

Promoting Pennycress Germination with Hormone Treatments

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Figure 4. Illustration of *Thlaspi arvense*, *perfoliatum* (also known as field pennycress and stink weed) from late 18th century [8].

Introduction

- Pennycress (*Thlaspi arvense* L.) → source of biofuel & provides extra revenues to farms while sequestering excess nitrogen in soil [1]
- Challenge of pennycress integration into corn-soybean rotation → innate seed dormancy [1]
 - Dormancy occurs when viable seeds do not germinate despite favorable conditions [2]
 - Pennycress has naturally uneven germination → reoccur as potential weed in successive years [1]
 - Fall establishment requires improved seed germination [1]
- Seed enhancement technologies, such as hormone-priming that modulate hormonal signaling networks, accelerate and synchronize germination [2, 3]
 - Gibberellic acid 4+7 (GA) stimulates cell division and regulates the induction of germination [4]
 - 0.01% GA solution used in previous research on pennycress germination [1]
 - 6-benzyladenin (BA) solution regulates adaptive response in plant to environment stressors [5]
 - 0.006% BA solution based on 60ppm treatment of *Nigella sativa* L. and *Allium cepa* L. [6]
 - BA solution seed yield of *Jatropha curcas*, another potential biofuel source [7]

Methods

- Seeds → MN106NS 2021 harvest (dark seed), tt8-t/ARV1 2021 & 2022 harvest (golden seed)
 - Control → no treatment
 - 9 experimental groups → 3 solutions with 3 soak times
 - Soaked in distilled water, 0.01% GA, or 0.006% BA for 30 minutes, 1 hour, or 4 hours
 - Vacuumed filtered and then washed with distilled water, dried for 48 hours before plated
- Germinated assay → 50 seeds per petri dish x 2 for each group
 - Condition → dark chamber at 20 °C ± 1°C
 - Observed seeds on day 4, day 7, day 10
 - Counted seeds as germinated when the radicle was twice the length of the seed

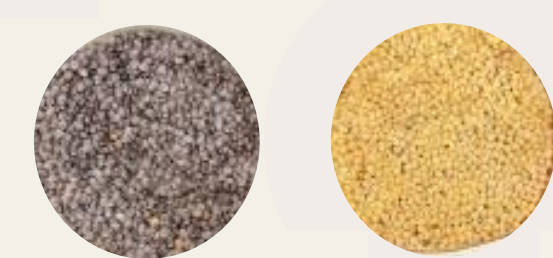


Figure 1. Black seed, specifically MN106NS 2021 harvest (right), and golden seed, specifically tt8-t 2021 harvested (left)



Figure 2. Stack of 10 petri dishes (one control and 9 treatments) of tt8-t 2022 harvest seeds

Results

Table 1. Results of the ANOVA for seed lot, treatment (trt), and their interaction for the germination tests at day 4, 7, and 10. The ANOVA was performed using the GLIMMIX procedure in SAS 9.4.

	Effect	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	Pr > F
Day 4	seed lot	2	58	41.3	<.0001
	trt	9	58	1.25	0.2855
	seed lot*trt	18	58	1.46	0.1415
Day 7	seed lot	2	58	35.27	<.0001
	trt	9	58	1.96	0.0616
	seed lot*trt	18	58	1.67	0.073
Day 10	seed lot	2	58	72.23	<.0001
	trt	9	58	3.37	0.0022
	seed lot*trt	18	58	1.8	0.047

Note: Day 4 and day 10 had statistically significant results. Day 7 did not have statistically significant results.

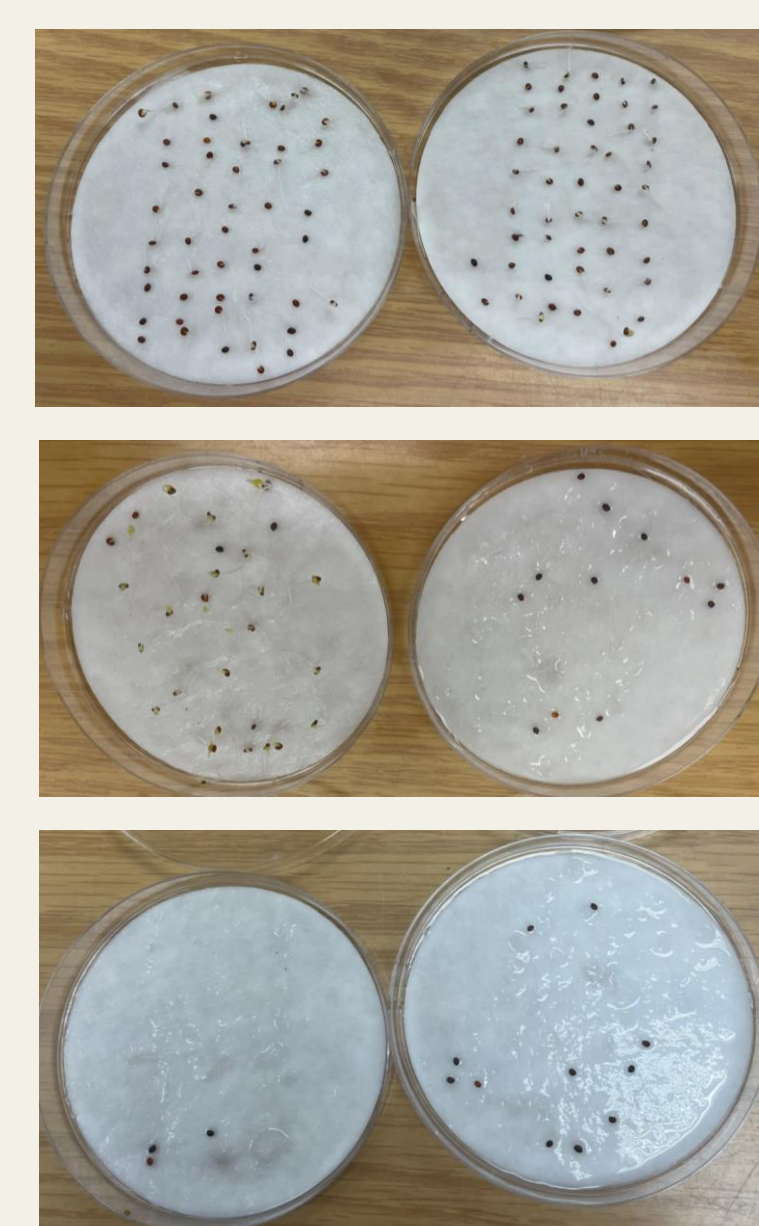


Figure 2. Day 4 (top), day 7 (middle), and day 10 (bottom) germination assay of MN106NS 30-minute soak in GA.

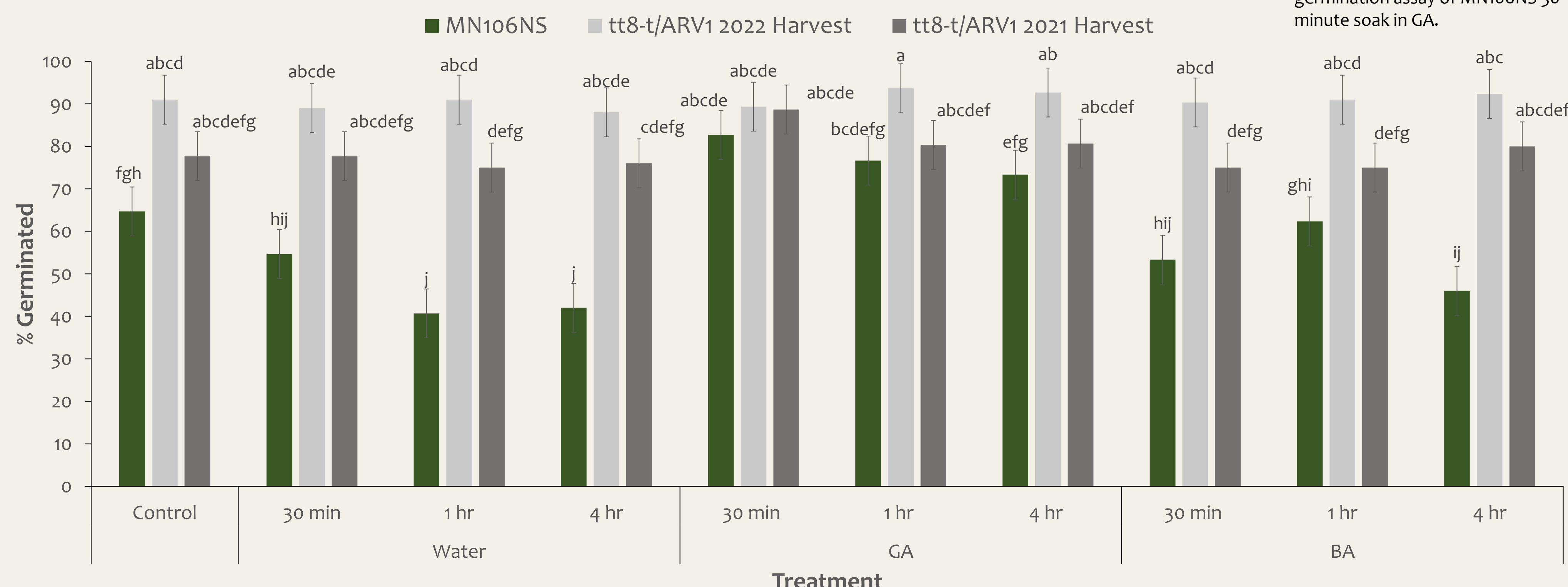


Figure 3. Treatment means and standard errors for control and 9 experimental groups for Day 10 germination results. The letters denote mean separation for the interaction of seed lot x treatment.

Research Question: What is the shortest duration of time needed to soak pennycress seeds in a hormonal solution to effectively break dormancy?

Discussion

- Germination of golden seed was not improved by any of the treatments shown by no mean separation (Figure 1)
 - tt8-t 2022 → statistically insignificant increase in germination rate in 4-hour soak in GA and 4-hour soak in BA compared to control by Day 10
 - tt8-t 2021 → statistically insignificant increase in germination rate in 30-minute, 1-hour, and 4-hour soak in GA and 4-hour soak in BA compared to control by Day 10
 - Both GA and BA did not significantly increase germination in comparison to the untreated seeds
- Germination of dark seed was improved by 30-minute soak in 0.01% GA illustrated by mean separation (Figure 1)
 - Day 7 → 30-minute, 1-hour, and 4-hour soak in GA all higher germination rates than control but not statistically significant evident by p value of interaction terms (Table 1)
 - Day 10 → 30-minute soak in GA statistically significant increase in germination rate evident by p-value of interaction term (Table 1)
 - Distilled water decreased dark-seed germination therefore not hydropriming effect but the hormones altering seeds
 - GA at low concentration and short soak time is successful hormonal treatment while BA at any concentration and soak time actually impeded germination

Conclusion

- Hormone treatment effective treatment for dark seed but no effect on golden seed
 - Corroborates with past research that GA solution increase germination for dark seed but not as effective for golden-seed [1]
 - Aligns with findings of higher germination rate in golden seeds (tt2) than dark seeds (MN106) [9]
 - MN106 treated with GA has comparable germinate rate to tt2 [9]
- In comparison to recommend treatment (1-hour soak in 0.01% GA), soak time can be halved [10]
- Most effective hormone solution and soak time → 30 min in GA for dark seed
- Best treatment for golden seed is no treatment

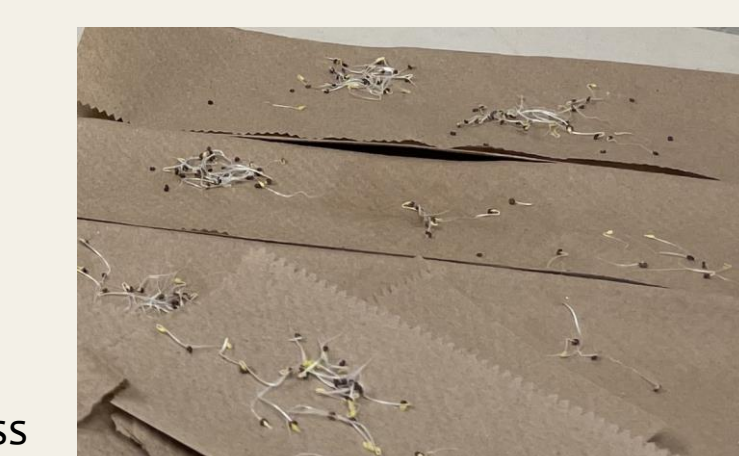


Figure 5. Discarded piles of germinated, dark seed pennycress

References

1. Koirala, N., Barker, D., Helfer, C., Phippen, W.B., Heller, N., Hard, A.W., Wells, S., Lindsey, A.J. (2022). A process to enhance germination of white pennycress variety. *Seed Science and Technology*, 50(2), 195-205.
2. Matilla, A.J. (2020). Auxin: Hormonal signal required for seed development and dormancy. *Plants*, 9, 705-722. doi:10.3390/plants9060705
3. Pedrini, S., Balestrazzi, A., Madsen, M.D., Bhalsing, K., Hardegre, S.P., Dixon, K.W., Kildisheva, O.A. (2020). Seed enhancement: Getting seeds restoration-ready. *Restoration Ecology*, 28(S3), S266-S275.
4. Sosnowski, J., Truba, M., Vaileva, V. (2023). The impact of auxin and cytokinin on the growth and development of selected crops. *Agriculture*, 13, 724-738.
5. Mangena, P. (2020). Effect of hormonal seed priming on germination allocation in soybean grown under induced drought stress. *Indian Journal of Agricultural Research*. 10.18805/IJAR.A-441
6. El-Ghamery, A.A. & Mousa, M.A. (2017). Investigation on the effect of benzyladenine on the germination, radicle growth and meristematic cells of *Nigella sativa* L. and *Allium cepa* L. *Annual of Agricultural Science*. 62, 11-21.
7. Pan, B. & Xu, Z. (2011). Benzyladenine treatments significantly increases the seed yield of the biofuel plant *Jatropha curcas*. *Journal of Plant Growth Regulation* 30, 166-174.
8. Rare Book Division, The New York Public Library. (1772 - 1793). *Thlaspi arvense, perfoliatum: maius = Tlaspi offic. [Field pennycress, Stink weed]* Retrieved from <https://digitalcollections.nypl.org/items/510d47dd-d50e-a3d9-e040-e00a18064a99>
9. Ott, M.A., Gardner, G., Rai, K.M., Wyse, D.L., Marks, M.D., Chopra, R. (2021). TRANSPARENT TESTA 2 allele confers major reduction in pennycress (*Thlaspi arvense* L.) seed dormancy. *Industrial Crops & Products*, 174, 114216, 10.1016/j.indcrop.2021.114216.
10. c. Aulbach, 2021, personal communication.

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