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Effect of NPK Fertilizer and Water Stress on Growth and Proline Content of Wild Elephant Grass (*Pennisetum polystachion*)

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Original Research Article

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Abstract: The greenhouse experiment was conducted to evaluate growth response and proline content of wild elephant grass (*Pennisetum polystachion*) to NPK fertilization and water stress. A factorial experiment in the base of completely randomized was performed design with 2 factors, replicated 3 time. Factors were (i) NPK fertilizer (0, 50, 100 and 150 kg^{-ha}), (ii) water stress (80, 60 and 40% of field capacity). Observed parameters were plant height, number of tiller, number of leaves, leaf area, shoot dry weight, root dry weight, and root to shoot ratio and proline content of wild elephant grass. Results showed that NPK fertilizer significant ($P>0.05$) increased plant height, number of tiller, number of leaves, leaf area and root dry weight, but did not affect ($P<0.05$) on shoot weight, root to shoot ratio and proline content. Water stress significant ($P<0.05$) root weight and proline content, but did not affect ($P>0.05$) plant height, number of tiller, number of leaves, leaf area, shoot weight and root to shoot ratio. No interaction ($P>0.05$) between NPK fertilization and water stress treatments on all observed parameter. The study showed that wild elephant grass was response to NPK fertilizer and resistance to water stress condition.

Keywords: Pennisetum polystachion, fertilizer, water stress, growth, proline.

INTRODUCTION

Water is very important for all living organisms including plants. Most plants are actively may contain nearly 90 percent water. Although, less than one percent of the total water used by plants is required for its metabolic activity, water plays a role in growth and development as well as crop production.

Limited water supply has a strong influence on the development, activity and duration of nutrient uptake by plants. Lack of water content in the soil causes a decrease in total dry matter, leaf production, and also leaf and root expansion rates both in shrubs and in woody plants [1]. Severe water stress in plants has a great impact on physiological and biochemistry process in plant. The response of plants to water pressure is usually seen in physiological parameters such as water potential, relative water content, stomata reactions, photosynthesis, or osmotic adjustment, which has been shown to be an indicator of drought in some studies [2].

Wild elephant grass (*Pennisetum polystachion*) is perennial plant has a high adaptation to drought conditions, but it is not tolerant of long dry season. In many countries, this grass also known as mission grass. Wild elephant grasses have a high competitive ability against other species and the grass is able to dominate the land in a short time. In Jambi province, wild elephant grass has been dominated agricultural lands in recent years. Small and very light seed sizes transported by wind or human factors cause of the rapidly spreading of this grass. Wild elephant grass has the potential to be used as ruminants feed. The easy growing and good quality of these grasses, being an important factor to consider as ruminants feed. Applying proper management to this grass should be considered for better yield and quality of the grass. The application of fertilization is an action that needs to be applied to improve the productivity and quality of this grass. Information on wild elephant grass growing management is still very limited. However, given the potential of this grass, it is necessary to conduct an in-depth study on the management of this lawn maintenance. The addition of nutrients in the form of fertilizer both organic and inorganic fertilizers need to be applied to determine the response of wild elephant grass to fertilization.

NPK fertilizer is a compound fertilizer that contains at least three main elements of nitrogen, phosphorus and potassium and other microelements. The three main elements have an important role in supporting the growth of plants. Previous studies have reported regarding to the effect of NPK fertilization on the growth of some grasses. NPK fertilization significant (P> 0.05) increased the production and quality of *Lolium perenne* L [3]. Other research results on some Bermuda grass cultivars (*Cynodon dactylon* L.) showed a positive effect of N fertilization on grass growth characteristics and quality.

The objective of this study was to evaluate the growth response and proline content of wild elephant grass (*Pennisetum polystachion*) to NPK fertilization and water stress.

MATERIALS AND METHODS

Experimental site and plant material

The greenhouse experiment was conducted at Pastures and Forages Research Centre, Faculty of Animal Science, University of Jambi, Jambi, Indonesia, from January 2015 to Mei 2015, located in $1^0$36'40.8" South, 103.$3^0$1'12.2" East. The soil was sandy loam soil, Ultisols Ordo contained 0.2 %, phosphorus, 19.0 mg/100 g soil and cation exchangeable capacity 4.1 me/100 g, as presented in Table 1. Drying of plant samples to determine plant weight and root weight performed at the Laboratory of the Faculty of Animal Husbandry, Jambi University. Determination of proline content was done in Basic and Integrated Laboratory, University of Jambi.

Tabel -1: Chemical properties of Ultisols

Properties	Values
pH H_2O	4.4
pH KCl	4.1
N - Organic (%)	0.2
C – Organic (%)	2.7
P_2O_5 (mg/100 gr)	19.0
K_2O (mg/100 gr)	61.06
K (me/100 gr)	0.09
Na (me/100 gr)	1.30
Ca (me/100 gr)	1.25
Mg (me/100 gr)	5.2
CEC (me/100 gr)	4.1
Al-dd (me/100 gr)	0.5

Experimental design and procedures

This experiment was performed as a factorial experiment in the base of completely randomized design with 2 factors, replicated 3 times. Factors were (i) NPK fertilizer (0, 50, 100 and 150 kg^{-ha}), (ii) water stress (80, 60 and 40% of field capacity).

Prior to the implementation of planting in growing, it was first determined the field capacity of soil used in research which was carried out by using gravimetric method (5). Each grass species were planted in growing bags containing soil weighing 10 kg. In this study used 36 growing bags. Stem clumps (pols) of wild elephant grass were used as planting material. All the plants received enough water (field capacity) for 30 day after planting and then, all plants were trimmed to obtain more uniform growing. Application of water stress performed two weeks later with three level of water stress. Fertilizer application performed after trimming (30 days after planting). Four levels of NPK fertilizer were applied ie. 0; 0.25 g; 0.50 g and 0.75 g, respectively that were equal to 0 kg^{-ha}; 50 kg^{-ha} and 150 kg^{-ha}. All plants were harvest at 60 days after plant to determine growth characteristics and proline content.

Measurement of growth parameters

The observed parameters were plant height, number of tillers, number of leaves, leaf area, shoot dry weight, root dry weight, and root to shoot ratio and proline content of wild elephant grass. Measurement and weighing of samples were determined at 60 days after trimming.

Pant height was measured from the soil surface to the top of the last leaf using the rule meter, and the mean of the measurement results were then calculated. Number of tillers and number of leaves also calculated for each polybag.

The leaf area was measured using IrfanView software. Three Leaves located at the middle of stem were cut to fit the Scanner device. Image of the results of the scanning, then converted into leaf.

Sample of fresh grass inserted into the paper bag for further drying. They were then oven-dried in the oven with a temperature of 70^0C for 48 h to determine dry weight of grass.

The grass roots were dismantled and cleaned by watering with water to remove the remaining of soil. The cleaned roots were then inserted into paper bags to be dried in the oven at a temperature 70^0 C for 48 h. Oven-dried roots were weighed to determine dry weight. Root to shoot ratio was obtained by dividing root dry weight by shoot dry weight.

Proline content was determined according to metode Bates [6]. Proline was extracted from 0.5 g of leaf sample by grinding in 10 ml of 3% sulphosalicylic acid and the mixture was then centrifuged at 10000 g for 10 min. Two ml of the supernatant was then added into test tubes towhich 2 ml of freshly prepared acid-ninhydrin solution and 2 ml of glacial acetic acid were mixed. The tubes were placed in a water bath for 1 h at 90oC and the reaction was terminated in ice-bath. The mixture was then extracted with 5 ml toluene and vortexed for 15 sec. After allowing standing at least for 20 min in darkness at room temperature to separate the toluene and aqueous phase, the toluene phase was then carefully collected into test tubes and the absorbance of the fraction was read at 520 nm with a Shimadzu UV-1700. The proline content in the sample was expressed as μmol^{-g} of fresh weight. The standard curve was prepared by employing L-proline.

STATISTICAL ANALYSIS

Data of growth parameters and proline content of wild elephant grass were analysed by Oneway-ANOVA using SPSS ver. 21 and Multpile Duncan Range Test (DMRT) was used to compared the treatments means [7].

RESUTS AND DISCUSSIONS

Growth characteristic of wild elephant grass (*Pennisetum polystachion*)

Growth characteristics of wild elephant grass (*Pennisetum polystachion*) under NPK fertilization and water stress presented in Table 2.

Table-2: Growth characteristics of wild elephant grass liar (*Pennisetum polystachion*) under NPK fertilizer and water stress

Treatments	Plant Height (cm)	No. of Tiller	No. of leaves	Leaf area (cm^2)	Shoot DW (g)	root DW (g)	Root to shoot rasio
NPK fertilizer (kg^{-ha})							
0 kg^{-ha}	103.67[a]	14.11[a]	73.78[a]	58.30[a]	59.00	10.27[a]	0.18
50 kg^{-ha}	110.89[ab]	17.44[b]	76.89[a]	60.84[ab]	68.11	10.82[ab]	0.16
100 kg^{-ha}	111.67[ab]	18.22[b]	84.78[ab]	64.84[b]	69.78	11.71[b]	0.17
150 kg^{-ha}	114.33[b]	20.89[c]	91.00[b]	65.45[b]	68.56	11.24[ab]	0.17
Water stress (% of FC)							
80 of FC	110.42	18.67	86.83	63.24	67.67	10.35[a]	0.16
60 of FC	110.83	17.25	79.08	62.59	65.58	10.88[a]	0.17
40 of FC	109.17	17.08	78.92	60.67	65.83	11.80[b]	0.19
P X W	ns	ns	ns	ns	ns	ns	ns
Values on the same column with different superscripts are different at 5% for each treatment							

NPK fertilization significantly ($P<0.05$) increased plant height, number of tillers, number of leaves, leaf area and root dry weight, but did not affect ($P>0.05$) shoot dry weight and root to shoot ratio. Decreasing water level significantly increased ($P<0.05$) root dry weight, but does not affect other variables. There was no interaction ($P0>0.05$) between NPK fertilization and water stress on all observed variables.

Increasing of plant height, number of tillers, number of leaves and leaf area of wild elephant grass (*Pennisetum polystahion*) indicated that *P. Polystachion* was tolerance to NPK fertilization. NPK fertilizer increase the availability and uptake of N, P, and K nutrients. Nitrogen, phosphorus and potassium have a major effect in increasing plant growth. Nitrogen (N) is an important element and has a major influence on a number of plant responses including pigmentation, shoot and root formation, tolerance to cold and dry condition, accumulation of aging process and growth recovery [8]. Sufficient phosphorus supply is an essential factor for the development of new plant cells and plays a role in transferring the genetic code from one cell to another when new cells are formed. Limited phosphorus supplies decrease plant production and phosphorus fertilization to ensure the availability of phosphorus to optimize plant production and maturity. However, the ability to absorb phosphates is different between plant species and between cultivars in the same species as well [9]. Increased growth of wild elephant grass due to fertilizer treatment also caused by the availability of potassium element in the fertilizer. Potassium plays a role in the metabolic processes of plants and helps the plant in absorbing nitrogen from the soil. Potassium is very important in many ways towards crop productivity. It not only performs important physiological functions, but also increases the efficiency of nitrogen use [10].

The similar results obtained in this study were not much different from those reported [11], that NPK fertilization significantly affects the number of leaves

per plant on Rhodes grass (*Chloris gayana* L. kunth.). Increasing doses of NPK fertilizer on lemon grass (*Cymbopogon flexuosus*) increased significantly plant height, number of tillers and number of leaves per hill [12]. Inorganic fertilization in the form of NPK fertilizer and organic fertilizer with compost gave a real effect on the number of tillers, number of leaves, root dry weight and canopy [13]. Number of grass seeds *Themeda triandra* Forssk. increased in line with increased doses of nitrogen and phosphorus fertilization [14]. NPK fertilization is also reported to significantly affect plant height, number of tillers and number of elephant grass leaves (*Pennisetum purpureum*) [15].

Increasing NPK fertilization did not affect the shoot dry weight of wild elephant grass (*Pennisetum polystachion*). NPK fertilization had no significant effect on fresh weight and dry weight rhodes grass (*Chloris gayana* L. Kunth) (11). Increased doses of N, P and K fertilizers did not affect the production of fresh yield and the production of dry elephant grass (*Pennisetum purpureum*) [16]. Different results reported, total dry production of Tanzanian grass [17] were positively related to nitrogen fertilization treatment. Without fertilizer treatment, obtained 750 g^{-sqm}, while with fertilization 320 kg N^{-ha}, 1.470 g^{-sqm}.

Water stress did not affect (P>0.05) plant height, number of tillers, number of leaves, leaf area and root to shoot ratio, but increase root dry weight of wild elephant grass (*Pennisetum polystachion*). Reseach results indicated that wild elephant grass is tolerant to drought conditions. Water stress through irrigation restrictions on maize did not affect the number of leaves of maize [18]. However, several other studies have shown different results. Water stress treatments had a significant effect on the height of *Chloris gayana*, *Paspalum dilatatum*, *Paspalum notatum* [19], *panicum maximum* and *Pennisetum purpureum* [20]. Water stress was also reported to affect plant height, number of tillers and number ofleaves of switchgrass (*Panicum virgatum*) [21].

There was no interaction between NPK fertilizer treatment and water stress of all observed variables. In general, although under water stress conditions, NPK fertilization was able to support the growth of wild elephant grass to optimize the utilization of growth factors. In general the increased efficiency of agricultural water use achieved through the application of sufficient dosage of fertilizer [22]. Drought stress greatly inhibits growth and development of maize. Growth and development consists of various component parameters that are estimated with different properties such as plant height, leaf area, root functional and functional character, plant biomass, fresh weight of plant, dry weight of plant and stem diameter. Plant height, stem diameter, plant biomass and leaf area reduce stress to drought [23, 24]. Leaves will naturally fold to reduce leaf area and light interception will decrease so it will decrease photosynthesis activity.

Proline content of wild elephant grass (*Pennisetum polystachion*)

Proline accumulation of is one of plant adaptations to water shortage and salinity conditon , so that can be used as parameters to determine the plants get the stress of both conditions [25]. Proline content of wild elephant grass under NPK fertilization and water stress treatments in the study presented in Table 3.

Table-3: Proline content of wild elephant grass *(Pennisetum polystachion*) under NPK fertilization and water stress

Treatments	Proline content (µmol^{-g})
NPK fertilizer (kg^{-ha})	
0 kg^{-ha}	20.55
50 kg^{-ha}	19.62
100 kg^{-ha}	19.30
150 kg^{-ha}	17.85
Water stress (% of FC)	
80 of FC	
60 of FC	18.76^{a}
40 of FC	19.77^{b}
NPK fertilizer (kg^{-ha})	20.10^{b}
P X W	ns
Values on the same column with different superscripts are different at 5% for each treatment	

NPK fertilization did not affect (P> 0.05) proline content of wild elephant grass (*P. polystachion*), but data in table above indicated that proline levels decreased in line with higher NPK fertilizer doses. Decreasing in proline levels due to NPK fertilizer contributes to maintaining the physiological condition of wild elephant grass, so as to maintained optimal growth. The increase of plant height, number of tillers, number and leaf area obtained in this study indicated that morphologically wild elephant grass gave a positive response to the increase of NPK fertilizer up to 150 kg^{-ha}. Similar result was found on bread wheat and durum wheat cultivars [26]. Proline content of Chinomaria chamomile (*Matricaria chamomilla* L.)

affected by phosphorus and potassium fertilization, but not affected by N fertilization [27]. The results of two wheat cultivars (*Triticum* ssp.) also showed differences in organic fertilization and inorganic fertilization on proline content [28].

Although the role of N, P and K elements related to the relationship and physiological mechanisms of nutrient availability to plant proline content has not been revealed in detail, but these three elements play a significant role in some of the plant's fisilogical processes. Nitrogen is the constituent element of all amino acids and has a central role in cellular metabolisms [29]. Phosphorus is a basic element involved in the transfer of energy in plants. Phosphorus also plays a role in the conversion of carbohydrates into hormones, proteins and energy to form new leaves and fruit. Phosphorus play a role in the process of photosynthesis, the basic ingredients of nucleic acids and phospholipids. The potassium element affects the absorption of plant cell water, acting as a catalyst in the absorption of iron and is an essential element in the formation and translocation of proteins, starches and sugars [30]. Potassium ions also aid chemical electrode gradients between cell mebranes and play a role in the transport of a number of chemical compounds [29].

Proline content of wild elephant grass was significantly increased ($P < 0.05$) due to water stress treatment. Water stress condition up to 40% of field capacity resulted in the highest proline content, significantly different ($P < 0.05$) with 80% of field capacity but not significantly different ($P > 0.05$) with 60% of field capacity. These results indicate that wild elephant grass is physiologically capable of maintaining biochemical conditions in leaf organs in response to low groundwater content. The content of wild elephant grass proline obtained in this study was lower than in other crops such as wheat [28], elephant grass [31] and Brachiaria grass [32], tall fescue (*Festuca arundinacea* (Schreb) [33]. The difference in proline content caused by each different species has different response to drought conditions. Significant differences in proline content also was found between the reproductive phase and the vegetative phase in wheat crops [34].

The absence of fertilizer interaction and water stress on elephant grass proliferation content indicates that in the water shortage to 40% field capacity, wild elephant grass roots are able to absorb nutrients, nitrogen, phosphorus and potassium availability, both in the soil and those given in the fertilizer treatment. The availability and absorption of elements N, P and K are capable of maintaining metabolic processes in wild elephant grass, even though under limited water availability. Nitrogen adsorbed by plants for the synthesis of amino acids, which are important components of proteins. Amino acids also play a role in the formation of protoplasm and cell division. If nitrogen availability for plants is low, plants are unable to produce proteins for metabolic processes and maintain their growth rates [35]. Phosphorus (P) is one of the important mineral nutrients and is involved in improving the negative effects of drought stress on plant growth and development [36]. Furthermore, it's stated that P improved the adverse effects of water stress and plays a role in the regulation of osmotic potential in the african violet plant (Saintpaulia) [37].

CONCLUSION

The finding of this study, it can be concluded that applying Fertilizing NPK to a dose up to 150 kg-ha increase the optimal growth of wild elephant grass (*Pennisetum polystachion*). Wild elephant grasses can sustain growth despite the limited availability of water. NPK nutrients in the fertilizer maintain wild elephants grass in regulating the metabolic process under in limited water condition.

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