United States Department of Agriculture

FINAL STUDY REPORT (February, 2026) **WAPMC - Pullman, WA**

Increasing Germination Efficiencies of Palouse Prairie Native Forbs using Oxygenated Water

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ABSTRACT

Improved plant propagation protocols can increase production efficiencies and the commercial availability of native seed and plants. Numerous native species exhibit physiological and/or physical dormancy that inhibit germination until appropriate ambient environmental conditions are met. Requirements to break dormancy in some native species can be lengthy and complex. Our objective was to improve germination efficiencies for eight Palouse Prairie native forb species. We evaluated germination after three treatments: 1) a control that followed published propagation protocols specific to each species, 2) a 10-day oxygenated water treatment, and 3) a 30-day cold-moist stratification followed by a 10-day oxygenated water treatment. We monitored seeds for 28 days after each treatment and recorded germination when radicles reached 2 mm in length. We planted germinated seeds into cone-tainers and recorded mortality rates after 30 days in the greenhouse. Not all species responded favorably to the oxygenated water treatment; however, some species responded well to a combination of cold-moist stratification and oxygenated water. For *Wyethis amplexicaulis* (WYAM) and *Eriogonum heracleoides* (ERHE2) this effectively reduced the pre-treatment requirements from 90 days to 40 days. Investigating new propagation protocols to improve the efficiency of native seed germination will provide valuable guidelines for native plant production.

INTRODUCTION

Conservation and restoration projects often require seeding native species. Successful plant establishment depends on the successful germination of those seeds. Poor germination rates limit the establishment and success of native species. Unlike invasive weeds, which germinate rapidly, many native species exhibit physiological and/or physical dormancy that inhibits germination until the proper environmental conditions are met (Kildisheva et al., 2020). In nature, dormancy ensures that germination occurs only when environmental conditions are favorable for successful maturation of the plant. Physiological dormancy involves embryo immaturity or hormonal inhibitors, while physical dormancy results from water-impermeable seed coats (Baskin & Baskin, 2014). For many native plant species, little is known about the requirements for breaking dormancy; for others, the requirements are well understood, but the process is lengthy and complex.

Many propagation protocols call for fall sowing in order to break physiological dormancy requirements with cold outdoor temperatures which can take 90-120 days or longer (Finch-Savage & Leubner-Metzger, 2006). Drawbacks of this method include seed loss to predation by rodents, insects, or fungi, and a limited planting window. Breaking dormancy prematurely in a laboratory

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setting allows earlier greenhouse propagation and, thereby, more mature plants for spring plantings, which may increase their survival rates (Tilley & Pickett, 2021). However, these processes can be time-consuming and labor-intensive for commercial production (Dumroese et al., 2009). Understanding native seed germination requirements and how to break dormancy prematurely is essential to increasing the success of future conservation plantings.

The Palouse region encompasses the rolling hills of eastern Washington and northern Idaho. Once blanketed in native vegetation, including bunchgrasses, shrubs, and wildflowers, the Palouse became a premier dryland agricultural region around the mid-19th century (Duffin, 2004). Less than 1.7 percent of the native Palouse Prairie remains intact, and those plant communities are fragmented across the landscape, mostly on private property (Davis, 2019). In recent decades, efforts to restore this native ecosystem have been led by private landowners and nonprofit organizations, with support from conservation districts, government agencies, and academic programs.

Eight species were selected for investigation based on seed availability, published propagation protocols for the control group, and their importance to the Palouse region. Our objective was to identify new germination methods that break dormancy for eight Palouse Prairie native forb species and to compare germination success across our treatments and the published propagation protocols. Improved native plant propagation protocols can increase production efficiency and the commercial availability of seed and plants.

MATERIALS AND METHODS

We evaluated eight forb species native to the Palouse Prairie region of Idaho and Washington using three different propagation strategies. 1) **Control**: published propagation protocols for each species per the Native Plant Networks' Propagation Protocol Database, 2) **Treatment 1**: a 10-day oxygenated water treatment per Tilley and Picket (2021), and 3) **Treatment 2**: a combination of 30-day cold-moist stratification followed by 10-day oxygenated water treatment. Table 1 shows the propagation protocol used for each species (Control). This experiment involved 25 seeds per species, 3 replications per trial, and 3 trials per treatment. Seeds were wild-collected in the Palouse region.

Table 1. Eight Palouse Prairie native forb species and their published propagation protocols (according to published protocol in the Native Plant Networks' Propagation Protocol Database) used as controls in the Increasing Germination Efficiencies of Palouse Prairie Native Forbs using Oxygenated Water study, 2025, Pullman, WA.

Symbol	Species	Control Propagation Protocol
BASA3	*Balsamorhiza sagittate*	90 days cold-moist stratification
COGR4	*Collomia grandiflora*	14 days cold-moist stratification
ERHE2	*Eriogonum heracleoides*	90 days cold-moist stratification
GAAR	*Gaillardia aristata*	30 days cold-moist stratification
LOTR2	*Lomatium triternatum*	24hr soak, 60 days cold-moist stratification
PEPR3	*Penstemon pruinosus*	90 days cold-moist stratification
POGR9	*Potentilla gracilis*	90 days cold-moist stratification
WYAM	*Wyethis amplexicaulis*	90 days cold-moist stratification

In the Control, seeds were placed between sheets of germination paper, moistened, and sealed in quart-sized bags. Cold-moist stratification was conducted in a controlled-environment growth chamber (Percival Scientific E-36VL, Perry, IA) at a constant temperature of 39°F for the designated time period for each species (Table 1). For Treatment 1, seeds were placed in cotton drawstring bags with glass marbles to keep the bag submerged. Each jar contained 500 mL of distilled water with a stone diffuser connected to an air pump. Jars were kept at 70°F with the air pump running continuously for 10 days. Treatment 2 was applied only to species requiring 90 days of cold-moist stratification (BASA3, ERHE2, PEPR3, POGR9, and WYAM) in accordance with published protocols. These species were cold stratified for 30 days, instead of 90, at 39°F, then placed in oxygenated water for 10 days.

Figure 1. Seeds in drawstring bags, in mason jars with distilled water, connected to an air pump adding oxygen to the water.

After each treatment, all seeds were placed on moistened germination paper in Petri dishes and incubated in a growth chamber. The growth chamber was set to a diurnal program: 70°F for 12 hours and 60°F for 12 hours. Germination was recorded at 4, 7, 14, 21, and 28 days after initiation. Seeds were considered germinated when radicles reached 2 mm in length. Germinated seeds were transplanted into cone-tainers, and mortality rates were recorded after 30 days in the greenhouse.

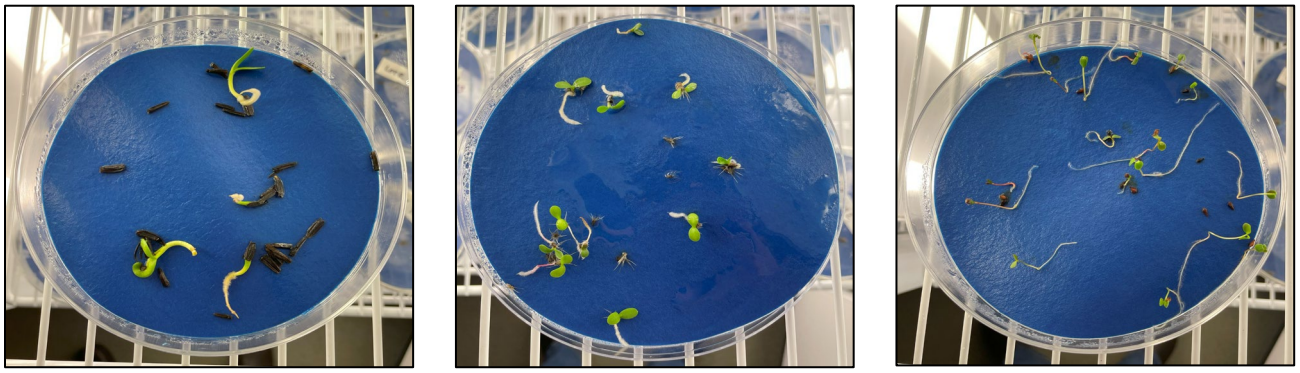

Figure 2. Seeds on Petri dishes in growth chamber, germinating after pre-treatment of published propagation protocols (Control).

We used chi-square tests of independence to compare germination and survival rates across treatments for each species. For species with significant overall treatment effects ($P < 0.05$), we conducted post hoc pairwise chi-square tests of all treatment combinations to identify specific treatment differences. We calculated efficiency scores as (number of survivors / total propagation days) × 100 to integrate germination success, survival, and time requirements into a single metric. All analyses were conducted in R version 4.3.1. Significance was assessed at $\alpha = 0.05$ for all tests.

RESULTS AND DISCUSSION

Germination and Survival

Germination and survival responses varied substantially among species and treatments (Figure 3). Chi-square tests revealed significant treatment effects on germination for six of eight species (BASA3, COGR4, ERHE2, LOTR2, PEPR3, WYAM; all $P < 0.05$), whereas survival showed significant treatment effects for two species (COGR4, WYAM; $P < 0.05$).

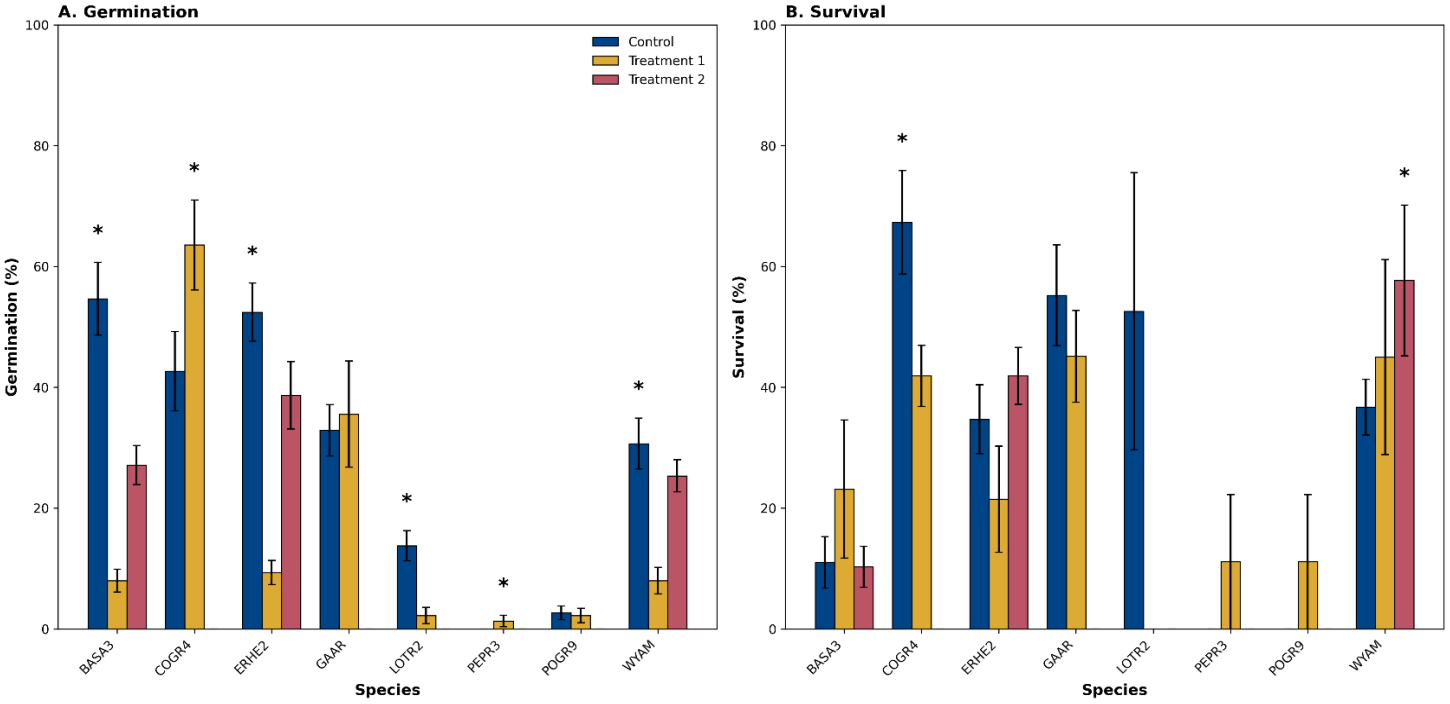

Figure 3. Germination and survival outcomes for eight Palouse Prairie native forb species across three propagation treatments. Panel A shows germination percentage at 28 days post-treatment. Panel B shows seedling survival percentages at 30 days post-transplant. Error bars represent standard error of the mean (SEM). Asterisks (*) denote species with significant overall treatment effects (chi-square test, P < 0.05). Control = published species-specific protocol; Treatment 1 = 10-day oxygenated water; Treatment 2 = 30-day stratification + 10-day oxygenated water.

For BASA3, all three treatments differed significantly (all $P < 0.001$), with Control producing the highest germination (54.7%), followed by Treatment 2 (27.1%) and Treatment 1 (8.0%). COGR4 showed superior germination with Treatment 1 (63.6%) compared to Control (42.7%, $P < 0.001$), though Control produced better survival (67.6% vs. 42.1%, P=0.016). For ERHE2, germination differed significantly among all treatments (P<0.001). Control showed the highest germination (52.4%), followed by Treatment 2 (38.7%), while Treatment 1 performed poorly (9.3%).

WYAM demonstrated the most promising results for protocol optimization. Germination did not differ significantly between Control (30.7%) and Treatment 2 (25.3%; P=0.248), indicating that reducing total pre-treatment time (from 90 to 40 days) did not affect germination success. Furthermore, Treatment 2 produced superior seedling survival (57.0%) compared to Control (34.7%, p=0.016), suggesting that oxygenated water treatment enhanced seedling vigor. Treatment 1 performed poorly (8.0% germination, $P < 0.001$), confirming that some stratification is necessary for this species.

LOTR2 and PEPR3 showed minimal germination across all treatments. LOTR2 achieved 13.8% germination with Control but essentially failed with Treatment 1 (1.8%), indicating the oxygenated water treatment was incompatible with this species' dormancy requirements. PEPR3 germinated poorly under all protocols (<2%), suggesting fundamental seed viability issues or unmet dormancy requirements. There were no significant differences between treatments for POGR9 and GAAR.

Germination Over Time

Germination kinetics revealed differences in treatment effectiveness over time (Figure 4). For BASA3 and ERHE2, Control and Treatment 2 showed rapid initial germination, with most germination occurring by day 7, whereas Treatment 1 produced minimal germination throughout the monitoring period. COGR4 exhibited the fastest germination response with Treatment 1, reaching maximum germination by day 7 (approximately 60%), demonstrating that this species responds particularly well to oxygenated water without extended stratification. WYAM showed more gradual germination kinetics, with Control and Treatment 2 reaching similar final germination percentages by day 28, though Treatment 2 exhibited slightly faster initial germination rates.

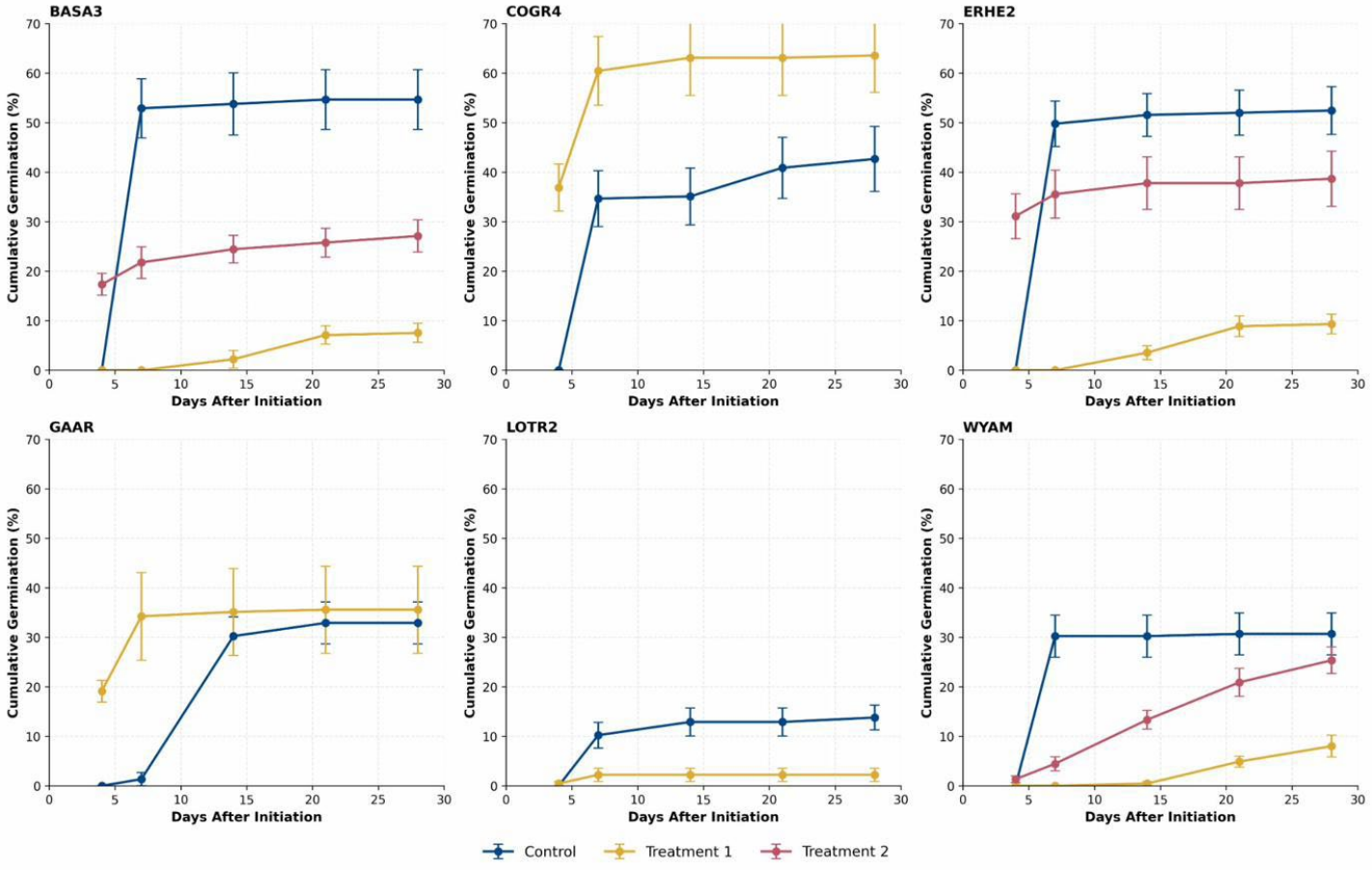

Figure 4. Temporal germination kinetics for six Palouse Prairie native forb species showing cumulative germination percentage over 28 days. Error bars represent SEM across nine replicates. Days after initiation do not include pre-treatment time.

Germination Efficiency

Efficiency scores, which integrate germination success, survival, and propagation time, identified optimal protocols for each species (Figure 5). COGR4 achieved the highest efficiency score with Treatment 1 (89.71), representing a 13% improvement over its Control protocol (79.17). This improvement resulted from increased germination and reduced propagation time. WYAM showed substantial efficiency gains with Treatment 2 (33.67) compared with Control (16.22), a 108% improvement attributable to time savings and improved survival. ERHE2 also showed efficiency improvement (25%) with Treatment 2 (34.69) compared to the Control (27.7). GAAR demonstrated a moderate improvement in efficiency with Treatment 1 (50.00) compared with Control (44.83), a 12% increase primarily attributable to time reduction. In contrast, BASA and LOTR2 showed the highest efficiency with the Control protocols (9.46 and 7.62, respectively), indicating that modified protocols provided no benefit for these species. PEPR3 and POGR9 exhibited minimal germination across all treatments (<3%), precluding meaningful comparisons of efficiency.

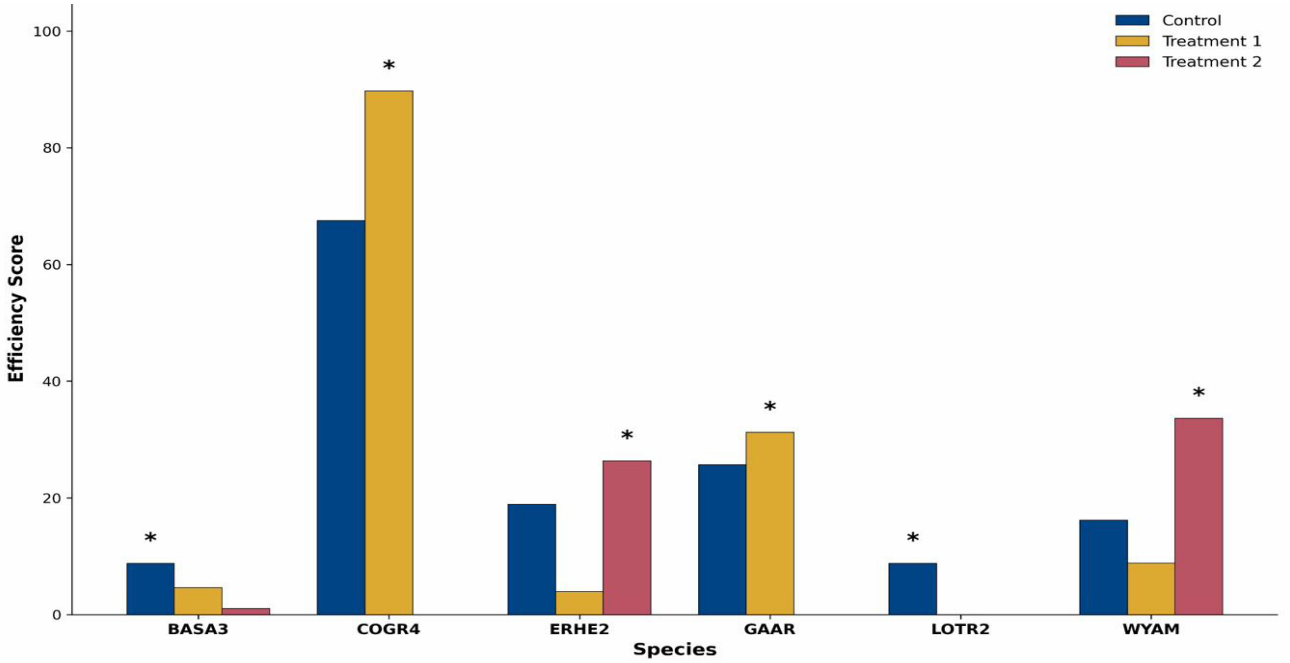

Figure 5. Comparison of propagation efficiency across treatments for six viable species. Efficiency score = (# Survived / Total Days) × 100, integrating germination success, survival, and time investment. Asterisks (*) denote the optimal treatment for each species based on the highest efficiency score. Higher values indicate greater efficiency in producing viable seedlings per unit time.

Protocol Recommendations

Based on an integrated assessment of germination success, seedling survival, time requirements, and efficiency scores, we concluded the following species-specific protocol recommendations. WYAM and ERHE2 are recommended for Treatment 2, offering major time savings (50 days, 34% reduction) and efficiency gains of 108% and 25%, respectively. COGR4 and GAAR are recommended for Treatment 1, providing efficiency gains of 13% and 12%, respectively, with time savings ranging from 4 to 20 days. BASA and LOTR2 performed best under the Control protocols, as modified treatments reduced overall propagation success.

CONCLUSION

Oxygenated water treatment, combined with reduced stratification periods, has significant potential to improve the efficiency of native forb propagation. WYAM (*Wyethia amplexicaulis)* and ERHE2 (*Eriogonum heracleoides)* demonstrated substantial time savings (50 days, 34% reduction) while maintaining or improving germination and survival outcomes. COGR4 (*Collomia grandiflora)* and GAAR (*Gaillardia aristata)* showed moderate gains in efficiency with oxygenated water treatment alone. However, species-specific responses underscore the need for tailored propagation protocols rather than universal approaches. BASA (*Balsamorhiza sagittate)* and LOTR2 (*Lomatium triternatum*) performed best under the published propagation protocols, indicating that some species possess dormancy mechanisms that cannot be overcome by oxygenated-water treatment alone. These findings directly address bottlenecks in native plant production for Palouse Prairie restoration. By shortening propagation times while maintaining or improving plant quality, these modified protocols can increase the availability of native plants for restoration projects.

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