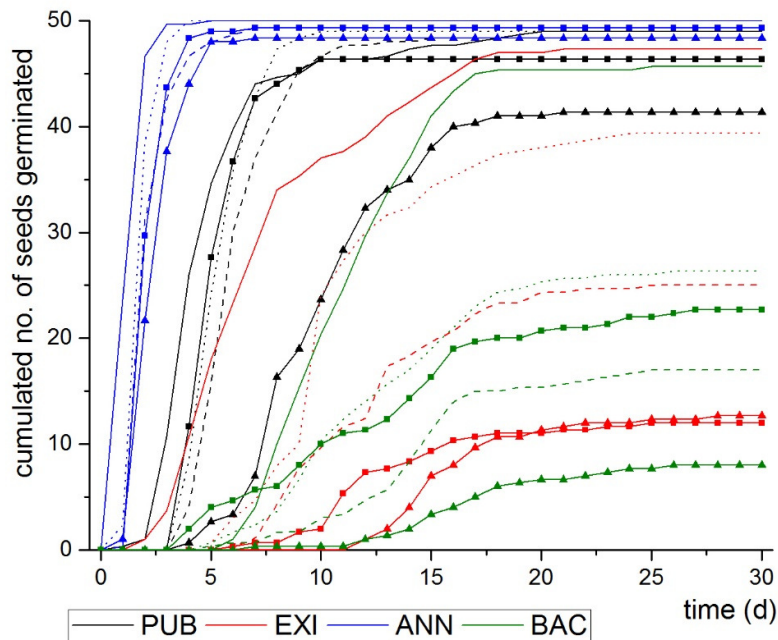


Figure 3. Cumulated number of germinated seeds over time. No priming (—■—), water (dashed ---), ASA (-▲-), KNO3 (dotted ...), GA-3 (solid —).

Germination strongly depends on the individual species. Using no priming agent or only water, both *C. annuum* and *C. pubescens* already have high germination rates above 90 %, while *C. eximium* and the wild type of *C. baccatum* have decreased germination rates around 30 to 50 %. Compared to no

priming, simply priming with water does not significantly enhance overall germination (except for EXI, see Table 2). Even the speed of germination expressed as MGT or G_{50} is not noticeably improved by using water priming for 24h. On the contrary, in cases of PUB and BAC, unprimed have slightly lower (although not significant) MGT and G_{50} values indicating faster germination.

Table 2. Results of ANOVA *post-hoc* Tukey test on significance. Comparison of overall germination and germination speed depending on priming medium vs. unprimed seeds.

Bold *P* values ($P < .05$) indicate significant enhancement of germination, **Bold** *P* values with **asterisk** ($P < .05$) indicate significant negative influence on germination.

		<i>P</i> values compared to unprimed seeds			
		water	ASA	KNO3	GA3
G	PUB	.917	.305	.802	.802
	EXI	.047	.999	<.001	<.001
	ANN	.999	.658	.886	.886
	BAC	.564	*.017	.852	<.001
MGT	PUB	.754	*.001	.997	.999
	EXI	.945	.756	.688	.073
	ANN	.999	.723	.700	.022
	BAC	.554	.306	.999	.992
G50	PUB	.875	*.002	.995	.905
	EXI	.998	.599	.559	.023
	ANN	.999	.752	.920	.063
	BAC	.680	.449	.977	.876

Priming with ASA leads to significantly reduced overall germination for PUB, ANN and BAC and unchanged values for EXI. Additionally, germination speed is to some extent significantly decreased for all species. This leads to the conclusion that ASA is not suitable as priming agent for pepper varieties.

Potassium nitrate as a common priming agent leads to improved germination rates for all investigated species, however, a significant increase is only observed for EXI due to the already high germination rates of ANN and PUB. For BAC and EXI lower MGT and G_{50} are also observed, confirming a positive effect on germination on seeds that take longer to germinate. This positive effect has previously been described by several groups [2,3,8,18], however, the present study contributes additional data for other varieties than *C. annuum*. Again, results for germination speed do not significantly differ from unprimed or water-primed seeds, although germination is more uniform and has a smaller variance.

Using gibberellic acid GA-3 as priming agent yields the highest overall germination rates. Even BAC and EXI now exceed 90 % overall germination. MGT and especially G_{50} are lower for all species indicating a faster and also more uniform germination. In cases of ANN and EXI, G_{50} of GA-3-treated seeds is reached almost twice as fast as compared to unprimed seeds. When sowing older seeds or species that seem to be harder to germinate, priming with GA-3 is a very effective method to enhance germination.

4. CONCLUSION

Priming of pepper seeds can markedly enhance both overall germination and germination speed, especially for seeds that take longer time to germinate such as wild types or older seeds. Priming with water or no priming at all show similar effects, indicating that water priming is just lost time, at least when sowing on filter paper. KNO_3 and gibberellic acid as priming agents are both enhancing germination, the latter being more effective but also less easy to handle. Acetylsalicylic acid prolongs germination and reduces overall germination and is thus not recommended.

REFERENCES

1. Taylor AG, Allen PS, Bennett MA, Bradford KJ, Burris JS, Misra MK. Seed enhancements. *Seed Sci. Res.* 1998; 8 (2): 245–256. doi:10.1017/S0960258500004141.
2. O'Sullivan J, Bouw WJ. Invigorating Pepper Seed. *Can. J. Plant Sci.* 1984; 64 387–393.
3. Amjad M, Ziaf K, Iqbal Q, Ahmad I, Atif M, Saqib ZA. Effect of Seed Priming on Seed Vigour and Salt Tolerance in Hot Pepper. *Pak. J. Agri. Sci.* 2007; 44 (3): 408–416.
4. Wijesinghe RE, Lee S-Y, Kim P, Jung H-Y, Jeon M, Kim J. Optical sensing method to analyze germination rate of *Capsicum annum* seeds treated with growth-promoting chemical compounds using optical coherence tomography. *J. Biomed. Opt.* 2017; 22 (9): 91502. doi:10.1117/1.JBO.22.9.091502.
5. Pandita VK, Anand A, Nagarajan S. Enhancement of seed germination in hot pepper following presowing treatments. *Seed Sci. Technol.* 2007; 35 (2): 282–290.
6. Yadav PV, Kumari M, Ahmed Z. Seed Priming Mediated Germination Improvement and Tolerance to Subsequent Exposure to Cold and Salt Stress in *Capsicum*. *Res. J. Seed Sci.* 2011; 4 (3): 125–136. doi:10.3923/rjss.2011.125.136.
7. Khan HA, Ayub CM, Pervez MA, Bilal RM, Shahid MA, Ziaf K. Effect of seed priming with NaCl on salinity tolerance of hot pepper (*Capsicum annum* L.) at seedling stage. 2009; 28 (1): 81–87.
8. Ermis S, Ozden E, Njie E, Demir I. Pre-treatment Germination Percentages Affected the Advantage of Priming Treatment in Pepper Seeds. *Am. J. Exp. Agric.* 2016; 13 (1): 1–7. doi:10.9734/AJEA/2016/26810.
9. Andreoli C, Khan AA. Matricconditioning integrated with gibberellic acid to hasten seed germination and improve stand establishment of pepper and tomato. *Pesqui. Agropecuária Bras.* 1999; 34 (10): 1953–1958. doi:10.1590/S0100-204X1999001000023.
10. Watkins JT, Cantliffe DJ. Mechanical Resistance of the Seed Coat and Endosperm during Germination of *Capsicum annum* at Low Temperature. *PLANT Physiol.* 1983; 72 (1): 146–150. doi:10.1104/pp.72.1.146.
11. Demir I, Okcu G. Aerated hydration treatment for improved germination and seedling growth in aubergine (*Solarium melongena*) and pepper (*Capsicum annum*). *Ann. Appl. Biol.* 2004; 144 (1): 121–123. doi:10.1111/j.1744-7348.2004.tb00324.x.
12. Georgiou K, Thanos CA, Passam HC. Osmoconditioning as a Means of Counteracting the Ageing of Pepper Seeds during High-temperature Storage. *Ann. Bot.* 1987; 60 (3): 279–285. <http://aob.oxfordjournals.org/content/60/3/279.abstract>.
13. Gerson R, Honma S. Emergence response of the pepper at low soil temperature. *Euphytica.* 1978; 27 (1): 151–156. doi:10.1007/BF00039130.
14. Demir I, Ermis S, Mavi K, Matthews S. Mean germination time of pepper seed lots (*Capsicum annum* L.) predicts size and uniformity of seedlings in germination tests and transplant modules. *Seed Sci. Technol.* 2008; 36 (1): 21–30. doi:10.15258/sst.2008.36.1.02.
15. Ellis RH, Roberts EH. The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol.* 1981;
16. Coolbear P, Francis A, Grierson D. The Effect of Low Temperature Pre-Sowing Treatment on the Germination Performance and Membrane Integrity of Artificially Aged Tomato Seeds. *J. Exp. Bot.* 1984; 35 (11): 1609–1617. doi:10.1093/jxb/35.11.1609.
17. Farooq M, Basra SMA, Ahmad N, Hafeez K. Thermal Hardening: A New Seed Vigor Enhancement Tool in Rice. *J. Integr. Plant Biol.* 2005; 47 (2): 187–193. doi:10.1111/j.1744-7909.2005.00031.x.
18. Rivas M, Sundstrom FJ, Edwards RL. Germination and crop development of hot pepper after seed priming. *HortScience.* 1984

ABBREVIATIONS

ANN – *Capsicum annum* L. cv. “Ancho Poblano”
 BAC – *Capsicum baccatum* var. *baccatum* L. cv. “Inca Surprise”
 EXI – *Capsicum eximium* Hunz. cv. “Ulupica from La Paz”
 PUB – *Capsicum pubescens* L. cv. “CAP357”
 MGT – Mean Germination Time
 G50 – Time to 50% Germination
 GA-3 – gibberellic acid

226 ASA – acetylsalicylic acid