Report on identifying a protocol to elicit flowering in *Brachiaria humidicola* with photoperiod management

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Summary

Two Genotypes of *Brachiaria humidicola* (A and B) were planted on the grounds of CIAT headquarters in Palmira during 2018 – 2019, 10 lamps were placed in the lot to evaluate 6 different photoperiods (1 - 6) with Light in 2 different wavelength range (W.R.) α and β, for this, 17 samples were carried out on the variables height, vigor, chlorophyll content and number of inflorescences; a total of 93 field work were carried out to support the trial, finding that the photoperiod 5 in the W.R. β and 3 photoperiod in the W.R. α for the B genotype show significant differences ($p < 0.05$, Tukey) with respect to the other treatments for height and number of inflorescences, performing the statistical analysis in the SAS software. As to the seed production, it was found that any light stimulus generates greater seed production, despite the conditions under which the crops were made and the method of harvest used. I order to refine the protocol and validate the results in bigger genotype sample another trial with the 2 most efficient treatments was proposed for 2020, focusing on number of inflorescences and seed production.

Objectives

Evaluate the induction of flowering in *Brachiaria humidicola* through exposure to different photoperiods.

Find the best photoperiod and Light wavelength range treatments that allow to induce flowering in *Brachiaria humidicola.*

Germplasm

Stolons of *Brachiaria humidicola* were taken, Genotype B which has a low amount of inflorescences and low height, and Genotype A which has a high amount of inflorescences and greater height, these genotypes are selected to look at the contrast with the treatments they undergo.

Essay approach

The essay is the FM1810 of the breeding program of *Brachiaria humidicola*, was designed to reduce flowering periods in *Brachiaria humidicola*. The effect of two different Light wavelength range will be tested, 6 photoperiods are being evaluated for each Light wavelength range, in which 1 is the control; in addition to the two contrasting genotypes explained above, this is a total of 24 treatments with 4 repetitions each, for a total of 96 experimental units.

The experimental units are established 1m^2 plots. In the field, 4 plots of each genotype are arranged under a lamp with a Light wavelength range and a photoperiod; These treatments are separated from each other by a live barrier of corn (*Zea maiz*) which prevents light contamination between treatments, as shown below (Fig. 1).

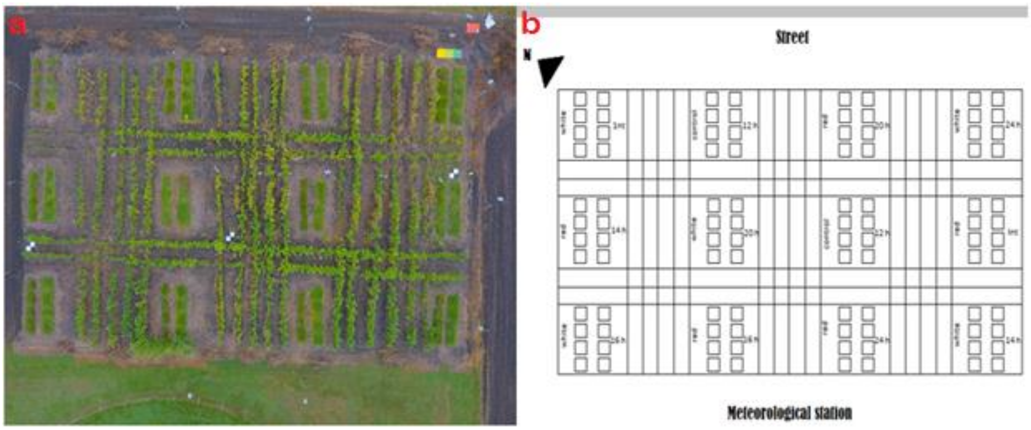

Fig. 1. Arrangement of field experiment FM1810 plots; *a)* photo of the trial taken in DRON, *b)* planting design planned for the trial.

Data collection

The data was collected in the different samples, a total of 17, in which the variables height, vigor, chlorophylls, number of inflorescences were evaluated, and the seed production data in grams calculated after harvesting. The variables are being measured as follows:

Height: The evaluation is carried out with the prairie measuring plate, which allows to obtain an average value, which better represents the behavior of the height in the plot, in addition to speeding up the measurement process.

Vigor: It is measured with the greenseeker, pointing it to the plot at a height of 1.20 m, and reports a value that represents the greenery that the plot has, in response to a normalized differential vegetation index (NDVI).

Chlorophylls: It is measured with the chlorofilometer in SPAD units, taking 5 representative points of each plot and reporting the average value of these points.

Number of Inflorescences: A 0.5 m x 0.5 m PVC frame is used as the sampling unit, placed on the plot and the inflorescences counted there are carried out taking into account any phenological state in which they are found.

Seed harvest: Plants were observed daily after flowering to determine the optimum time for harvesting, after the cohort was harvested cutting the whole inflorescence and stored until the ripening was complete. Collected seed was subjected to cleaning and weighting in the seed lab followed by storage at cold room.

Statistical analysis

The data were analyzed with the SAS software, after checking the normality assumptions, making an ANOVA Table, the means of each variable were compared by Tukey test (a = 0.05).

Results

The results obtained are reported based on the statistical analysis for each of the variables.

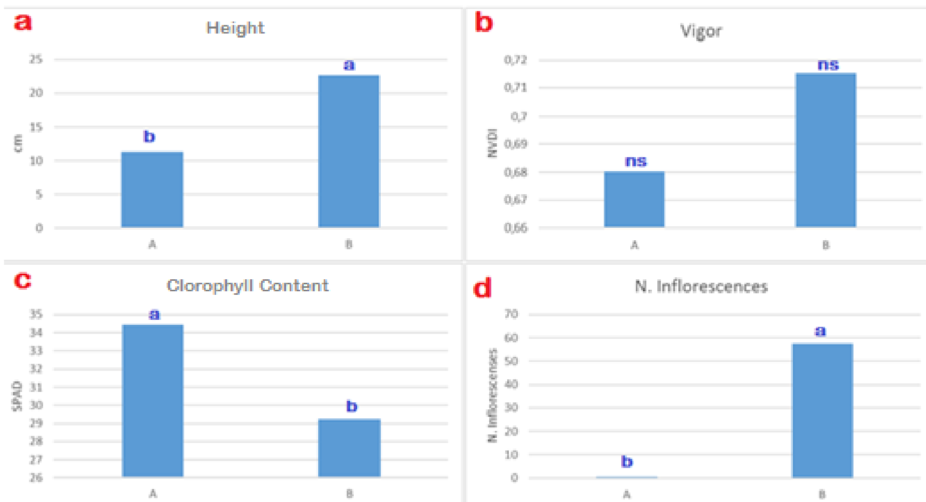

Fig.2. Comparison among Genotypes (A, B) for each one of the variables; *a)* Height for each Genotype in cm, *b)* Vigor for each Genotype in NDVI, *c)* Chlorophyll content for each Genotype in SPAD, *d)* N. Inflorescences for each Genotype in 0.25 m^2. Letters indicate differences between treatments ($p < 0.05$, Tukey).

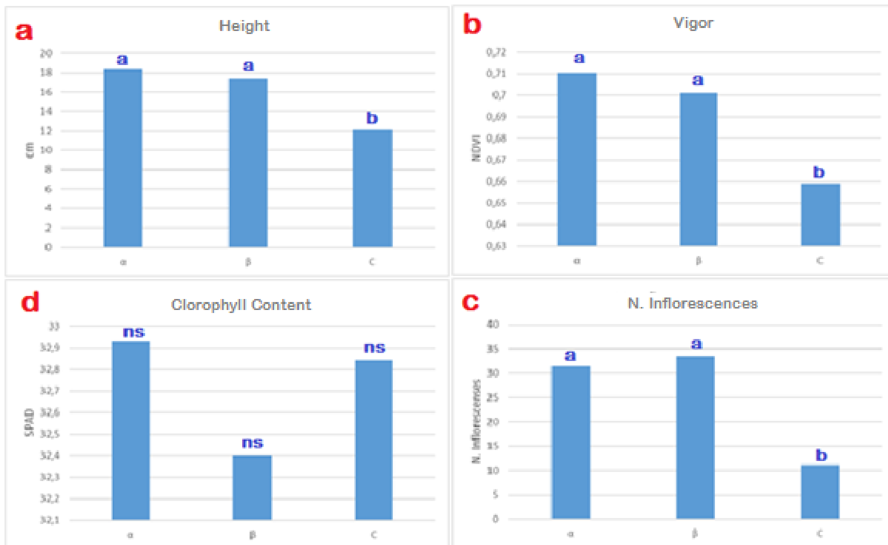

Fig.3. Comparison different W.R for each of the measured variables. *(α, β, C)*; *a)* Height for each W.R. in cm, *b)* Vigor for each W.R. in NDVI, *c)* Chlorophyll content for each W.R. in SPAD, *d)* N. Inflorescences for each W.R. in 0.25 m^2. Letters indicate differences between treatments (p <0.05, Tukey).

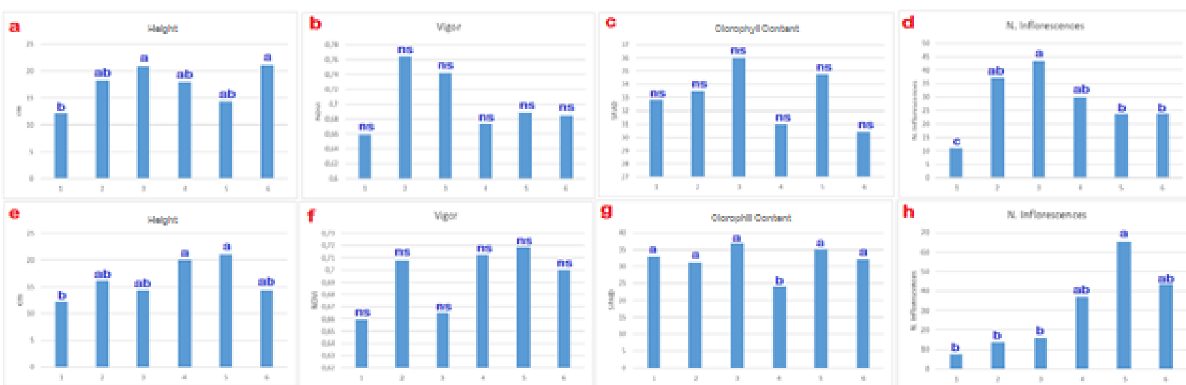

Fig.4. Comparison among different photoperiod treatments (1 – 6) in the α and β W.R. *a)* Height for each photoperiod in cm in the α W.R., *b)* Vigor for each photoperiod in NDVI in the α W.R., *c)* Chlorophyll content for each photoperiod in SPAD in the α W.R., *d)* N. Inflorescences for each photoperiod in 0.25 m^2 in the α W.R., *e)* Height for each photoperiod in cm in the β W.R., *f)* Vigor for each photoperiod in NDVI in the β W.R., *g)* Chlorophyll content for each photoperiod in SPAD in the β W.R., *h)* N. Inflorescences for each photoperiod in 0.25 m^2 in the β W.R., Letters indicate differences between treatments (p <0.05, Tukey).

It is observed in Fig. 2. that Genotype A shows better results in height, vigor and number of inflorescences / 0,25 m^2 in contrast to Genotype B. It is also evident in Fig. 3. that the W.R. α and β induce the plants to have a greater height, vigor and N. Inflorescences/ 0,25 m^2, however chlorophylls are not affected by this factor.

Fig. 4. shows the positive effects of photoperiod stimulation on *Brachiaria humidicola* plants, for the height and chlorophyll content variables, as for the N. of inflorescences (which is the one of greatest interest in this trial). We observe that for treatments with W.R. α, the number of inflorescences

increases progressively until photoperiod 3 and then decays, while for W.R. β the courve remains linear until photoperiod 5, these being the photoperiods that presented the greatest number of inflorescences in the trial.

For the factor interactions, it was found that Genotype B * W.R. α * Photoperiod 3 and Genotype B * W.R. β * Photoperiod 5, produced the highes amount of inflorescences, therefore those were selected and used in the following trial.

Seed production:

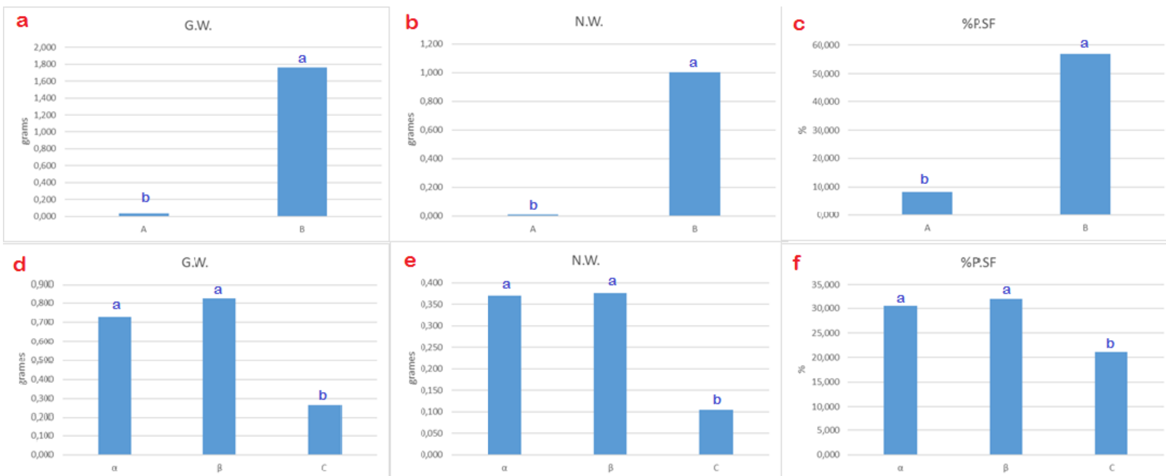

Fig.5. Comparison among genotypes for seed production traits: *a)* Gross weight for each Genotype in grams, *b)* Net weigth for each Genotype in grams, *c)* Seed filling percentage for each genotype, *d)* Gross weight for each W.R. in grams, *e)* Net weigth for each W.R. in grams, *f)* Seed filling percentage for each W.R., Letters indicate differences between treatments (p <0.05, Tukey).

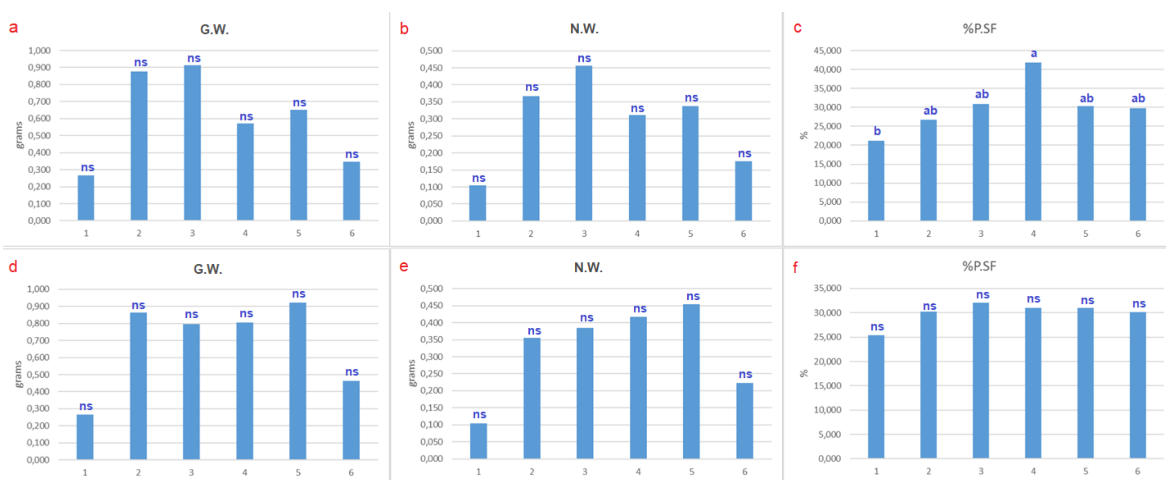

Fig.6. Comparison among treatments for Seed production traits, G.W., N.W. and %P.SF vs Photoperiod *a)* Gross weight for each Photoperiod in grams in the α W.R., *b)* Net weight for each Photoperiod in grams in the α W.R., *c)* Seed filling

percentage for each Photoperiod in the α W.R. *d)* Gross weight for each Photoperiod in grams in the β W.R., *e)* Net weight for each Photoperiod in grams in the β W.R., *f)* Seed filling percentage for each Photoperiod in the β W.R., Letters indicate differences between treatments ($p < 0.05$, Tukey).

For the seed production, there are certain doubts regarding the veracity of the analysis performed, as there were a number of events that could interfere with the proper development of the trial, watching the Fig.5. it becomes clear that the control is the one with the lowest production of seeds in terms of gross weight, net weight and percentage of seed filling.

By separating the photoperiods in W.R., according with the Fig.6. We noticed that there are only significant differences in %F.SP. However, this behavior is not representative since the harvest method chosen for testing was not the most appropriate.

Next steps

An essay with the best photoperiod treatments and bigger amount of genotypes will be developed, in order to determine the most optimal protocol for the induction of flowering in *Brachiaria humidicola.*

Harvest method used must be changed and perform it more carefully, the most appropriate would be "ordeñado".

Annexed

Visual appreciation of the trial

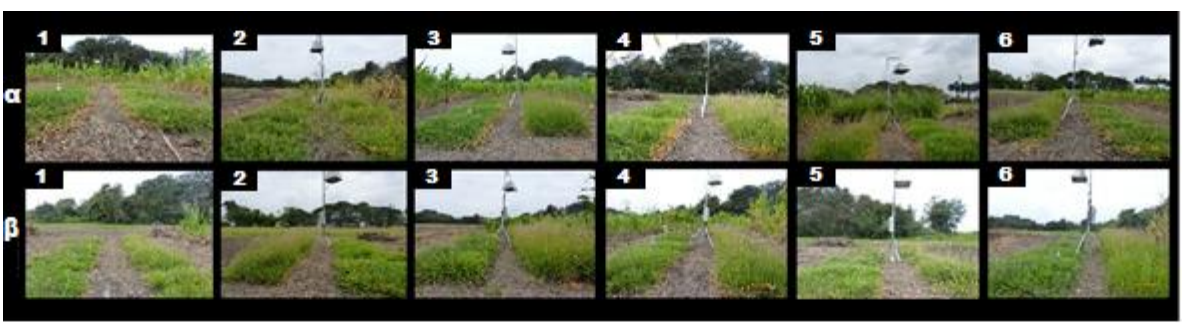

Fig.7. Visual behavior of the treatments in field.

Activities performed

In order to keep the test in an adequate state, optimize the sampling of variables and integrity of the artifacts arranged there, the following activities have been carried out to date (which are recorded in the test field book):

Table 1. Activities performed in the trial (FM1810)

#	DATE	ACTIVITIE
1	*19/12/2018*	*Sowing*
2	*11/01/2019*	*Lamps adecuation*
3	*-*	*Irrigation*
4	*15/01/2019*	*Sowing*
5	*11/01/2019*	*Lamps instalation*
6	*28/03/2019*	*Siembra de maiz*
7	*25/04/2019*	*Weed control*
8	*26/04/2016*	*Evaluation1*
9	*27/04/2019*	*Weed control*
10	*29/04/2019*	*Evaluation1*
11	*-*	*Estandarization*
12	*-*	*Labeling*
13	*6/05/2019*	*Evaluation1*
14	*18/05/2019*	*Pesticide application*
15	*13/05/2019*	*Flowering evaluation1*
16	*20/05/2019*	*Harvest 2*
17	*20/05/2019*	*Labeling 2*
18	*21/05/2019*	*Pesticide application*
19	*22/05/2019*	*Harvest*
20	*27/05/2019*	*Seed benefit*
21	*30/05/2019*	*Estandarization cut*
22	*30/05/2019*	*Fertilization a pound of the corn*
23	*5/05/2019*	*Fertilization of the plots*
24	*6/06/2019*	*Evaluation2*
25	*12/06/2019*	*Evaluation3*
26	*14/06/2019*	*Zadocks sampling*
27	*19/06/2019*	*Evaluation4*
27	*20/06/2019*	*Zadocks sampling*
27	*25/06/2019*	*Zadocks sampling*
27	*27/06/2019*	*Evaluation5*
27	*28/06/2019*	*irrigation*
27	*3/07/2019*	*Evaluation6*
27	*5/07/2019*	*Zadocks sampling*
28	*9/07/2019*	*Irrigation*
29	*10/07/2019*	*Irrigation*
30	*11/07/2019*	*Evaluation7*
31	*12/07/2019*	*Harvest*
32	*16/07/2019*	*Irrigation*
33	*17/07/2019*	*Irrigation*
34	*18/06/2019*	*Seed benefit*
35	*19/07/2019*	*Irrigation*

36	*22/07/2019*	*Corn sowing*
37	*22/07/2019*	*Plots cut*
38	*23/07/2019*	*Irrigation*
39	*24/07/2019*	*Irrigation*
40	*25/07/2019*	*Irrigation*
41	*26/07/2019*	*Irrigation*
42	*29/07/2019*	*Irrigation*
43	*30/07/2019*	*Irrigation*
44	*31/07/2019*	*Irrigation*
45	*1/08/2019*	*Irrigation*
46	*2/08/2019*	*Evaluation8*
47	*2/08/2019*	*Coverage implementation*
48	*2/08/2019*	*Irrigation*
49	*5/08/2019*	*Irrigation*
50	*5/08/2019*	*Report of lamps which don´t work*
51	*6/08/2019*	*Irrigation*
52	*8/08/2019*	*Irrigation*
53	*9/08/2019*	*Irrigation*
54	*9/08/2019*	*Evaluation9*
55	*12/08/2019*	*Irrigation*
56	*13/08/2019*	*Irrigation*
57	*14/08/2019*	*Coverage implementation*
58	*14/08/2019*	*Irrigation*
59	*15/08/2019*	*Irrigation*
60	*15/08/2019*	*Evaluation10*
61	*20/08/2019*	*Irrigation*
62	*21/08/2019*	*Irrigation*
63	*23/08/2019*	*Evaluation11*
64	*27/08/2019*	*Irrigation*
65	*28/08/2019*	*Irrigation*
66	*30/08/2019*	*Evaluation12*
67	*6/09/2019*	*Irrigation*
68	*13/09/2019*	*Irrigation*
69	*16/09/2019*	*Irrigation*
70	*17/09/2019*	*Irrigation*
71	*18/09/2019*	*Irrigation*
72	*19/09/2019*	*Irrigation*
73	*25/09/2018*	*Weeding*
74	*26/09/2018*	*Weeding*
75	*1/10/2019*	*Plots cut*
76	*2/10/2019*	*Corn sowing*
77	*4/10/2019*	*Lamps revition*
78	*10/10/2019*	*Evaluation13*

79	*11/10/2019*	*Plots fertilization*
80	*17/10/2019*	*Evaluation14*
81	*24/10/2019*	*Evaluation 15*
82	*28/10/2019*	*Delimit plots*
83	*29/10/2019*	*Delimit plots*
84	*1/11/2019*	*Scythe*
85	*8/11/2019*	*Pesticide application*
86	*12/11/2019*	*Plots fertilization*
87	*14/11/2019*	*Evaluation16*
88	*22/11/2019*	*Evaluation17*
89	*22/11/2019*	*Remove old corn plants*
90	*25/11/2019*	*Corn fertilization*
91	*29/11/2019*	*Harvest*
92	*19/12/2019*	*Remove lamps*
93	*19/12/2019*	*Seed benefit*