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Research paper

Coupling effects of water availability and pH on switchgrass and the optimization of these variables for switchgrass productivity determined by response surface methodology

Hongjuan Zhang [a], Quanzhen Wang [a, *], Yuan Liu [a], Jian Cui [b], Xiaomin Ma [a], Muyu Gu [a], Maolin Xia [c]

[a] Department of Grassland Science, College of Animal Sci. and Techn., Northwest A&F University, Yangling 712100, Shaanxi Province, PR China
[b] Department of Plant Science, College of Life Science, Northwest A&F University, Yangling 712100, Shaanxi Province, PR China
[c] Grassland Department, Extension Center of Technology for Livestock in Tibet, Lhasa, 850000, Tibet, PR China

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ABSTRACT

Perennial grasses can be planted in marginal lands, and the perennial grass species switchgrass (*Panicum virgatum*) has emerged as an ideal candidate for bioethanol production. To choose the optimal soil water content and pH value for bioethanol production using switchgrass, a greenhouse experiment was conducted with five pH levels (4.9, 6.3, 7.0, 7.7, and 9.1) and five soil water contents (8%, 16%, 20%, 24%, and 32%). The coupling effects of water and pH stresses on switchgrass were investigated by evaluating fresh weight and dry weight, biomass composition (acid detergent fiber, neutral detergent fiber, acid detergent lignin, cellulose, hemi-cellulose, and lignin) and physiological response (soluble sugar content, proline content, MDA content, chlorophyll content and relative conductivity) in Alamo switchgrass. The results demonstrate a significant and positive correlation between the cellulose and acid detergent fiber content in switchgrass biomass ($R^2 = 0.959$). The results of the variance analyses demonstrate that water and pH stress yielded a coupling effect on switchgrass, and this finding was significant for fresh weight and neutral detergent fiber and proline content. By response surface methodology, the optimal combination of soil water content (SWC) and pH was SWC = 31.968%–44.424% and pH = 6.328–8.123. These results provide basic evidence for planting switchgrass in marginal lands.

1. Introduction

Water deficit and pH stress are important environmental factors restricting plant growth and photosynthesis in many regions of the world, and these two stresses often occur simultaneously. Drought is one of the leading abiotic stresses that cause a decrease in plant production [1]. Globally, the water available for crops is limited, covering only 20% of the total area used for agriculture [2]. With the changes in global climate and environment and the uneven distribution of seasonal and regional precipitation, water deficiency is becoming more obvious, and soil with efficient water content is decreasing, which seriously affects the growth, development and breeding of plants [3]. Acid soil, which is soil with a pH of 5.5 or lower, is one of the most important limitations to agricultural production worldwide [4]. Acid soil covers 30% of the ice-free land worldwide [5]. Approximately 50% of the world's potentially arable soils are acidic, and approximately 60% of arable acid soils are distributed in tropical and subtropical regions where agriculture is the dominant business [4]. Moreover, inappropriate irrigation and fertilization, consistent tillage and overgrazing cause the pH of lands to decrease as their time of agricultural use increases [6]. Soil alkalization is usually caused by Na_2CO_3 and $NaHCO_3$, and $0.56*10^9$ ha (37%) of the cultivated areas of the world are sodic [7]. For example, in northeast China, more than 70% of the land area is alkaline grassland [8].

Acid and alkali soils and soil water deficits severely limit crop production. At present, these stresses are becoming even more prevalent as the intensity of land use increases throughout the world. The amount of arable land is limited worldwide, but there is a considerable amount of marginal lands. Marginal lands are not

* Corresponding author. Department of Grassland Science, College of Animal Science and Technology, Northwest A&F University, Yangling, Shanxi Province, PR China.
E-mail address: wangquanzhen191@163.com (Q. Wang).
URL: http://www.nwsuaf.edu.cn/

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considered as farmland because of their high alkalinity and salinity, and their water deficiency. There are more than 100 million hm^2 of marginal lands, which is far more than the amount of arable land worldwide [9].

Energy crops that are called C4 crops, such as switchgrass (*Panicum virgatum* L.), have broad environmental tolerance, low nutritional requirements and generate high biomass yields, even in infertile land [10–14]. Switchgrass (*P. virgatum* L.), a C4 plant that is a warm-season perennial characterized as a tall-growing bunch grass [15], can produce 5.4 times more renewable energy than other plants [16], and less greenhouse gas emissions than row-crops [17], and improve soil quality by sequestering carbon below ground [18]. Due to the above mentioned characteristics, switchgrass has received special attention in many countries and is one of the main crops being promoted for growth in marginal lands for bioethanol production, as the use of marginal lands avoids competition for arable lands being used for food production.

Alkalinity, acidity and water deficiency of soil can directly disrupt the growth, development and breeding of plants [5,19,20], and the degree of these effects depends on the impacts of their soil characteristics on the physiological and biological processes of the plant and on the capability of the plant to adapt to water and pH stresses. To survive in adverse environments, plants have evolved a number of physiological and biological adaptations to deleterious stresses, which include changes in physiological indices and biomass composition [19].

Many previous reports have clearly demonstrated that alkaline stress limits plant growth. For example, the following have been observed for plants under alkaline stress: a decrease in *Cynodon dactylon* biomass [21], a decrease in seedling or shoot growth [8,22], a decrease in chlorophyll content [19], a sharp increase in wheat electrolyte leakage [22], a decrease in radicle elongation in the halophyte *Spartina alterniflora* (Poaceae) [23], an accumulation of proline content [7,22]. However, some studies have obtained different results; for example, shoot growth in *Puccinellia tenuiflora* was stimulated under low alkali stress ($P < 0.05$) and was markedly decreased under high alkali stress ($P < 0.01$) [7]; *Medicago ruthenica* shoot growth was stimulated under moderate pH levels below 7.26 [24]; and *Lotus tenuis* proline accumulation was not affected by high pH in the absence of NaCl [25]. At present, there are only a few studies regarding the effect of alkali stress on switchgrass [26], and they found that the seedling growth of switchgrass is sharply reduced at high pH values.

There are also some reports of acid treatment in plants. Wu et al. [27] studied the effects of six pH treatments, i.e., 7.8, 7.0, 6.5, 6.0, 5.5, and 5.0, in vitro on *Vicia faba* and found that the relative water content of the leaves was the highest under pH 6.5; proline content was higher under acidic treatment; and MDA content was the highest under pH 5.0 treatment. Nie [28] conducted a study regarding the effect of acid rain on peanut seed germination and seedling growth and concluded that the chlorophyll content decreased sharply at pH = 2.5, which is the pH of acid rain. Fan [29] studied the effects of simulated acid rain on the seedling growth of five hardwood species growing in China and concluded that the growth of all the species was retarded, chlorophyll content declined at pH 2.0, and seedling growth was stimulated at pH 3.5 and pH 5.0. Liang J [30]. reported that acid rain caused the content of crude fat and soluble sugar in rape (*Brassica napus* L.) to decrease, and those decreases were greater as acidity increased. In contrast, Huang Y. et al. [31] exposed cabbage to different concentrations of oxalic acid for six periods of time (0 h, 12 h, 24 h, 36 h, 48 h, 60 h); they found that the soluble sugar content of cabbage increased with oxalic acid content and time and that the MDA content was highest for 1 mmol/L oxalic acid exposure for 24 h and 2 mmol/L oxalic acid exposure for 36 h compared to the other oxalic acid concentrations and time periods of exposure tested.

Similarly, several reports have demonstrated the effect of water on plant growth. Flooding stress increases the MDA content and relative electrical conductivity of the cell membrane of leaves in ginkgo [32]. Water deficiency decreases rice yield [33], decreases the net plant growth and total dry mass of *Jatropha curcas* (*J. curcas*) [3,32,35], decreases the total internode length of rice [11,36], decreases plant height [3,37], decreases tiller number and biomass production [11], decreases chlorophyll content [38], decreases plant height [39,40]; increases the lignin content of white clover [41]; increases the MDA content and relative electrical conductivity of cell membrane [42], and increases proline content [38,42,43]. However, contradictory results have been reported; for example, Kee [44] found that differences in soil moisture did not affect the aboveground biomass of reed canarygrass (*Phalaris arundinacea* L.). Previous studies on water deficiency in switchgrass have shown that plant height, tiller number, biomass accumulation, concentrations of cellulose and hemi-cellulose were decreased and lignin concentration was increased with increased water stress [45].

Most previous studies on abiotic stresses in switchgrass have been focused principally on alkali and salt tolerance [26] or water deficiency tolerance individually [44–47]. However, to our knowledge, no study thus far has addressed the effects of soil water content and pH stress on the fresh weight, dry weight, biomass composition and physiological response of switchgrass, especially the effects of water and pH stress in combination. Fresh weight, dry weight, biomass composition and physiological response are important for the development and acclimation of plants; they can directly influence the productivity and fitness of agricultural crops and grasses [19]. Thus, the main objectives of the present study were as follows: (1) to test the correlations of the various indices of switchgrass growth with soil water content and pH; (2) to determine how the integrative effects of water and pH influence switchgrass yield and quality; and (3) to choose the optimal soil water content and pH value for bioethanol production. These characterizations will enrich our knowledge of the effects of soil water content and pH on switchgrass growth, may help predict the performance of switchgrass in natural environments, and may improve the potential for seedling plantation in marginal lands.

2. Materials and methods

2.1. Experimental outline

This experiment was conducted in a greenhouse without temperature controlling equipment at the Faculty of Grass Science, Northwest A&F University, Yangling, Shaanxi province, China (34°28′ N, 108°07′ E) from April to September, 2013. During the experimental period of 8:00–19:00, the mean temperature ranged from 25 to 38 °C, and the relative humidity ranged from 30 to 55%.

2.2. Experimental method

Each pot (25 cm in diameter, 15 cm in depth) was filled with 3 kg soil through a 2 mm sieve, and three holes (1 cm in diameter) were evenly distributed at the bottom of the pots to provide better aeration. The experiment material was vegetative "Alamo" switchgrass obtained by rhizome reproduction. The switchgrass buds were planted in PVC pots, which were placed outside the greenhouse. The average diurnal temperature was 28 °C during the day and 20 °C at night. After two weeks, seven seedlings with uniform growth were selected for each pot, and they were transplanted on 15 May, 2013. The cultivation medium was soil that has been passed through a 20 mash soil sieve. After 15 days, irrigating was complete, and all of the seedlings survived. The irrigation and