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RESEARCH ARTICLE

SEED HYDROPRIMING TO ALLEVIATE DROUGHT STRESS IN GERMINATION OF TWO SENSITIVE COTTON (*Gossypium hirsutum* L.) VARIETIES

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ABSTRACT

Drought is a serious threat to cotton growing. This study aims to evaluate effect of hydropriming on seed germination of two varieties of cotton, STAM 129A and STAM 190, under drought stress. Seeds are primed for 3, 6, 9, 12, 15 and 18 hours. The germination test was carried out in 90 mm Petri dishes, at 0, -3, -6 and -9 bar adjusted by PEG-6000, and lasted 7 days. Germination percentage (GP), mean germination time (MGT), germination index (GI) and the relative PEG injury rate (RPIR) was then calculated. The results show that drought stress damage on seed germination was significantly reduced by priming seeds for 12 hours for both cotton varieties. Under drought stress, 12H-hydropriming increased the GP by 206.25% for STAM 129A and 179.26% for STAM 190. Germination, previously nil at -6 bar and -9 bar for unprimed seeds, becomes possible with priming and better with 12H-hydropriming. Increases of 250.76% and 289.55% of GI for respectively STAM 129A and STAM 190 are noted, when MGT did not vary significantly from the control. RPIR was reduced 35.89 % for STAM 129A and 27.10 % for STAM 190. Thus, seed hydropriming of STAM 129A and STAM 190 for 12 hours can be recommended to maintain germinative vigor whatever rainfall conditions may arise.

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INTRODUCTION

The cotton plant (*Gossypium hirsutum* L.) is a widely distributed textile plant in the world's tropical belt. In West Africa, particularly in Togo, Benin, Burkina Faso and Mali, cotton contributes 5-9% of GDP and accounts for 30-40% of export revenue in these countries (UEMOA, 2006). In many cases, the revenue from its cultivation constitutes the main source of secure income for producers (MAEP, 2013). Unfortunately, in Togo, its production has been steadily declining in recent years, from 1.326 kg/ha in 1997/98 to 695 kg/ha in 2017/2018, a decline linked to soil impoverishment and environmental constraints such as drought. In fact, Togo has been suffering for several consecutive years from a number of major natural and anthropogenic risks, among which drought ranks high (Adewi *et al.*, 2010). Agricultural production, which is essentially rainfed, is characterized by an increasingly unfavorable environment marked by rising temperatures, irregular rainfall, decreasing rainfall and the number of rainy days, and a decrease in the rainfall/potential evapotranspiration ratio (DGMN, 2015; Djaman *et al.*, 2017). For example, during the 2020/2021 season, the rains started timidly in March with an average height of 55.83 mm in 4 days compared to 53.23 mm in 3 days in the same month in 2019 (NSCT, 2021). They gradually settled down over the months with variable amounts from one region to another.

However, the months of May, June, and July, which are the months of cotton planting, had lower rainfall amounts than the previous season, with 130.13 mm, 136.17 mm and 137 mm, respectively, compared to 137.33 mm, 183.21 mm and 192.19 mm. In these particularly exceptional climatic conditions, efforts made did not allow the planting of the area to the extent of the objectives set. As a result, multiple reseeded and high rejections of areas of about 14.213 hectares, or 12% of the 119.739 hectares sown, thus reducing the area finally sown to 105.526 hectares (NSCT, 2021). Many of the plots prepared to receive cotton were no longer plowed due to drought. Also, the sudden cessation of rains at the end of October had repercussions on late plantings, whose bolls did not all reach maturity. Thus, maintaining rapid and health field germination is necessary to give a high yield of good quality. To cope with drought stress, the breeding of tolerant varieties, exogenous application of growth regulators, osmoprotectants and plant mineral nutrients, and alteration in cropping patterns, etc. are being followed (Venkateswarlu and Shanker, 2009; Vurukonda *et al.*, 2016). However, most of these practices are highly technical and the breeding of drought tolerant varieties is quite difficult due to the complex genetic nature of drought stress, inadequate knowledge on drought responsive genes/QTLs, and involvement of multidimensional stresses (Khan *et al.*, 2019). In recent years, various biotechnological approaches such as QTL mapping, characterization of drought-responsive genes, genome-wide association studies, and genetic engineering are being followed to mitigate the effects of

drought stress, but the risk assessors still face challenges in assessing the food and environmental safety of genetically modified crops (Liang, 2016). On the other hand, agronomic management strategies such as surface tillage, spraying of anti-transpirants, selection of water-use efficient genotypes, and reducing the evaporation by mulching are considered as the static tools in managing the drought stress, but these practices increase the cost of cultivation and are often inadequate on reconsideration for controlling crop performance (Ferrante and Mariani, 2018). Seed hydropriming is a simple, easy, low-cost, practical and environmentally friendly technique, which involves the soaking of seeds in normal water and dehydrating them to their original moisture content before sowing (Singh et al., 2015). It has emerged a precise technology used frequently to assure synchronized seed germination with high seedling vigor in field under the adverse ambient (soil /water) as well as environmental conditions (Kumar et al., 2018; Singhal et al., 2019; Kumar et al., 2020). The purpose of this study was to evaluate the impact of hydropriming on germination of two varieties of *G. hirsutum* under drought stress.

MATERIALS AND METHODS

Plant material: The plant material consisted of linted seeds of two varieties of *G. hirsutum* currently cultivated in Togo: variety STAM 129A covers essentially the Savannah and Maritime regions, and variety STAM 190 covers the Krara, Central and Plateaux regions (Table 1). These varieties are sensitive to water deficit (Koffi et al., 2021). The seeds were supplied by Wet Savannah Agronomic Research Center in Anié (Togo) (N 7°47'56"; E 1°17'38"). This center is interested, among other things, in the creation of high-performance varieties that meet the ever-increasing requirements of producers (high field yield, resistance to pests and water deficit), development companies (high ginning yield) and the international market (good technological quality).

Table 1. Characteristics of the different varieties of *G. hirsutum* studied

Varieties	Origin	Year of creation	Cotton-seed yield (kg/ha)	Precocity	Port
STAM 190	Togo	2012	1046	Early	Elongated-arborescent
STAM 129A	Togo	2002	1062	Early	Elongated-arborescent

Source: Wet Savannah Agronomic Research Center, Anié (Togo)

Seed imbibition kinetics to set hydropriming delays: Seed imbibition kinetics allows to identify the reversible phase of seed germination which is used to set the priming delay. It was determined by measuring the cumulative percentage of water absorbed (Le Deunff et al., 1989). 100 seeds are put to soak in water at 25 °C and weighed every 2 h during 36 h.

$$\% \text{ of water absorbed at time } t = \frac{[\text{Weight}(t) - \text{Weight}(\text{dry})]}{\text{Weight}(\text{dry})} \times 100$$

This preliminary study showed that kinetic of imbibition of cotton seeds is characterized by a triphasic pattern (Figure 1), which is similar to the pattern in most seeds. The germination irreversibility threshold is around 18 hours for the two cotton varieties studied. Thus, the priming time should not exceed 18 hours.

Seed hydropriming and experimental design: Hydropriming consisted of soaking the seeds in distilled water at 25 °C, followed by drying under ventilation, until the seeds regained their initial weight (7-8 %). In order to precisely define the desired level of hydration of primed seeds and thus prevent radicle protrusion, several seed priming durations were tested: 3 hours (3H-hydropriming), 6 hours (6H-hydropriming), 9 hours (9H-hydropriming), 12 hours (12H-hydropriming), 15 hours (15H-hydropriming) and 18 hours (18H-hydropriming). The effects of priming on germination were evaluated

at the optimal moisture conditions (0 bar) and under drought stress (-3 bar, -6 bar and -9 bar) by using PEG 6000 according to Micheland Kaufmann (1973):

$$\Psi_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$

Ψ_s : osmotic potential of PEG-6000 solution in bar; C : concentration of PEG-6000 in g/kg H₂O ; T : temperature in degrees C.

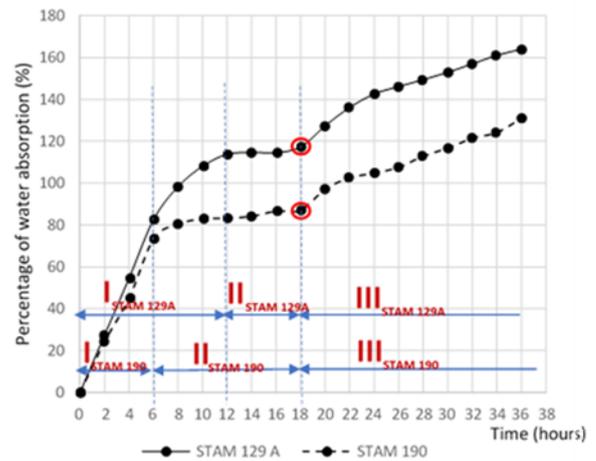


Figure 1. Kinetic of imbibition of seeds. I: imbibition phase; II: germination phase “in the strict sense” (reversible phase); III: growth phase (irreversible phase); in this figure, these phases are identified by comparison with the control batch; O: end of the reversible phase

For germination tests, carried out in 90 mm Petri dishes, four repetitions of 25 seeds were placed in paper rolls soaked in solution in a ratio (volume / paper weight) of 3:1, and incubated at 25°C. The substrate was replaced 48 and 96 hours after beginning the assay to avoid fungal growth due to moisture. The assay was repeated twice and completed following a randomized statistics design. Seeds were considered to have germinated upon the emergence of radicles (≥ 1 mm). After 7 days, the growth parameters were measured by International Seed Testing Association (ISTA) standard method.

Measurement of seedlings-related parameters: Germination percentage (GP) which is an estimation of the viability of a population of seed was assessed according to following formula:

$$GP = \frac{\text{Number of germination seed}}{\text{Total number of seed sown}} \times 100$$

Mean germination time (MGT) which represented the mean time a seed requires to initiate and end germination was calculated based on the following equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum Ni \cdot Ti}{\sum Ni}$$

Where Ni is the number of seeds which germinated on the day Ti

The germination index (GI), which is a measure of the percentage and speed of germination. Higher values for this measure indicate a

greater rate of germination. It was calculated as describe in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$GI = \sum Gt / Dt$$

where Gt = is the number of germinated seeds on day t and Dt = is the time corresponding to Gt in days

Relative PEG injury rate (RPIR) was the measurement of cumulative effect of PEG treatment at different concentration on germinating percentage. It was calculated based on following method:

$$RPIR = \frac{GP \text{ in control} - GP \text{ in PEG treated seeds}}{GP \text{ in control}}$$

Statistical analysis: Data were analyzed as a factorial experiment (7 priming treatments × 4 osmotic potential values). The equality of variances was first tested using Hartley's test. This test was non-significant (rejection of the equality of variances), which prompted us to resort to the angular transformation ($Y = 2\text{ArcSin}\sqrt{X}$) of the various germination indices calculated, before analyzing them using ANOVA and the mean Newman-Keuls test with the statistics software R version 4.3.1 at the 5% significance level. Regression equations between the various parameters measured are generated to highlight any existing correlations.

RESULTS

Effect of hydropriming on cotton seed germination: Seed germination parameters were significantly affected by priming time, irrespective of germination conditions. In addition, all calculated germination attributes were affected by the addition of PEG, but to a lesser extent when seeds were primed. In both cotton varieties studied, final germination showed a curvilinear response to priming time, both in normal and drought stress conditions, with optimum values at 12H-hydropriming (Figures 2, 3, 5). Indeed, 12H-hydropriming provided the highest values for germination percentage and germination index, while showing the lowest values for the relative PEG injury rate. For STAM 129A, 12H-hydropriming increased the germination percentage by 34.70% under normal hydrous conditions, and by 206.25% under drought stress (average of values measured at -3 bar, -6 bar and -9 bar) (Figure 2). STAM 190 showed a 27.90% increase in germination percentage under normal hydrous conditions, and 179.26% under drought stress. Germination, previously nil at -6 bar and -9 bar for unprimed seeds of both cotton varieties, becomes possible with priming and better with 12H-hydropriming. With 12H-hydropriming, STAM 129A achieved a maximum of 32 germinated seeds per day at 0 bar, compared with an average of 9 under drought stress, representing increases of 154.32% and 250.76% respectively, compared with 148.43% and 289.55% for STAM 190 (Figure 3). The addition of PEG hindered cotton seed germination, given the relative PEG injury rate values calculated. However, this damage was reduced 35.89 % (for STAM 129A) and 27.10 % (for STAM 190) with 12H-hydropriming (Figure 5).

In general, under normal hydrous conditions, primed seeds germinated one day earlier than unprimed seeds, with a mean germination time of 4.7 days (unprimed seeds) and 4.06 days (primed seeds) for STAM 129A, compared with 4.72 days (unprimed seeds) and 4.02 days (primed seeds) for STAM 129A (Figure 4). However, under drought stress conditions, mean germination time did not vary significantly from the control, approximately 4.56-4.77 days (rounded to 5 days). Longer priming, 18H-hydropriming, had a repressive effect on seed germination, although the few seeds that germinated did so earlier, at around 3.83-4.02 days under normal hydrous conditions, and 4.33-4.46 days under drought stress conditions. On the basis of the analysis of average measurements, 12H-hydropriming

of seeds appears to be, for the two cotton varieties studied, the best treatment for maintaining germinative vigor under drought stress. It can therefore be recommended for the cultivation of STAM 129A and STAM 190 cotton varieties in Togo, in order to maintain germinative vigor during the sowing period, without worrying about a possible drought pocket.

Relationships between different measured parameters

Figures 6 and 7 show the regression equations between the different germination parameters calculated under drought stress. Germination percentage (GP), germination index (GI) and relative PEG injury rate (RPIR) are linearly correlated with each other, defining proportional relationships. GP and GI are positively correlated with each other, but negatively with RPIR. For the two cotton varieties, the weighted points of 12H-hydropriming, and to a large extent 9H-hydropriming of seeds, are always positioned in favor of increasing GP and GI, and against increasing RPIR.

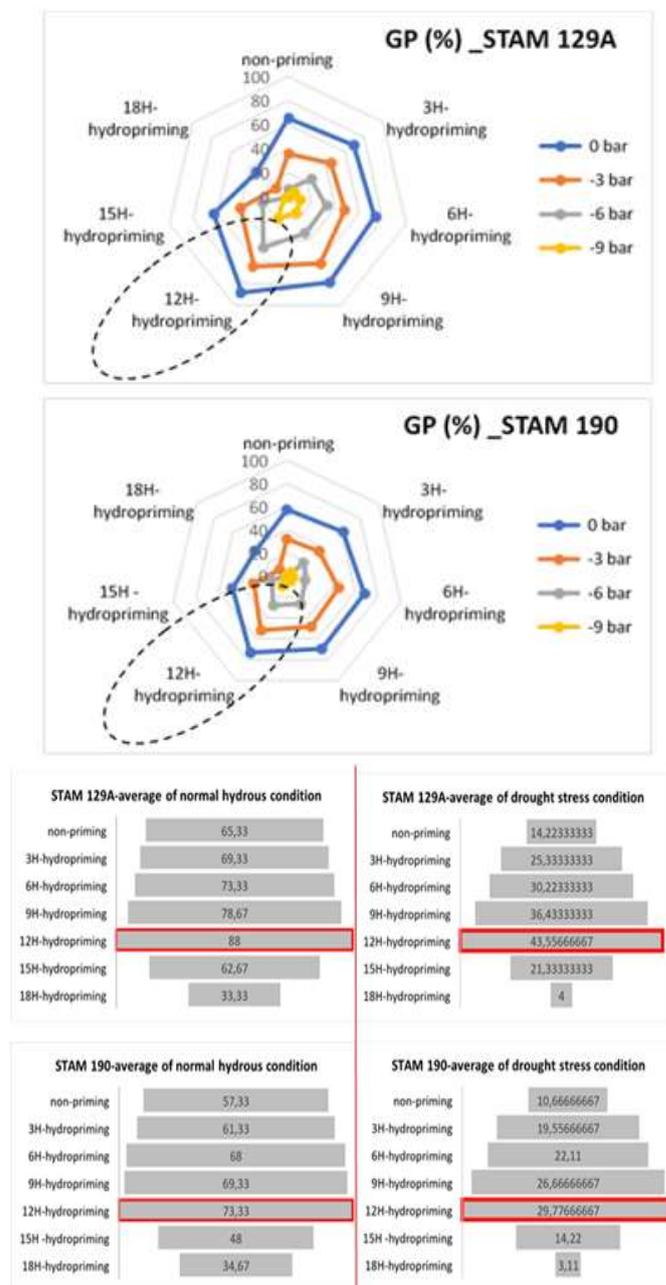


Figure 2. Effect of cotton seed hydropriming on Germination percentage (GP)

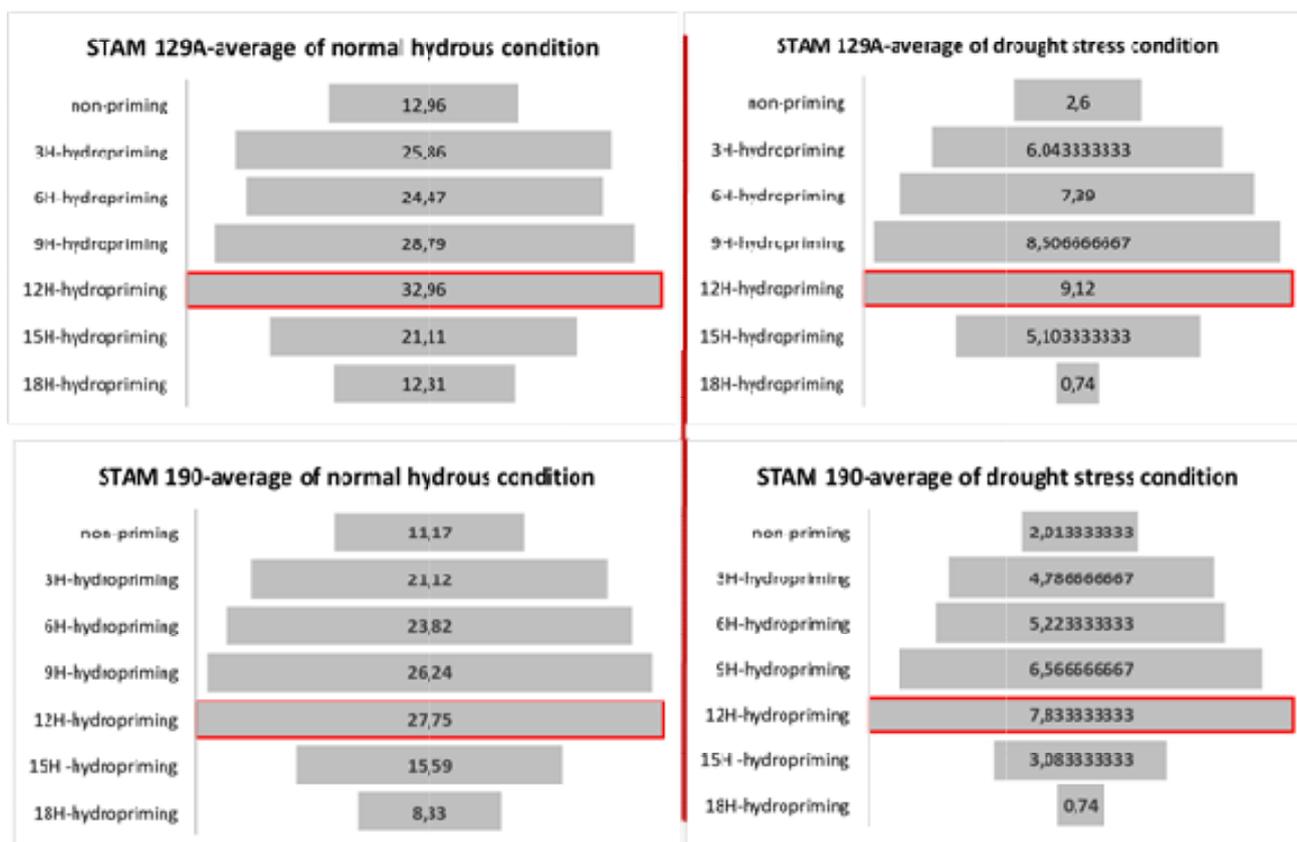
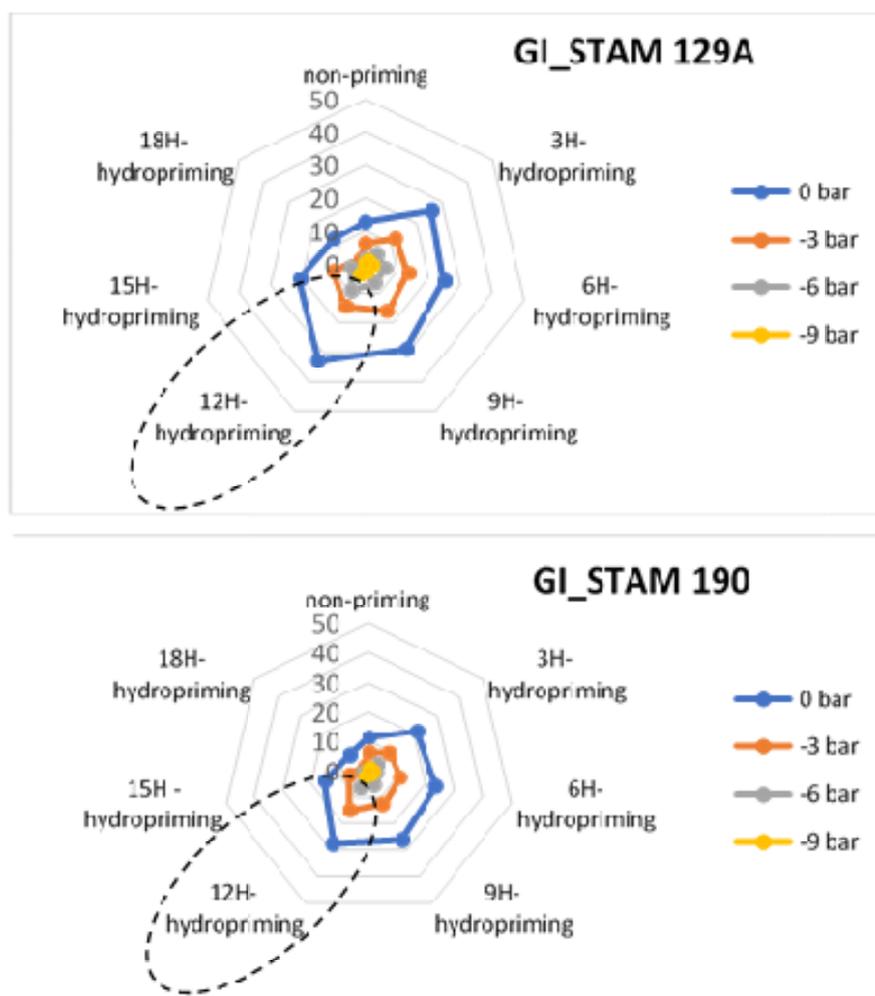
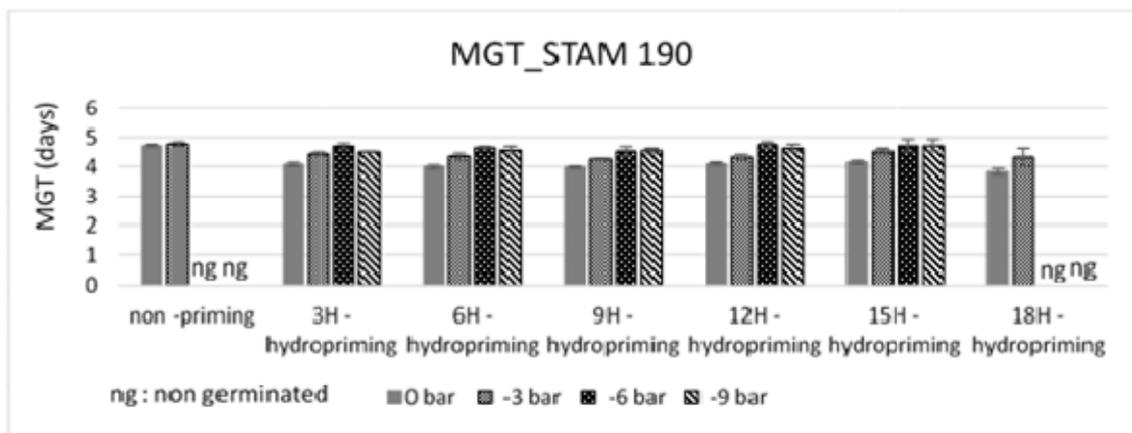
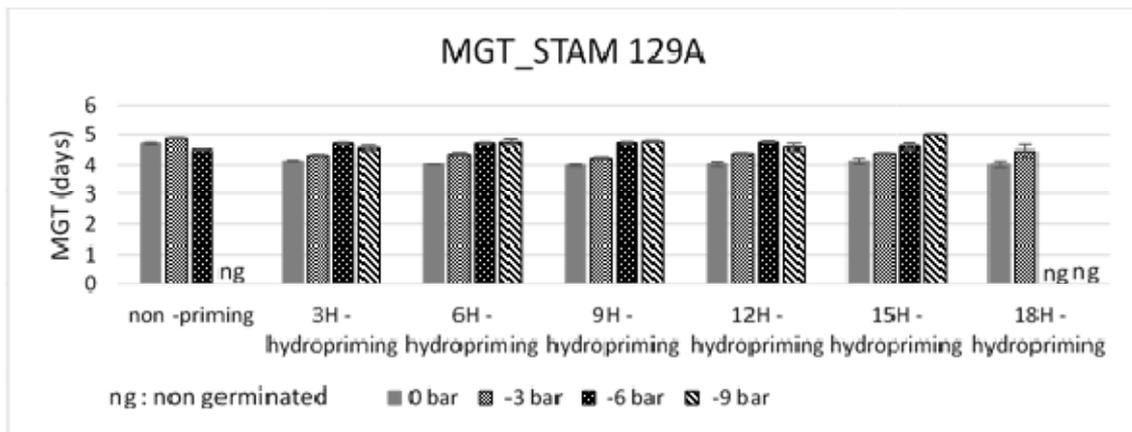


Figure 3. Effect of cotton seed hydropriming on Germination index (GI)



STAM 129A-average of normal hydrous condition

non -priming	4,7
3H -hydropriming	4,13
6H -hydropriming	4,04
9H -hydropriming	4,01
12H -hydropriming	4,04
15H -hydropriming	4,13
18H -hydropriming	4,02

STAM 129-average of drought stress condition

non -priming	4,68
3H -hydropriming	4,523333333
6H -hydropriming	4,6
9H -hydropriming	4,566666667
12H -hydropriming	4,566666667
15H -hydropriming	4,663333333
18H -hydropriming	4,46

STAM 190-average of normal hydrous condition

non -priming	4,72
3H -hydropriming	4,08
6H -hydropriming	4
9H -hydropriming	3,99
12H -hydropriming	4,1
15H -hydropriming	4,15
18H -hydropriming	3,83

STAM 190-average of drought stress condition

non -priming	4,77
3H -hydropriming	4,546666667
6H -hydropriming	4,523333333
9H -hydropriming	4,443333333
12H -hydropriming	4,57
15H -hydropriming	4,66
18H -hydropriming	4,33

Figure 4. Effect of cotton seed hydropriming on Mean germination time (MGT)

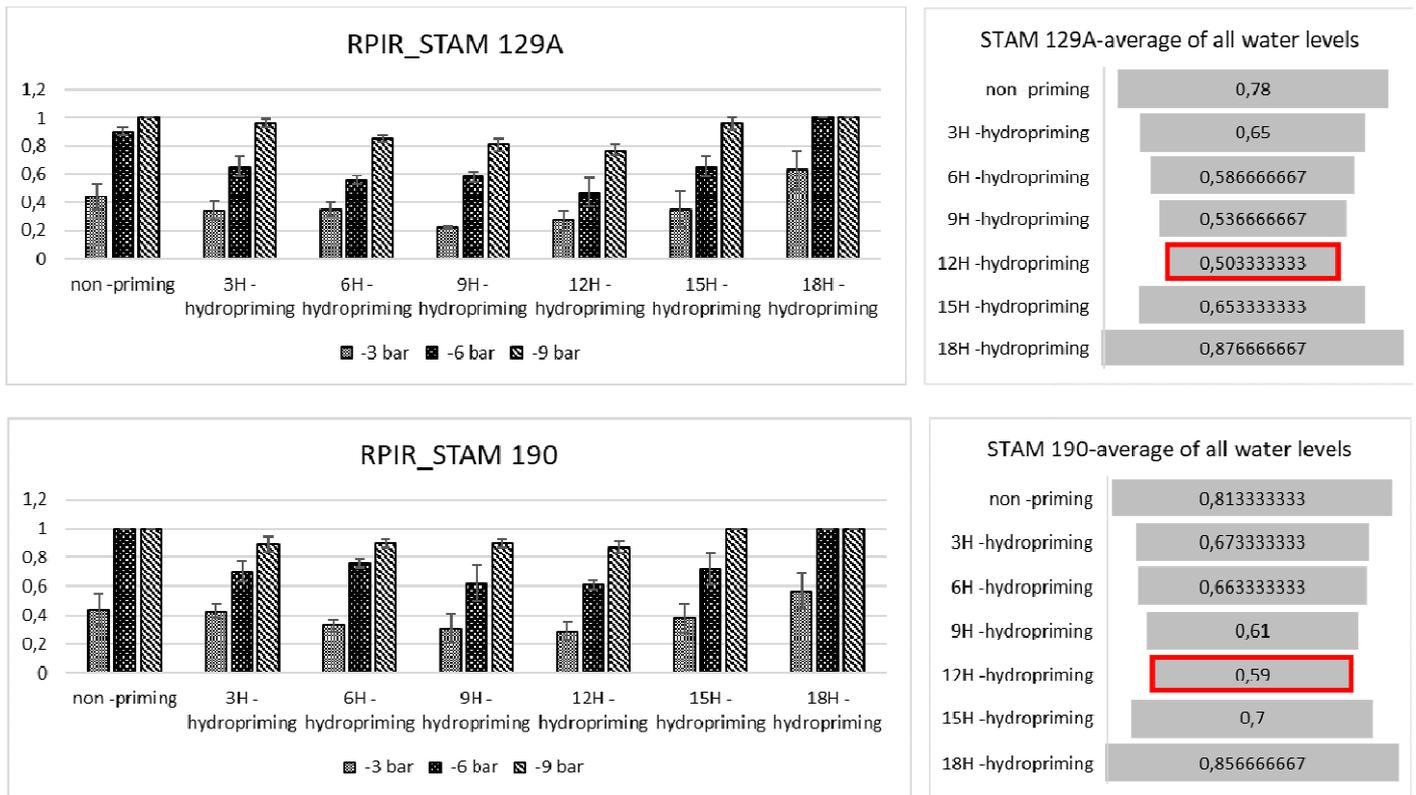


Figure 5. Effect of cotton seed hydropriming on Relative PEG injury rate (RPIR)

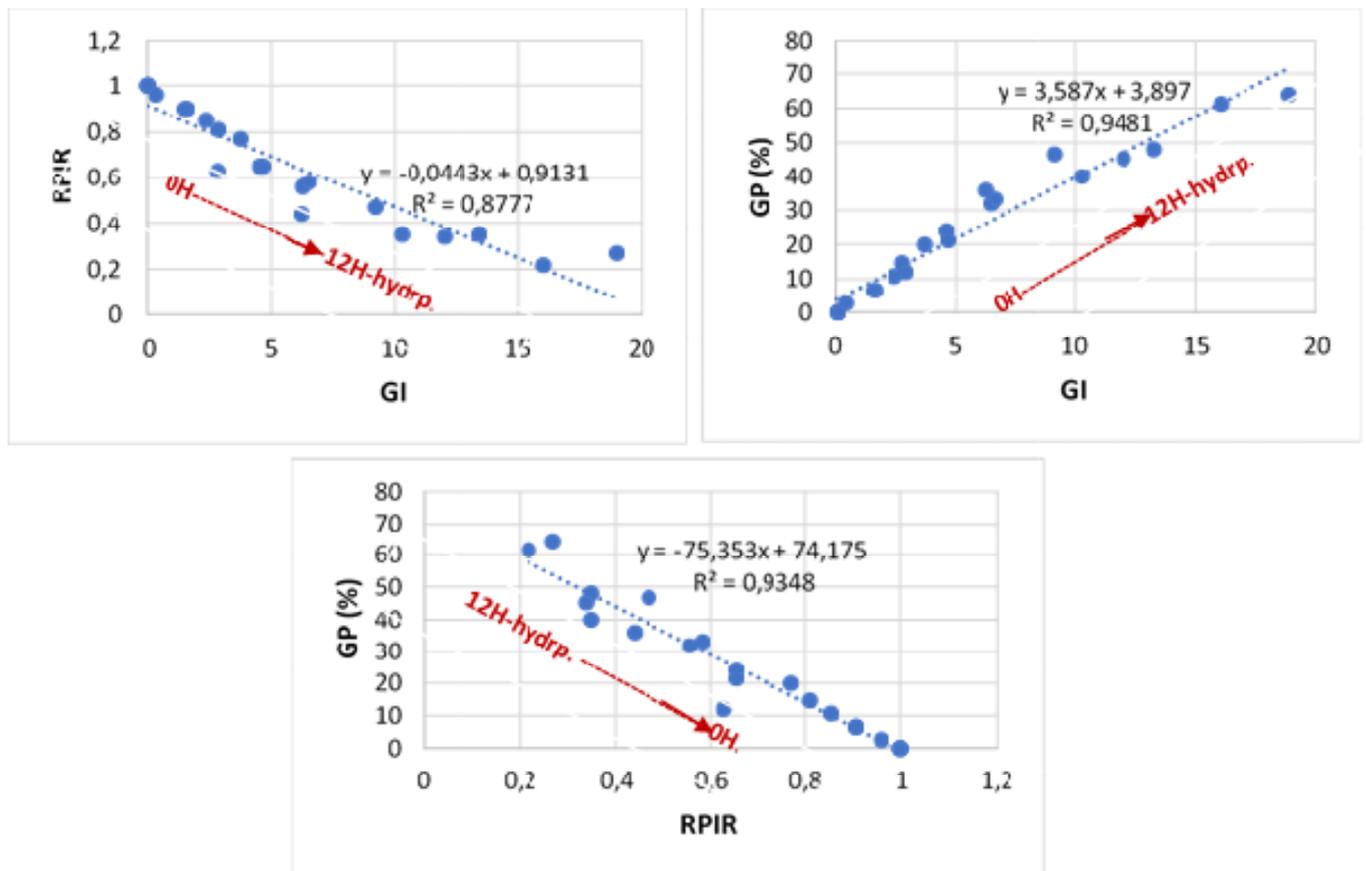


Figure 6. Regression equations under drought stress between parameters measured (STAM 129 A). GP: germination percentage; GI: germination index; RPIR: Relative PEG injury rate; 0H: non-priming; 12H-hydrp.: 12H-hydropriming.

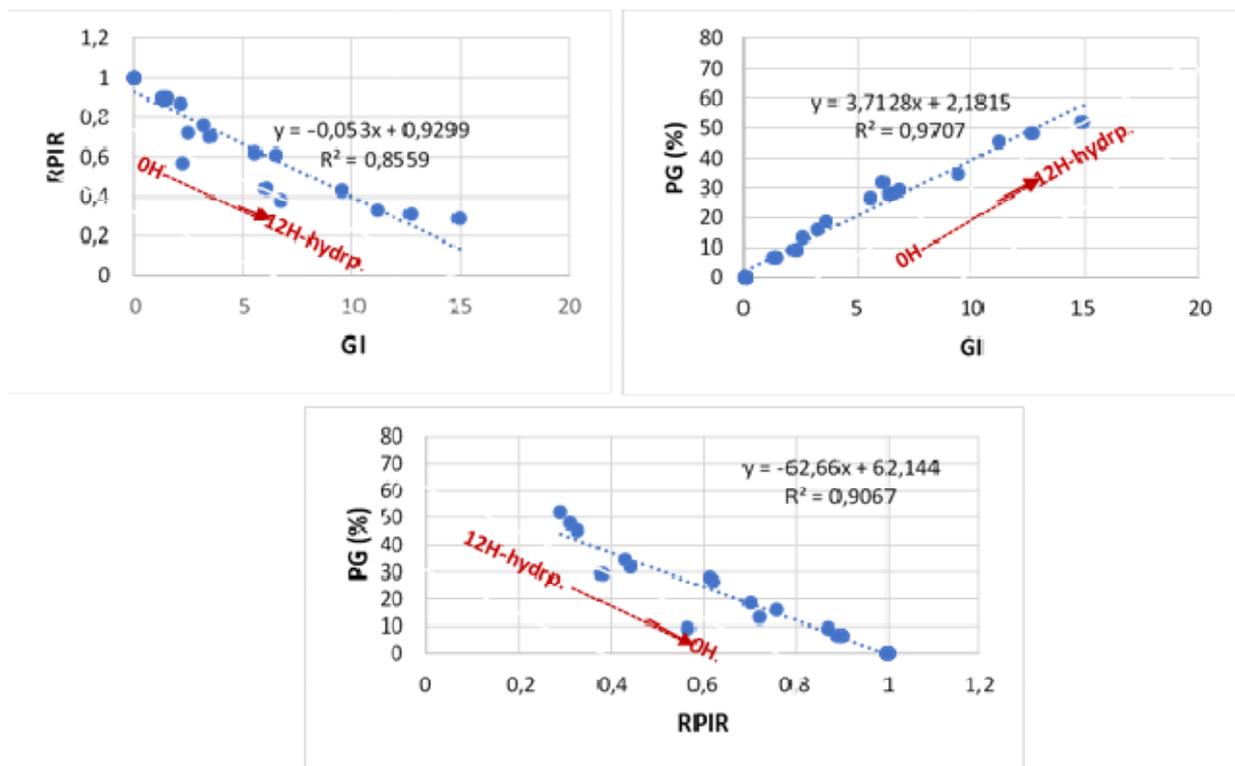


Figure 7. Regression equations under drought stress between parameters measured (STAM 190). GP: germination percentage; GI: germination index; RPIR: Relative PEG injury rate; OH: non-priming; 12H-hydrp. 12H-hydropriming

DISCUSSIONS

According to the results, hydroprimed seeds indicated higher germination percentage and germination index, and lower mean germination time and relative PEG injury rate than non-primed seeds under drought treatments. 12H-hydropriming resulted in a significant advantage in terms of germination capacity and speed, whatever the water conditions. Alvarez and Perna (2018) also found that 12H-hydropriming promoted better germination of cotton seeds of the Fiber Max 1830, Fiber Max 2334 and Sinuana M 137 genotypes, under normal conditions. Previous results confirmed that water uptake and then germination traits are improved by priming treatment under drought stress in cotton (Casenave and Toselli, 2007; Naguib, 2019; Awan *et al.*, 2022).

Pre-sowing treatment improves the pre-germination metabolic processes through imbibe sufficient water and makes the seed ready for radicle emergence (Ibrahim, 2016). Hydropriming is one of the seed enhancement methods that might be resulted in increased seed performance (germination and emergence) under stress conditions (Rambod *et al.*, 2016; Hussain *et al.*, 2017). During priming the seed is taken through the first biochemical processes within the initial stages of germination bringing the seed close to the point of germination (Mustafa *et al.*, 2017). Seed priming causes an advancement of metabolic status during the prolonged phase of imbibition that prepares the radicle protrusion (Naguib, 2019). It augments moisture content with continuous oxygen supply and induces the activation of pregermination enzymes (amylase, cellulase, and xylanase), to convert stored food products (carbohydrates, proteins and lipids) into simpler forms (ATP) for pre-germinative metabolic processes. Seed priming also induces enhancing metabolite production, protein synthesis and osmotic adjustment (Damalas *et al.*, 2019; Lemmens *et al.*, 2019; Mirmazloum *et al.*, 2020; Chakma *et al.*, 2021), antioxidant activity and storage protein solubilization and minimizes lipid peroxidation (Iseri *et al.*, 2014).

Mainly during seed priming portion of the seed endosperm is hydrolyzed that permits faster embryo growth (Afzal *et al.*, 2012). Sung *et al.* (2008) reported that the positive impact of primed seeds on germination rate is triggered by cell cycle regulation and cell elongation processes. Priming significantly increases the quantity of mitochondria and upregulation of proteins for cell division (α - and β -tubulin) (Mustafa *et al.*, 2017). Rehydration through seed priming brings major cellular changes in seeds such as de novo synthesis of nucleic acids and proteins, activation of sterols and phospholipids, and repairing DNA damaged during threshing (Padgham, 2009; Zulueta-Rodríguez *et al.*, 2015). Priming reduced germination time by two days for both varieties. Faster seedling emergence from primed seeds would result in better resource acquisition and utilization and more time for optimal growth and seed filling, resulting in higher yield (Murungu *et al.*, 2010).

The process of seed germination is a tri-phase phenomenon which includes an initial phase of rapid early water uptake, followed by a plateau phase with little change in water content; and subsequently, the last phase which coincides with radicle emergence and resumption of growth. Primed seed completed first two phases during the priming process so immediately germinate after sowing (Ahmadvand *et al.*, 2012). The repressive effect of 18H-hydropriming indicates that cotton seed priming as a method of improving germinative vigor should occur in a controlled manner up to a well-defined water point, just before radicle emergence (Lutts *et al.*, 2016). Too long a priming would affect seed structure and physiology, due to anatomical damage, enzymatic imbalance, oxidative stress, defective solubilization and transport of metabolites and nutrients from reserve digestion (Çokkizgin and Bölek, 2015). This would be linked to the fact that cotton, from sowing to harvesting, is a very sensitive plant to oxygen deficiency caused by excess water (Wang *et al.*, 2017). For all the germination parameters measured, the results are broadly similar for the two cotton varieties studied. STAM 190 is a descendant of the same complex cross as STAM 129A grown in Togo. This would explain the similarity in germination behavior between the seeds of the two varieties.

CONCLUSION

This study provides useful information on the germination of cotton seeds of the STAM 129A and STAM 190 varieties grown in Togo, and their performance when hydroprimed. In general, seed hydropriming of the two cotton varieties studied improved germination parameters, in agreement with previous studies on other cotton varieties. Seed priming increased the attributes of germinative vigor (GP and GI) and tolerance (RPIR) to dry conditions. The magnitude of the response is associated with the duration of priming, and is more evident under constrained water conditions. For both varieties, 12H-hydropriming remains the most effective treatment under both normal and stress conditions. For sowing cotton seeds of STAM 129A and STAM 190 varieties, we therefore recommend soaking the seeds for 12 hours on the eve of planting, from 6:00 or 7:00 p.m. to 6:00 or 7:00 a.m. on the day of sowing.

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