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Influence of Drought Stress Removal on Alkaloid and
Nitrate Concentrations in Pearl Millet

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SUMMARY

Alkaloid and nitrate concentrations of pearl millet leaves and stems from plants which were allowed to develop drought stress then irrigated to remove the water deficit were monitored over time. After 25 days without irrigation, moderately drought-stressed plants had a higher concentration of total alkaloids and a lower concentration of nitrate than irrigated plants. Irrigation after drought stress tended to decrease alkaloid levels and increase nitrate levels.

Introduction

Previous studies with pearl millet have shown that both alkaloid and nitrate concentrations are sensitive to drought stress. In addition, unpalatable pearl millet has been reported to be directly related to drought-like conditions. The objectives of this study were to determine the effect of plant water status and the removal of drought stress on alkaloid and nitrate levels in pearl millet; and to analyze millet leaves and stems for site accumulation of alkaloids and nitrates.

Procedure

Studies were conducted in the field during the dry hot summer of 1980 on an upland Darco soil (Grossarenic Paleudult; loamy, siliceous, thermic) in East Texas. Hybrid pearl millet 'Millex 24'¹ was sown at 20 lbs/acre in 10 inch rows on 2 June 1980.

The experimental design was a randomized block with four replications. Treatments were the following three levels of drought stress: 1) plants were not irrigated and allowed to develop drought stress; 2) plants were sprinkler irrigated to prevent drought stress; and 3) plants were not irrigated until drought stressed, then irrigated to remove stress. Mean alkaloid and nitrate levels were separated using Duncan's New Multiple Range Test. Plants received a blanket application of 224 kg N, 112 kg P₂O₅, and 112 kg K₂O per hectare on 15 June 1980. The plants received 2 in. of rain from sowing date through 21 June 1980. Plants in Treatment 1 received no rain or irrigation past 21 June 1980. Plants in Treatment 2 received water on 7, 9, 11, 14, and 16 July. Plants in Treatment 3 received no water from 21 June 1980 until moderate stress developed, then plants were irrigated on 14 and 16 July. Plants were sampled on 17 July 1980 from all three irrigation treatments.

¹'Millex 24' hybrid pearl millet was provided by the Northrup-King Seed Company.

Pearl millet plants were sampled 42 days past emergence, prior to flag leaf emergence. Samples were taken in the afternoon, frozen at -18°C , and later separated into two components: leaf blades and stems with leaf sheaths attached. These components were chopped and a portion of the plant tissue was analyzed for total alkaloid content. Another portion was oven dried and used for dry weight determination and nitrate analysis. Nitrate concentrations were determined by shaking dried plant tissue with deionized water, filtering, and measuring the nitrate content of the filtrate with an Orion nitrate ion electrode (model 92-97). Total basic alkaloids were extracted from frozen grass and measured by titration with p-toluene-sulfonic acid. Nitrate and total alkaloid content were calculated and reported on a dry weight basis. "Whole plant" refers to the total aboveground plant sample. Whole plant nitrate and alkaloid levels were calculated proportionately from leaf and stem concentrations and leaf to stem ratios.

Leaf elongation rate, leaf xylem water potential, and soil water pressure were used as indicators of plant and soil water status in the studies conducted. Soil moisture and leaf xylem water potential were measured at sampling time. Leaf elongation rate is reported from 6 July through sampling date.

Results and Discussion

Table 1 shows differences in plant and soil water status between the three irrigation treatments in this study at sampling time. Irrigated and drought removed treatments were not significantly different as far as soil water pressure and leaf elongation rate are concerned. The nonirrigated treatment was significantly different from both ($P < 0.05$). In terms of leaf xylem water potential, irrigated and nonirrigated plants were significantly different ($P < 0.05$) from each other while plants removed from stress were intermediate between the irrigated and nonirrigated plants.

Table 2 shows that at sampling time alkaloid concentrations in both leaf and whole plant tissue were significantly higher in nonirrigated than in irrigated plants, while alkaloid levels in drought removed plants were intermediate and not significantly different from either irrigated or nonirrigated plants. Stem alkaloid levels were fairly constant under all treatments. Table 3 shows the same comparisons for plant nitrate concentration. While leaf nitrate concentration was fairly constant under all treatments, both stem and whole plant nitrate levels were significantly higher in irrigated than in nonirrigated plants. Plants removed from drought stress had a stem nitrate concentration nearly as high as irrigated plants. Whole plant nitrate levels in drought removed plants were intermediate and not significantly different from either irrigated or nonirrigated plant nitrate concentrations.

Because leaf alkaloid concentration and stem nitrate concentration were the higher and more active of the plant parts investigated for the respective antiquality agents, concentrations in these parts are used for illustration in the following figures. Figures 1 and 2 show leaf

alkaloid and stem nitrate concentration for the three irrigation treatments over time as drought stress is removed. Leaf alkaloid levels are lowest in irrigated plants, highest in nonirrigated plants, and decreasing in plants removed from drought stress. Conversely, stem nitrate levels are lowest in nonirrigated plants, but highest, though decreasing, (probably due to dilution by growth) in irrigated plants and rapidly increasing in plants removed from drought stress. Lower nitrate levels in nonirrigated plants may in part be due to water-deprived plants pushing their roots below the nitrate-containing zone of soil. The restricted amount of rain or applied water to these nonirrigated plots may have prevented leaching of the applied fertilizer N into this lower root zone. Alkaloid levels were low in this study relative to those previously reported for pearl millet in East Texas, possibly because the millet hybrid used in this study may be an inherently low alkaloid line (1) and is genetically different from 'Millex 23' which was the line previously reported to become unpalatable. Whole plant nitrate concentrations did not enter the potentially toxic range (which begins at 15,000 to 20,000 ppm nitrate) in this study.

Figures 3 and 4 show the regression of leaf alkaloid and stem nitrate concentrations on pearl millet drought status as indicated by leaf elongation rate. Thirty-nine percent of the variability in leaf alkaloid content and 71 percent of the variability in stem nitrate content is associated with water status of the plant. Table 4 shows similar regression equations for the other plant parts.

Literature Cited

1. Krejsa, Beverly B., F. M. Rouquette, Jr., L. R. Nelson, E. C. Holt, and B. J. Camp. 1982. Total alkaloid and nitrate content of eleven pearl millet lines. Forage Research in Texas CPR 4024:67-69.

Table 1. Plant and soil water status in irrigated, nonirrigated, and drought removed Millex 24 pearl millet during the drought stress removal study.

Treatment	Water status measurements		
	Soil water pressure (kPa) ¹	Leaf xylem Water potential (kPa)	Leaf elongation rate (cm/48 hr)
Irrigated	-121 a ²	-273 a	41.6 b
Nonirrigated	-1520 b	-423 b	17.3 a
Drought removed	-474 a	-317 ab	33.8 b

¹One kPa is equivalent to 0.145 psi.

²Means within a column followed by the same letter are not significantly different at the 0.05 level by Duncan's New Multiple Range Test.

Table 2. Millex 24 pearl millet leaf, stem, and whole plant alkaloid concentrations under three irrigation treatments in the drought stress removal study.

Treatment	Alkaloid concentration (ppm)		
	Leaf	Stem	Whole Plant
Irrigated	37 b ¹	15 a ¹	29 b ²
Nonirrigated	67 a	11 a	47 a
Drought removed	49 ab	10 a	35 ab

¹Means within this column followed by the same letter are not significantly different at the 0.05 level by Duncan's New Multiple Range Test.

²Means within this column followed by the same letter are not significantly different at the 0.10 level (means were separated at the 0.05 level by Duncan's New Multiple Range Test, but differences in whole plant alkaloid content due to irrigation treatment were significant only at the 0.10 level).

Table 3. Millex 24 pearl millet leaf, stem, and whole plant NO_3 concentrations under three irrigation treatments in the drought stress removal study.

Treatment	Nitrate concentration (ppm)		
	Leaf	Stem	Whole plant
Irrigated	3,049 a ²	9,398 a ¹	5,431 a ²
Nonirrigated	2,889 a	4,236 b	3,354 b
Drought removed	3,280 a	8,369 a	4,970 ab

¹Means within this column followed by the same letter are not significantly different at the 0.05 level.

²Means within this column followed by the same letter are not significantly different at the 0.10 level.

Table 4. Regression equations, coefficients of determination, and significance levels for the regression of alkaloid and NO_3 concentration with decreasing drought stress as indicated by increasing leaf elongation rate.

Concentration vs. leaf elongation rate	$\hat{Y} =$	R^2	Significance level
Leaf alkaloid	$80.4 - 0.965x$.39	0.05
Stem alkaloid	$8.9 + 0.101x$.04	0.10
Whole plant alkaloid	$53.7 - 0.544x$.30	0.10
Leaf NO_3	$2584.5 + 15.80x$.15	0.10
Stem NO_3	$1171.6 + 199.44x$.71	0.001
Whole plant NO_3	$2014.9 + 83.19x$.67	0.01

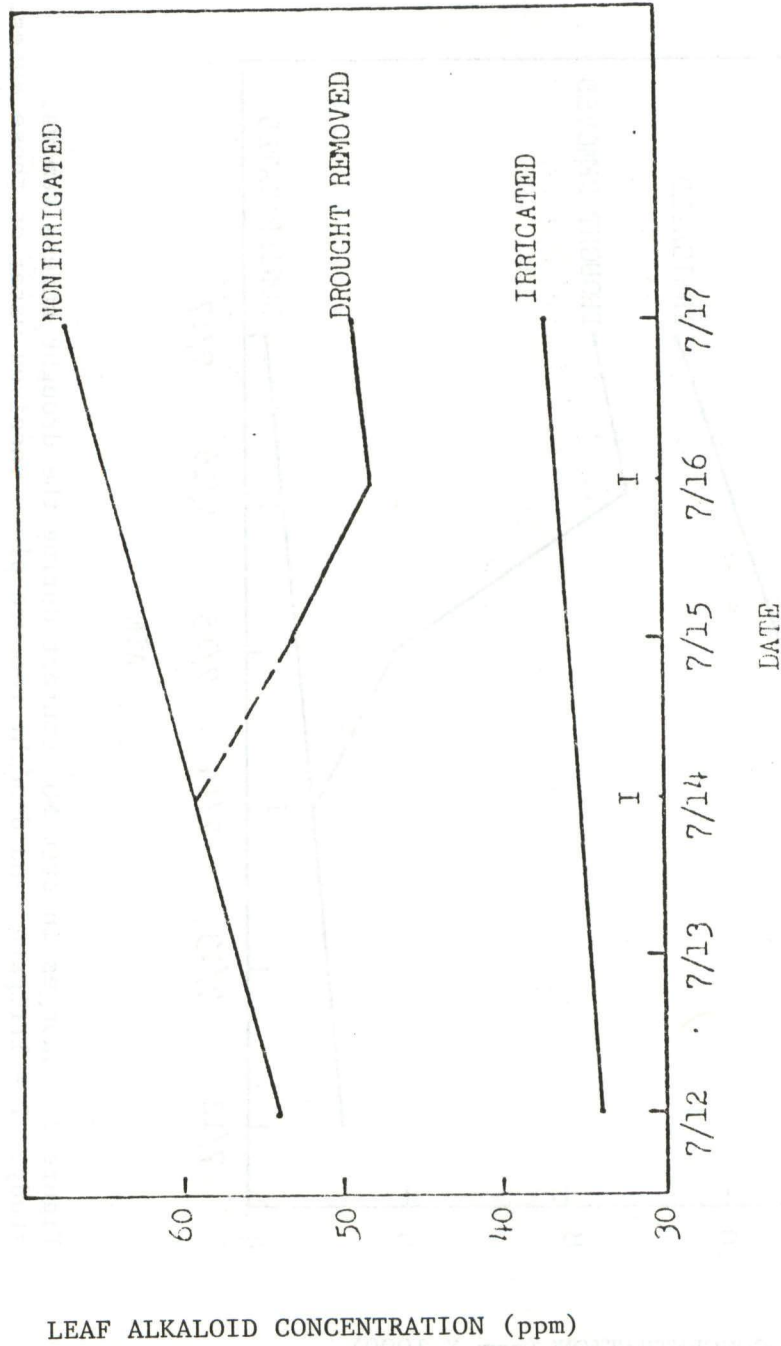


Figure 1. Changes in leaf alkaloid content during the drought stress removal study. I = Irrigated and drought removed plots received water on these dates.

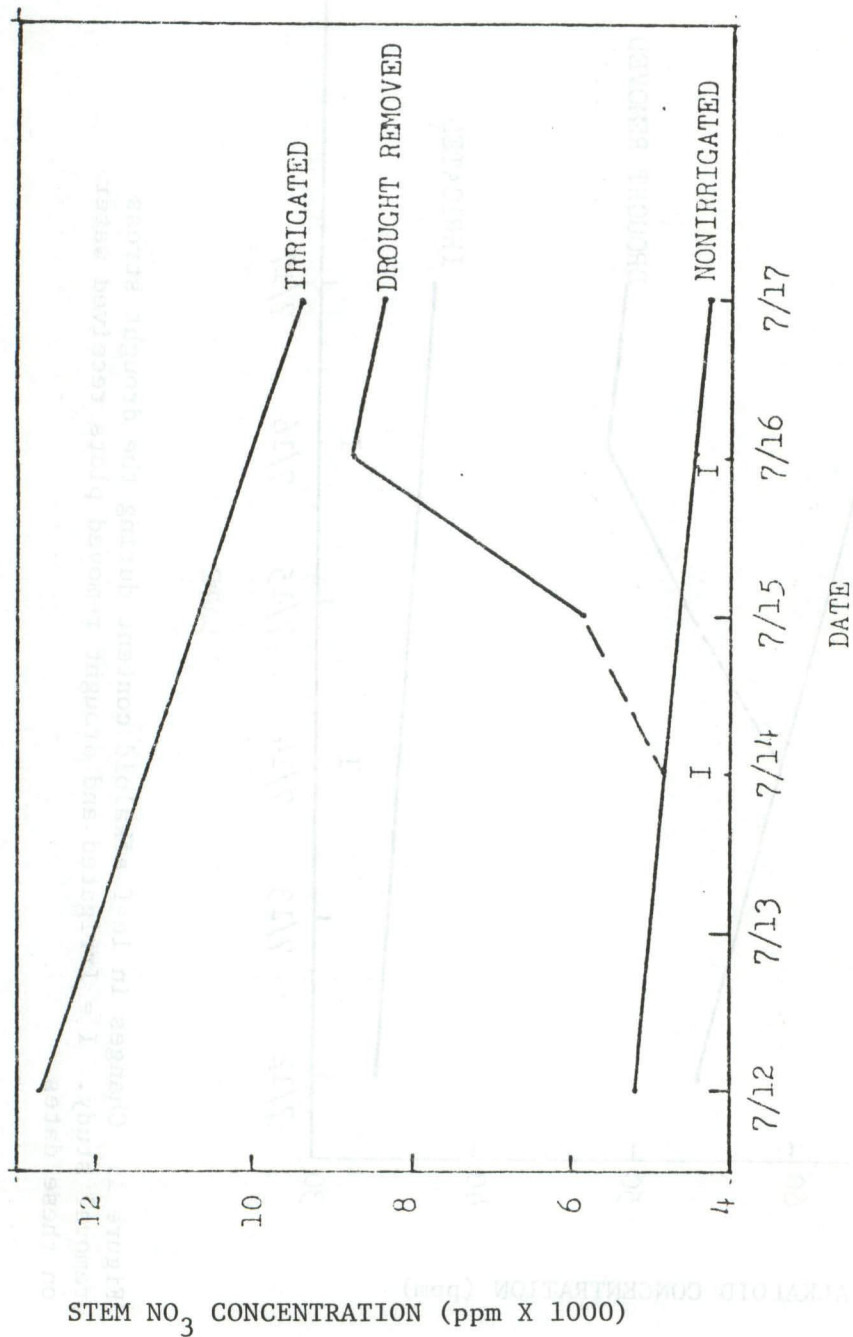


Figure 2. Changes in stem NO₃ content during the drought stress removal study. I = Irrigated and drought removed plots received water on these dates.

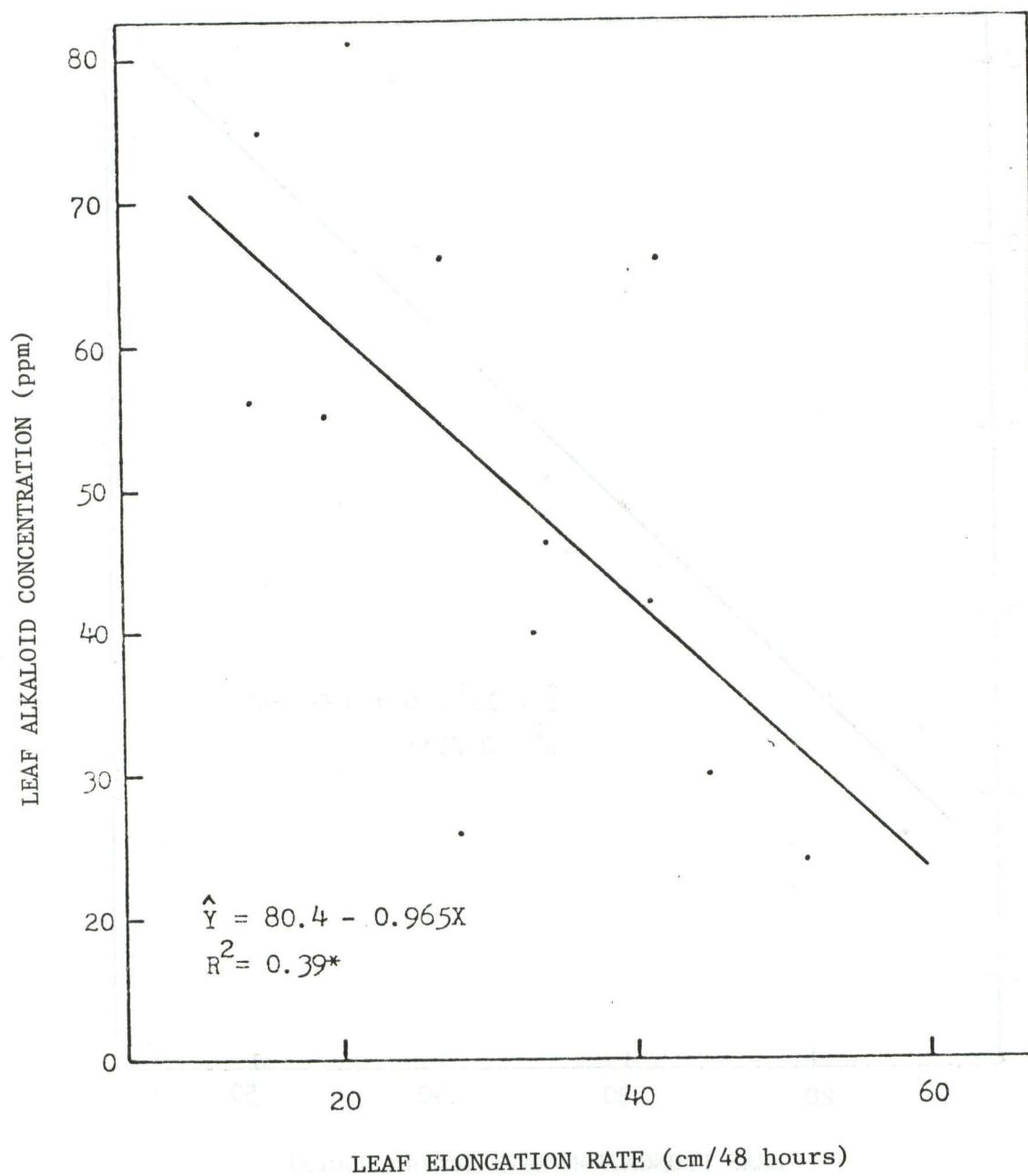


Figure 3. Regression of leaf alkaloid concentration on pearl millet drought status (leaf elongation rate).

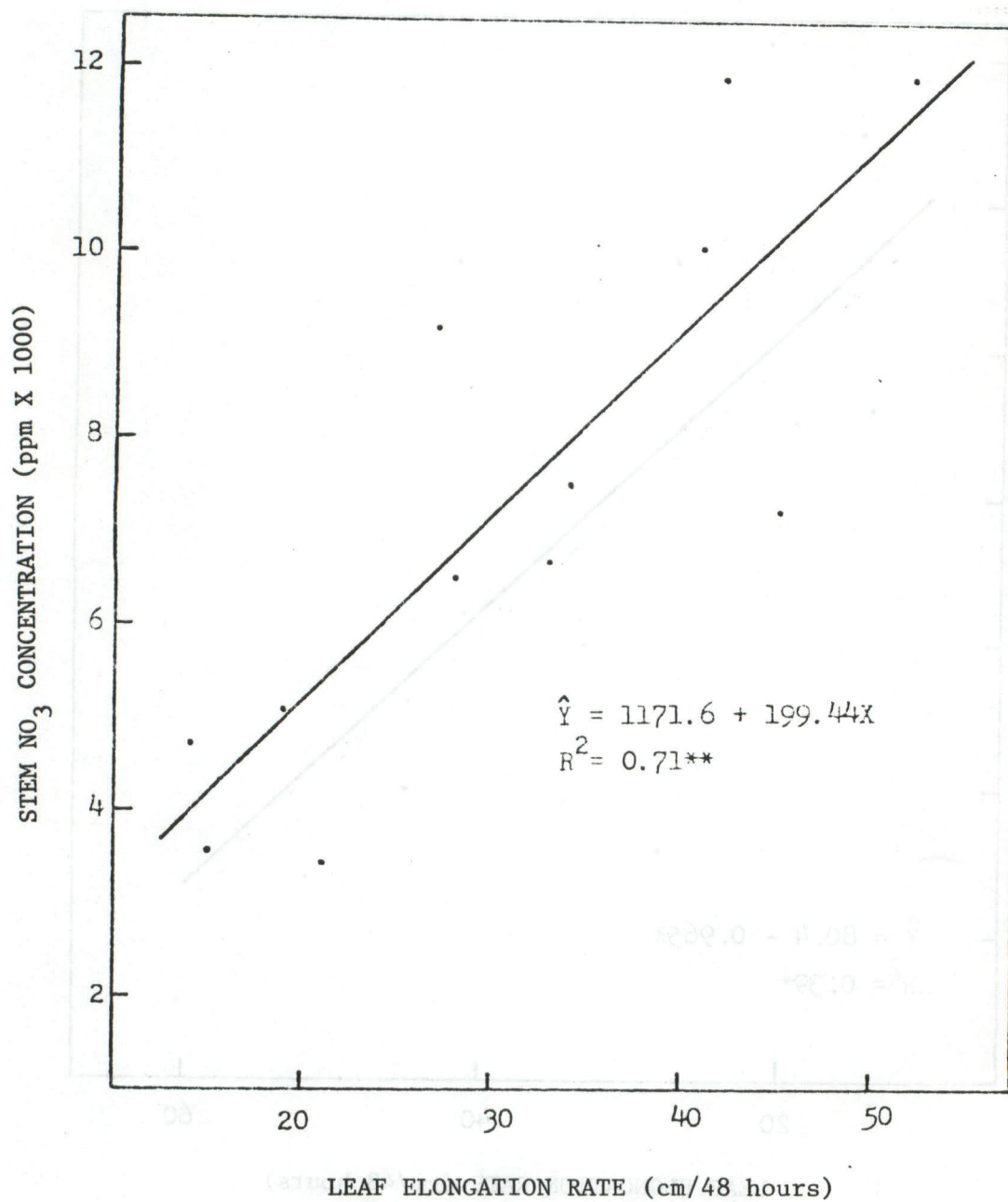


Figure 4. Regression of stem NO₃ concentration on pearl millet drought status (leaf elongation rate).