EFFECTS OF THE COUPLING OF WATER AND NITROGEN ON THE YIELD AND QUALITY OF KING GRASS (*PONNISETUM PURPUREUM* SCHUMACHER × *P. GLAUCUM* (LINNAEUS) R. BROWN)

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Abstract. Using the method of pot culture, the effect of different water and nitrogen conditions on the growth of King grass [*Pennisetum purpureum* Schumacher × *P. Glaucum* (Linnaeus) R. Brown] and its physiological response mechanisms were examined. The results showed that under the soil moisture conditions designated as W1 (35%-45%), W2 (55%-65%) and W3 (75%-85%), the application rate of nitrogen corresponded to maximum yields of 271.78, 355.05 and 329.06 kg hm⁻², and protein contents of 361.54, 505.44 and 647.88 kg hm⁻². With the increase of water stress, the proline content of King grass increased, while the CAT enzyme significantly decreased, and the CAT enzyme increased significantly with the nitrogen content under the same moisture condition. Soil moisture conditions had a greater effect on the yield and quality of King grass than the application rate of nitrogen, but there was a synergistic effect between them. Therefore, if water and nitrogen are properly matched, higher yield and quality can be obtained. The best conditions for water and nitrogen were found to be a soil moisture content of 75%-85% of field capacity and an application rate of 329.06 kg hm⁻² for nitrogen.

Keywords: nitrogen, field capacity, proline, catalase activity, Hainan

Introduction

King grass [*Pennisetum purpureum* Schumacher × *P. Glaucum* (Linnaeus) R. Brown] is a perennial grass, also called Napier, in Colombia or Pennisetum and elephant grass or Africa Pennisetum (Xu et al., 2006). King grass has a short growing season, high yield, more tillers, regeneration capacity and other characteristics (Zhao et al., 2015). Under moderate water and fertilizer conditions, King grass can grow for up to 9 months each year (Xie et al., 2012), fresh yield of 2.25×10⁵ kg hm⁻², stump tiller 50 to 80, the second year of high fresh yield (3.0-3.75)×10⁵ kg hm⁻². King grass not only provides high yield, but nutritious protein, the per hectare of which can reach that of 8-10 hm² corn. In addition, King grass is soft and juicy, highly palatability, and is one of the best feeds for herbivorous livestock and fish (Li et al., 2015).

Irrigation and application of chemical fertilizer are two major factors for agricultural production, and they are two important technical measures that also offer productivity control. In crop production, irrational use of water and chemical fertilizer resources can waste water and fertilizer and pose a serious threat to the environment (Zhou et al., 2016). Therefore, a study of rational application of water and chemical fertilizer aspect cannot be ignored. For the purpose of detect the effects of the coupling of water and nitrogen on the yield and quality of King grass, soil moisture and nitrogen fertilizer

were used as dependant variables in trial pot experiments to determine the impact of water and nitrogen coupling on King grass growth traits. In addition, the response of proline and catalase (CAT) and other physiological traits under different water and nitrogen conditions was also examined (Meng et al., 2019).

Material and methods

Materials

The *P. purpureum* × *P. Glaucum* CV. Reyan No. 4 was selected as test materials. The latsol granite was applied for pot culture of King grass. Soil texture is sandy loam soil, and the main physical and chemical properties shown in *Table 1*.

Table 1. Soil physical and chemical characteristics

pH	Organic matter g kg ⁻¹	Alkaline hydrolysis N mg kg ⁻¹	Available P mg kg ⁻¹	Available K mg kg ⁻¹
6.12	6.9	101.5	7.2	47.1

Experimental design

The soil was air-dried and passed through 2 mm mesh. Each pot, which was 31 cm × 29 cm (D × H), fitted 20 kg soil.

Set three soil moisture levels, namely: W1: saturation field capacity of 35%-45%; W2: saturation field capacity of 55%-65%; W3: saturation field capacity of 75%-85%. Soil moisture of each pot was measured using a moisture meter (DELMHORST) at 4:30pm each day, and water was added to maintain the preset moisture level in each pot. To the pots containing these three soil moisture conditions, four nitrogen contents were applied as follows: N0: 0 g kg⁻¹; N1: 0.0675 g kg⁻¹; N2: 0.135 g kg⁻¹; N3: 0.2025 g kg⁻¹. These applications were equivalent to the following amount of nitrogen fertilizer per hectare 0 kg hm⁻², 151.875 kg hm⁻², 303.75 kg hm⁻², 455.625 kg hm⁻². All the treatments were conducted with completely randomized block design and three replicates. In addition, each pot was treated with 0.0668 g P₂O₅ kg⁻¹ and 0.0668 g K₂O kg⁻¹. Phosphate fertilizer was applied as basic fertilizer. Potassium fertilizer was applied in two batches, 50% as basic fertilizer and 50% used as additional fertilizer after 60 days of planting. Nitrogen fertilizer dissolved in water and was applied in three batches, which were after planting 30, 60 and 90 days. Fertilizer types were: urea nitrogen, phosphate with calcium magnesium phosphate and potassium chloride.

These tests were conducted in August 10th to 20th December, in 2017 at greenhouse of Danzhou County (Hainan, China), Hainan University, College of Agriculture campus bases.

Analytical methods

Fresh yields and dried yields above ground were weighted after planting 130 days (Bao, 2000). The field capacity was determined by Wilcox method (Jiang et al., 2006). All crude fiber contents of King grass were determined with acid elimination decomposition method. The crude grass protein contents were converted from total

nitrogen contents, which was determined by semi-micro Kjeldahl method (He, 1983). Proline content was measured using ninhydrin colorimetric method (Wang, 2011). Catalase activity was determined by potassium permanganate titration method (Wang, 2011).

Statistical analysis

Description statistics, Anova test were performed by SAS 9.0 on Windows. And the none linear regression model was applied to estimate the optimized soil moisture and application rate of nitrogen in King grass. The figures were conducted by Sigmaplot 10.0 on Windows.

Results

Effects of different water and nitrogen treatments on King grass yield

As shown in *Figure 1*, at a constant level of nitrogen fertilizer, the yield of grass was directly related to the soil water content. This is confirmed by the fact that the W1, W2, W3 moisture conditions, resulted in yields of 37.33%-62.41%, 56.69%-76.42%. At W3 each fertilization yields are the highest and significantly higher than other water treatment levels, indicating that W3 is optimum moisture content of the soil for growing King grass. As can be seen from the figure at the W1, W2 and W3 water level, the correlation coefficient of nitrogen fertilizer rates and yield were: 0.998, 0.994 and 0.998 ($n=12$, $P<0.001$). According to nitrogen contents at different water levels, it can be seen that the corresponding moisture conditions Nitrogen yield the highest point of the King grass were 2.42 g pot⁻¹, 3.16 g pot⁻¹, 2.92 g pot⁻¹, equivalent hectare fertilizer were: 271.78 kg hm⁻², 355.05 kg hm⁻² and 329.06 kg hm⁻².

Figure 1. Effect of application rate of N on the production of King grass in different moisture conditions

Effects of different water and nitrogen treatments on the crude protein content

The contents of crude protein in King grass with decreasing soil moisture content at a fixed nitrogen level (*Fig. 2*). At a fixed nitrogen content, the King grass crude protein content under moderate water (W1) treatment was significantly higher than the other

two soil moisture content levels and the mild water treatment (W2) is slightly higher than the appropriate water treatment (W3). Within a range, the Nitrogen content can result in higher protein yields from King Grass. In the W1, W2 and W3 water levels, the correlation coefficient between nitrogen fertilizer rates and crude protein were: 0.937, 0.868 and 0.939 ($n=12$, $P<0.001$). At different soil water contents the crude protein content of the highest levels of nitrogen were 3.21 g pot^{-1} , 4.49 g pot^{-1} and 5.76 g pot^{-1} , equivalent hectares of $361.54 \text{ kg hm}^{-2}$, $505.44 \text{ kg hm}^{-2}$ and $647.88 \text{ kg hm}^{-2}$.

Effects of different water and nitrogen treatments on crude fiber content

Figure 3 shows that at a constant moisture content, the crude fiber content of King grass increased as the with nitrogen soil content decreased. Beyond a minimum nitrogen content the grass crude fiber content increased, but there is an optimum soil nitrogen content as the data show. When nitrogen levels were in the range of N1-N2, the crude fiber content of the King grass was in the range of 28.06%-30.93%. Therefore, the optimum application rate of nitrogen is between 28.17%-34.08%.

Figure 2. Effect of application rate of N on the crude protein of King grass in different moisture conditions

Figure 3. Effects of water and nitrogen coupling on the crude fibre content of King grass[†]
[†]Among columns followed by different miniscule (capital) letters are significantly different at $P < 0.05$ ($P < 0.01$)

Effects of different water and nitrogen treatments on Proline content

As the water stress on the grass increased, there was a significant increase in the accumulation of proline in the King grass (Fig. 4). Under moderate water deficit level W1, proline content was between 109.02-215.91 $\mu\text{g g}^{-1}$. Conditions W2 and W3 led to average increase of 54.64% and 135.73%, The water level in W1 caused the King grass proline content to increase with increasing nitrogen rate. As can be seen, compared with N0, N1, N2, N3, resulted in, an increase of 6.38%, 60.63%, 98.04% of proline content. Moisture levels in W2 caused, the King grass proline content to be in the range of 75.22-125.61 $\mu\text{g g}^{-1}$ also exhibiting increases with the amount of nitrogen fertilizer. This increase for N, N1, N2, N3, respectively, was 23.91%, 38.70%, 67.00% proline content. Proline accumulation was lowest in the W3 water treatment condition, its content was between 50.76-81.73 $\mu\text{g g}^{-1}$, significantly lower than the other two under each N treatments moisture levels.

Figure 4. Effects of different water and nitrogen on the proline of King grass[†]
[†]Among columns followed by different miniscule (capital) letters are significantly different at $P < 0.05$ ($P < 0.01$)

Effects of different water and nitrogen treatments on catalase activity

The effect of water and nitrogen on CAT enzyme activity in the King grass is shown in Figure 5. As water stress increased, CAT activity in the King grass decreased, while at constant soil moisture content CAT activity increased gradually with increasing amounts of nitrogen. CAT activity was the highest at the highest level of nitrogen, N2. Under moderate water deficit situation W1, the CAT activity was the lowest, between 1.76-1.99 $\text{mg g}^{-1} \text{min}^{-1}$. At the W3 water treatment, the CAT activity exhibited the highest performance, between 1.98-2.12 $\text{mg g}^{-1} \text{min}^{-1}$, but at the N2, N3 levels it was 2.12 $\text{mg g}^{-1} \text{min}^{-1}$, significantly higher than the other treatments.

King grass yield and fertilizer coupling model building

With the King grass yields as the dependent variable, the relative soil moisture content, and nitrogen rate as the independent variables, regression analysis, yields the following regression model:

$$Y = -48.3549 + 203.1436X_1 + 0.1547X_2 + 0.3259X_1X_2 - 142.6219X_1^2 - 0.000545X_2^2 \quad (\text{Eq.1})$$

where: X_1 for soil moisture (%), X_2 is nitrogen rate (kg hm^{-2}), Y is yield of King grass (t hm^{-2}). Upon examination, the F value is 130.25, the coefficient of determination R^2 is 0.99 ($P < 0.001$), indicating that the regression equation was highly significant. Factor levels are the dimensionless linear encoder substitution, standardized partial regression coefficient, it can affect the primary and secondary status on yield trial judge according to their size factors, factors which characterize the role of sign direction. Considering the partial regression coefficient 1, 2 times the item, we can see the level of soil moisture (X_1) the degree of influence on the King grass yield is higher than the amount of nitrogen fertilizer (X_2) the degree of influence.

A term X_1 (soil moisture), X_2 (nitrogen) coefficients 203.1436, 0.1547 are positive, indicating increased soil moisture and nitrogen fertilizer applied were significantly increased yield, soil moisture coefficient is higher than nitrogen rate coefficient, indicating that the soil water is the more important factor affecting the King grass production; independent variables X_1 , X_2 interaction term coefficient of 0.3259 is greater than zero, indicating that there are obvious synergies between soil moisture and nitrogen fertilizer application, so a reasonable match between the two, the King grass can obtain a higher yield; since squared variable coefficients are negative, indicating that the King grass yield and moisture content and the amount of nitrogen fertilizer as a function of diminishing returns, that is, when the moisture content and the amount of excess nitrogen can lead to lower yields.

Figure 5. The effect of different water and nitrogen treatments on activity of CAT of king grass[†]
[†]Among columns followed by different miniscule (capital) letters are significantly different at $P < 0.05$ ($P < 0.01$)

Discussion

Water and Nitrogen Conditions relationship with King grass yield and nutrition

This study shows that under different moisture conditions, although King grass yields increase with increasing nitrogen, the increase in production gradually reduced, showing a decreasing rate fertilizer law (Si et al., 2017). Under this condition, the moisture content is a major factor limiting the growth of the King grass. The soil moisture at a mild deficit (W2) condition, results in an inadequate supply of water so that increases in nitrogen are needed to compensate (Luo et al., 2019). This weakens the degree of production caused by inadequate water supply; while under appropriate moisture (W3) condition, an insufficient supply of nitrogen fertilizer can compensate for low soil moisture content demonstrating the synergistic effect required to achieve the

"water promote fat" effect, reducing the King grass yield caused by the lack of nitrogen to reduce hazards (Wang et al., 2019).

At various levels of soil moisture content, the highest crude protein content of King grass required fertilizer inputs that were higher than the required amount of nitrogen to maintain the highest yield. So simply increasing nitrogen to increase the proportion of crude protein in the grass, will reduce yield, but will also lead to reduction in the utilization of nitrogen and eventual nitrogen leaching which will present environmental risks (Liu et al., 2012). Soil moisture levels and nitrogen inputs are the main limiting factor affecting crop yield and quality (Huang, 2003), and are the two factors that offer significant synergies (Hajaji et al., 2013). Therefore, the King grass yield can be based on the actual production to adjust the ratio of water and nitrogen.

Physiological response to different water and nitrogen conditions

Osmotic adjustment is an important physiological mechanism of plant adaptation to drought stress. Increasing the intracellular concentration of solute plants through metabolic activity, lowers osmotic potential and maintains turgor pressure, so a variety of physiological processes are related normal turgor (Xu et al., 2016). Currently crop drought resistance is closely related to the penetration of regulating substances of which there are three, namely, proline (Pro), betaine and soluble sugars. Often in the free state of proline widespread in plants, when the plants are exposed to environmental stress, free proline increasingly accumulates in plants (Li et al., 2017), the largest accumulation of proline results from drought stress. For the King grass, under moderate water deficit (W1) cases, the highest accumulation of proline, is when nitrogen increases, which is consistent with findings Nadzariah et al. (2018). The moisture deficit (W2) condition, causes proline accumulation which is secondary only to the W1 condition, but also exhibited an increase with nitrogen rate rise phenomenon. As shown under water stress, proline accumulation is closely related to the ability of King grass to react to water stress. But on the other hand, studies have shown that nitrogen levels can improve the ability of the plant to adjust to osmotic changes while strengthening the crop's sensitivity to drought, this aspect needs further study (Zhang, 2008; Li et al., 2014).

Conclusions

Within the scope of this study, under W1, W2 and W3 water level, the highest yield of the corresponding amount of nitrogen fertilizer were 271.78 kg hm⁻², 355.05 kg hm⁻² and 329.06 kg hm⁻². The impact of soil moisture on grass yield is higher than the influence of the King grass amount of nitrogen, and there are significant synergies between the two facots, both with a reasonable, King grass can get a higher yield, to meet the needs of herbivorous livestock in order to maintain the balance of ecological environment. Under the experimental conditions, when soil moisture is W3 (75%-85%), nitrogen rate was 329.06 kg hm⁻², the King grass can significantly increase yields and improve its quality. The nitrogen can be applied by water solution, the concentrations and application volumes should be studied in future.

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