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Research article

Effects of PEG-induced osmotic stress on growth and dhurrin levels of forage sorghum

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ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench) is a valuable forage crop in regions with low soil moisture. Sorghum may accumulate high concentrations of the cyanogenic glucoside dhurrin when drought stressed resulting in possible cyanide (HCN) intoxication of grazing animals. In addition, high concentrations of nitrate, also potentially toxic to ruminants, may accumulate during or shortly after periods of drought. Little is known about the degree and duration of drought-stress required to induce dhurrin accumulation, or how changes in dhurrin concentration are influenced by plant size or nitrate metabolism. Given that finely regulating soil moisture under controlled conditions is notoriously difficult, we exposed sorghum plants to varying degrees of osmotic stress by growing them for different lengths of time in hydroponic solutions containing polyethylene glycol (PEG). Plants grown in medium containing 20% PEG (−0.5 MPa) for an extended period had significantly higher concentrations of dhurrin in their shoots but lower dhurrin concentrations in their roots. The total amount of dhurrin in the shoots of plants from the various treatments was not significantly different on a per mass basis, although a greater proportion of shoot N was allocated to dhurrin. Following transfer from medium containing 20% PEG to medium lacking PEG, shoot dhurrin concentrations decreased but nitrate concentrations increased to levels potentially toxic to grazing ruminants. This response is likely due to the resumption of plant growth and root activity, increasing the rate of nitrate uptake. Data presented in this article support a role for cyanogenic glucosides in mitigating oxidative stress.

1. Introduction

Drought, salinity and high temperatures are major causative factors leading to reduced crop yields worldwide, threatening sustainable agriculture and global food security [1]. With a high natural tolerance to hot dry conditions, Sorghum is an important forage crop in areas of low or intermittent rainfall. It also serves as a model for studying drought resistance in other agriculturally important grasses [2,3]. Typically, as drought conditions become more extreme, the older leaves senesce whilst younger leaves maintain a high photosynthetic rate and stomatal conductance *via* elevated rates of osmotic adjustment [4]. The net effect is continued growth long after most other pasture crops have died, providing a valuable source of animal feed during periods of sustained drought.

Cyanogenic glucosides occur in many plant species, including some important crop plants [5]. Sorghum produces the cyanogenic glucoside, dhurrin ((*S*)-4-hydroxymandelonitrile-β-D-glucopyranoside) in all vegetative tissues. During maceration and ingestion of the plant tissue, dhurrin is hydrolysed by the action of dhurrinase, a specific β-glucosidase also present in the plant tissue. This process results in the release of hydrogen cyanide (HCN) [6], a respiratory toxin, with a lethal dose ranging from 0.5 to 3.5 mg HCN kg^{-1} body weight for various mammals [5]. In cattle, dhurrin can also be hydrolysed by microbes in the rumen, independently of the plant's endogenous dhurrinase [7]. Cyanogenic glucosides are thought to have evolved as a herbivore defence mechanism [8] but may subsequently have gained a role in transport of reduced nitrogen [9]. More recently it has been proposed that cyanogenic glucosides may play a role in mitigating oxidative stress [10,11].

Abbreviations: HCN, hydrogen cyanide; LAR, leaf area ratio; MeJa, methyl jasmonate; NAR, net assimilation rate; PEG, polyethylene glycol; RGR, relative growth rate; RWC, relative water content; SA, salicylic acid; SLA, specific leaf area.

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The HCN potential (HCNp) of plant tissue is defined as the amount of HCN released following complete hydrolysis of the cyanogenic glucosides that are present. Typically HCNp is highest in ontogenetically young tissues [12,13] and in plants experiencing drought stress or exposed to high levels of nitrogenous fertilizer [13–15]. In agricultural settings, fields of forage sorghum experiencing drought stress have been reported as having concentrations of dhurrin in excess of 750 ppm HCNp [16,17], making them unsuitable for cattle grazing. Replication of such field associated drought stress effects under controlled experimental conditions has proved difficult [7,16] possibly due to confounding effects of a range of variables including plant age, intensity or duration of the stress and alterations in the availability of nutrients. For example, drought stressed plants often have high concentrations of tissue nitrogen and are also stunted, both factors independently associated with higher HCNp [12,14]. Wheeler et al. [7] concluded that in sorghum, a severe drought stress of short duration may lead to a decrease in HCNp of plants whilst, in contrast, long, chronic exposure to drought stress can induce higher levels of HCNp.

In addition to potentially toxic levels of dhurrin in drought stressed sorghum, increased levels of nitrate may be problematic as rapid conversion to nitrite can occur in the rumen [18,19]. An inverse relationship between dhurrin and nitrate concentration has been observed in field-grown sorghum experiencing drought stress [20]. This makes it particularly difficult for farmers to judge the safety of a sorghum forage crop under such conditions. Ideally, a measurement of both HCNp and nitrate in plant tissues should be obtained in order to estimate toxicity. Few studies of drought-stressed sorghum have included measurements of both HCNp and nitrate in leaf tissue (e.g. [21]), and the interaction between HCNp and nitrate concentration has not been studied in any detail. Moreover, no studies have included an analysis of HCNp or nitrate in roots, or assessed plants carefully in the period immediately after the alleviation of drought stress.

The aim of this study was to measure the effect of osmotic stress on the concentration of dhurrin and nitrate in sorghum plants to simulate chronic drought stress. Different degrees of osmotic stress were achieved by growing plants in hydroponic media containing varying concentrations of polyethylene glycol (PEG-8000). PEG modifies the osmotic potential of nutrient solution culture and thus induces plant water deficit in a relatively controlled manner. Physiological indicators of plant health were used to verify that sorghum plants were indeed experiencing stress. Plants were harvested at various time points to account for ontogenetically-related changes in HCNp and to compare the impact of both short and long term exposure to stress. HCNp and nitrate concentrations were also measured in roots and shoots after the osmotic stress had been removed, and compared with tissue nitrogen concentration.

2. Results

2.1. Variable-PEG-exposure experiment

In order to determine the concentration of PEG required to induce a stress response, sorghum plants (4-leaf stage) were grown for 4 weeks in nutrient solution containing a range of PEG concentrations. Plant height decreased linearly with each increase in PEG from an initial 20 ± 1 cm for the 0% PEG control plants to 10 ± 1 cm for the 20% PEG-treated plants ($r^2 = 0.77$, $P < 0.001$; Fig. 1A; Supplementary Table S1). The total leaf area of the control plants was 300 ± 20 cm^2 compared with 140 ± 10 cm^2 and

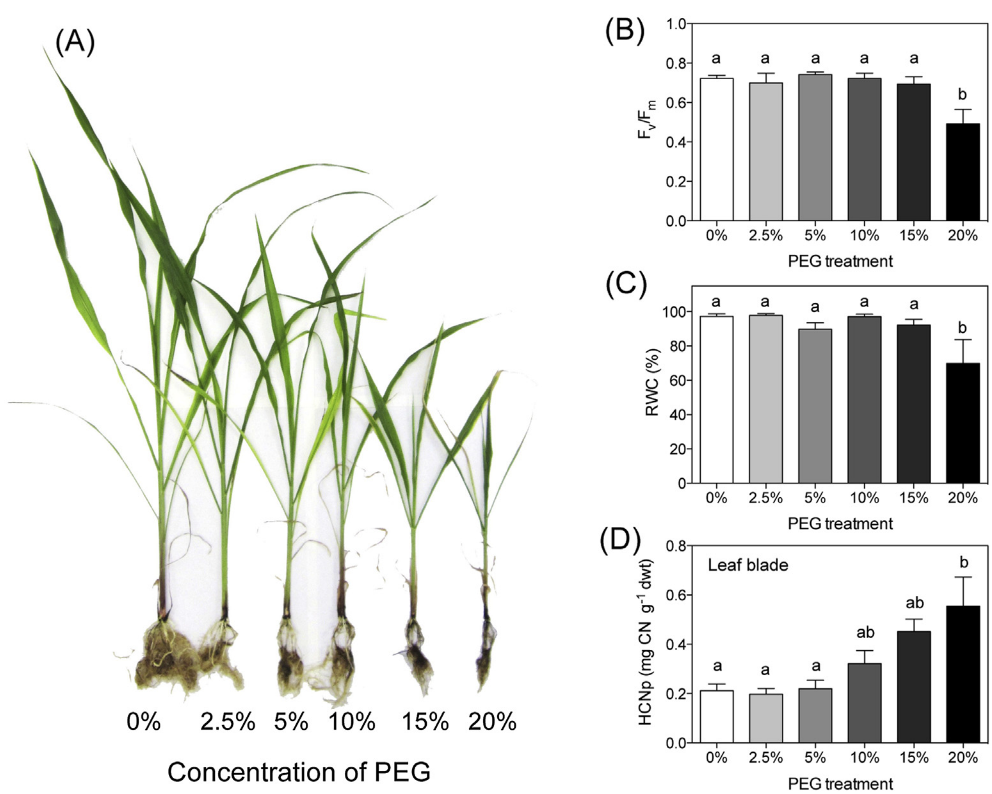

Fig. 1. Phenotype of sorghum plants from the variable-PEG-exposure experiment. Plants were transplanted into nutrient solution containing up to 20% PEG at the 4-leaf stage. (A) Plants at the final harvest, 4 weeks after the imposition of the PEG treatments. (B) Efficiency of photosystem II measured by Fv/Fm ratios. (C) Relative water content (RWC%) of the first fully-expanded leaf. (D) HCNp of the leaf blade. Values are means ± 1 SE ($N = 5$ plants). Columns marked with identical letters at the top are not significantly different at $P < 0.05$ (Full data set is supplied in Supplemental data, Table S1).