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BRIEF COMMUNICATION

Influence of acidity on growth and biochemistry of *Pennisetum clandestinum*

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Abstract

Hydroponics were used to study the impact of acidity on growth, nutritive properties and metabolic changes in kikuyu grass (*Pennisetum clandestinum* Hochst). Four treatments (pH 6.0, 5.0, 4.0, and 3.0) were compared for effects on biomass, leaf and root length, crude protein, amino acid content and key enzymes of sugar metabolism. Reduction in biomass, root and leaf length, amino acid contents, glucose-6-phosphate dehydrogenase (G6PDH) and pyruvate kinase (PK) content was observed only at pH 3.0, in association with increased leaf proline content. Kikuyu grass is able to grow normally under mild acidity (down to at least pH 4.0).

Additional key words: amino acids, enzymes, proline, sugar metabolism.

Kikuyu (*Pennisetum clandestinum* Hochst.) is a perennial grass, native to the higlands of Central Africa (Kenia, Zaire) (Skerman and Riveros 1990), but now common in many areas (Rumball 1991). The attention of researchers to this grass is due to its easy runner growth, to its root development. It is often used as an erosion-controlling ground cover, and has high nutritive properties as pasture. In fact, kikuyu is a highly digestible palatable grass, with high protein and low fibre content (Butler and Bailey 1973, Marais *et al*. 1992). Many reports show that kikuyu is tolerant to salinity (Russel 1976), drought (Whiteman 1990), waterlogging (Dale and Read 1975) and acidity (Miles 1991).

Soil acidification constraints productivity of many crops and damages both quality and quantity (Von Uexkull *et al*. 1995). Acidity has damaging effects *per se*, but generally it causes poor growth indirectly, for example by inducing deficiencies of K and Ca, or other nutrients such as P and Mo (Adams 1981, Fageria *et al*. 1989). In addition, toxic contents of Al and Mn ions, in acid soil solutions, can stunt growth and/or kill roots, resulting in the inability of plants to take up nutrients and extract water from the soil (Delhaize and Ryan 1995). In this paper, we identify the relative extent to which kikuyu growth and nutritive properties were affected by acidity. The biomass, leaf and root length, crude protein, amino acid content and key enzymes involved in the main pathways of sugar metabolism were determined. This study may provide information for kikuyu grass utilization as pasture in acid soil where the growth of other forage species is markedly reduced.

Seedlings of *Pennisetum clandestinum* were obtained from cuttings and were grown in water culture in a plexiglas box, in a growth chamber at irradiance of 80 W m^{-2} (in a 16-h photoperiod), relative humidity of 70 %, and temeprature of 25 °C. After 20 d of rooting, the cuttings were transferred into growing units containing an aerated Hoagland nutrient solution with 1 mM NH_4NO_3. The pH was adjusted to 6.0 with 0.1 M KOH (controls). More acidic conditions were obtained by adding 1 M HCl to the nutrient solution, to generate pH values of 3.0, 4.0 or 5.0. During treatment, the hydroponic medium was renewed every 2 d. All reagents used were analytical grade (*Sigma Chemical Co*., St. Louis, MO, USA). Fifteen days after the beginning of the treatments leaf and root length, fresh mass of leaves and roots were measured for six plants for each pH value.

Crude protein from leaves was measured according to

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Abbreviations: GK - glucokinase; GPI - phosphoglucoisomerase; G6PDH - glucose-6-phosphate dehydrogenase; MDH - malate dehydrogenase; PEPCK - phosphoenolpyruvate carboxykinase; PK - pyruvate kinase; SAI - soluble acid invertase.

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Nelson and Sommers (1980) and Martillotti *et al.* (1987). Free amino acids were extracted from leaves by a water in the ratio of 1:5 (m/v). The samples were filtered and acidified to pH 2.2 with 0.1 M HCl. Qualitative and quantitative determinations were carried out with a *Beckman* amino acid analyzer (*Model 118 CL*) using a lithio buffer. Amino acid *Standard H* (*Pierce Chemical Company*, Rockfort, IL, USA) was used as a blank.

Plant tissue was extracted in 1:3 (m/v) 100 mM Hepes-NaOH pH 7.5, 5 mM $MgCl_2$, and 1 mM dithiothreitol (DTT) using a mortar and pestle. The extract was filtered through two layers of muslin and clarified by centrifugation at 20 000 *g* for 15 min. The supernatant was used for enzymatic analysis. All steps were performed at 4 °C. Glucokinase (GK, NTP:D-glucose-6-phosphotransferase, EC 2.7.1.1) (Huber and Akazawa 1986), phosphoglucoisomerase (GPI, D-glucose-6-phosphate ketoisomerase, EC 5.3.1.9) (Tsai *et al.* 1970), glucose-6-phosphate dehydrogenase (G6PDH, D-glucose-6-phosphate:NADP 1-oxidoreductase EC 1.1.1.49) (Doelhert *et al.* 1988), pyruvate kinase (PK, EC 2.7.1.40) (Bergmeyer *et al.* 1983), soluble acid invertase (SAI, β-fructofuranosidase, β-fructofuranoside fructohydrolase, EC 3.2.1.26) (Zhu *et al.* 1997), phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.49) (Walker *et al.* 1995), malate dehydrogenase (MDH, EC 1.1.1.37) (Bergmeyer *et al.* 1983) activities were measured spectrophotometrically at A_{340} (spectrophotometer *UV-VIS 2100*, *Shimadzu*, Kyoto, Japan).

At pH 3.0 the decrease of kikuyu leaf and root biomass was stronger compared to the other treatments and the root length was reduced by 40 % although leaves were much less affected (Table 1). The amounts of crude proteins and free amino acid pool extracted from leaves of kikuyu grown at pH 4.0 and 5.0 were similar to that detected in control plants. High acidity (pH 3.0) reducted concentrations of crude protein and total amino acid markedly, but amount of proline increased by more than 100 % (Table 2). The SAI activity detected in roots and leaves of kikuyu grown at pH 5.0 did not differ from that of control plants. The exposure to pH 4.0 and 3.0 caused a progressive and significant increase in soluble invertase content (Table 3). The activity of GK, first enzyme of glycolytic pathway, slightly increased in kikuyu leaves up to pH 3.0 compared to control, whereas in roots a decrease in activity was measured (Table 3). PGI activity

Table 1. Effect of different acidity on fresh mass [g plant^{-1}] and length [cm] of kikuyu grass roots and leaves.

		pH 3	pH 4	pH 5	pH 6
Roots	length	13.00 ± 0.5	22.50 ± 0.4	24.00 ± 0.8	23.00 ± 0.5
	fresh mass	1.33 ± 0.22	3.17 ± 0.20	3.27 ± 0.61	3.42 ± 0.44
Leaves	length	25.00 ± 0.8	30.10 ± 0.5	32.00 ± 0.5	30.00 ± 0.5
	fresh mass	1.85 ± 0.31	3.88 ± 0.22	4.17 ± 0.50	4.00 ± 0.24

Table 2. Amino acids and crude protein [% of dry mass] contents detected in leaves of kikuyu grass grown under different pH (n.d. - not detectable).

Amino acids	Control	pH 5	pH 4	pH 3
Aspartic acid	1271.6 ± 4.4	1273.1 ± 5.2	1260.4 ± 4.8	750.7 ± 3.4
Threonine	755.0 ± 7.6	735.0 ± 6.5	728.0 ± 4.6	358.0 ± 6.0
Serine	1342.3 ± 9.5	1321.7 ± 6.5	1318.1 ± 5.8	658.6 ± 6.4
Glutamic acid	585.0 ± 2.8	535.0 ± 4.0	540.2 ± 1.8	387.6 ± 3.2
Proline	679.6 ± 6.0	670.0 ± 3.5	678.6 ± 4.2	1350.0 ± 5.3
Glycine	890.0 ± 11.5	878.0 ± 8.9	870.3 ± 6.8	457.0 ± 5.9
Alanine	673.3 ± 12.0	650.7 ± 8.3	644.0 ± 7.5	327.9 ± 6.3
Cystine	650.0 ± 5.7	630.0 ± 7.9	628.4 ± 6.8	n.d.
Valine	34.7 ± 0.8	25.5 ± 1.8	27.0 ± 1.7	16.6 ± 1.8
Methionine	131.0 ± 4.5	127.0 ± 1.5	123.6 ± 2.6	77.0 ± 3.8
Isoleucine	575.0 ± 2.8	577.6 ± 3.0	576.0 ± 5.8	287.4 ± 3.4
Leucine	528.3 ± 4.4	526.5 ± 7.9	518.0 ± 8.7	262.0 ± 3.0
Tyrosine	1183.3 ± 16.6	1166.4 ± 12.8	1172.0 ± 5.2	576.4 ± 6.9
Phenylalanine	480.0 ± 5.7	470.0 ± 3.5	458.6 ± 4.1	241.0 ± 3.0
Histidine	635.0 ± 7.6	649.6 ± 8.9	628.3 ± 7.2	244.4 ± 5.2
Lysine	675.0 ± 7.6	665.5 ± 8.0	634.3 ± 11.9	356.0 ± 5.9
Arginine	85.0 ± 1.5	80.0 ± 3.7	84.0 ± 2.9	38.0 ± 1.7
Crude protein	12.1	12.9	10.2	5.9

Table 3. Activities of soluble acid invertase (SAI) [µg(glucose) g^{-1}(f.m.) min^{-1}], glucokinase (GK), phosphoglucoisomerase (PGI), pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G6PDH), phosphoenolpyruvate carboxykinase (PEPCK) and malate dehydrogenase (MDH) [nmol(NADPH) g^{-1}(f.m.) min^{-1}] detected in leaves and roots of kikuyu grass exposed to different acidity.

Treatments		SAI	GK	PGI	PK	G6PDH	PEPCK	MDH
Leaves	control	63.0 ± 0.9	14.27 ± 0.31	0.618 ± 0.006	197.40 ± 4.21	16.61 ± 0.73	162.3 ± 1.1	0.95 ± 0.03
	pH 5	66.5 ± 0.2	14.99 ± 0.20	0.624 ± 0.001	249.74 ± 4.02	13.42 ± 0.34	169.1 ± 0.9	1.21 ± 0.08
	pH 4	104.0 ± 2.0	15.08 ± 0.30	0.660 ± 0.008	250.47 ± 2.53	13.87 ± 0.25	178.1 ± 2.0	2.18 ± 0.19
	pH 3	185.5 ± 3.0	15.22 ± 0.34	0.700 ± 0.009	0.17 ± 0.01	3.26 ± 0.09	258.8 ± 2.9	2.43 ± 0.32
Roots	control	18.3 ± 0.9	19.84 ± 0.35	0.895 ± 0.008	41.29 ± 0.96	6.45 ± 0.10	104.1 ± 2.0	1.12 ± 0.01
	pH 5	20.0 ± 0.5	19.13 ± 0.09	1.075 ± 0.009	40.10 ± 0.70	7.10 ± 0.32	106.7 ± 1.3	1.10 ± 0.02
	pH 4	33.8 ± 1.5	18.74 ± 0.01	1.290 ± 0.030	39.20 ± 1.06	6.45 ± 0.24	110.4 ± 0.8	1.28 ± 0.08
	pH 3	73.5 ± 1.8	17.26 ± 0.01	1.680 ± 0.010	0.10 ± 0.01	3.06 ± 0.12	150.3 ± 2.0	0.45 ± 0.03

was enhanced in leaves and roots of kikuyu grass grown in nutrient solution with pH 3.0 and 4.0. Higher PK activity was in leaves compared to roots, and it was enhanced by increasing acidity of culture medium in leaves up to pH 4.0, but was almost abolished at pH 3.0 (Table 3). The PK activity in roots of kikuyu grown in nutrient solution with pH 5.0 and 4.0 was similar to that detected in control plants, but at pH 3.0 it was almost undetectable. The G6PDH, the main enzyme of oxidative pentose phosphate pathway (OPPP), declined with increasing acidity and at pH 3.0 showed an 80 % reduction in leaves and a 50 % reduction in roots (Table 3). PEPCK activity in kikuyu grass grown at pH 5.0 was essentially the same as in control plant, but increased sligtly at pH 4.0 and more sharply at pH 3.0 (Table 3).

The MDH activity increased in leaves of kikuyu grass by decreasing the pH value of nutrient solution. In roots exposed to pH 5.0 and 4.0, the MDH activity was similar to that detected in control plants but it significantly decreased at pH 3.0.

Our results show that kikuyu growth and nutritive properties were affected only when acidity was increased to pH 3.0. Proline is an important parameter to measure the stress tolerance capacity of the plants (Delauney and Verma 1993). Proline accumulation, detected in leaves of kikuyu grown at the lowest pH, could be considered a sympton of acidic stress injury. Increases in proline are often involved in stress resistance mechanisms, although its precise role still remains a controversial subject. Under our experimental treatments, the growth of roots was more adversely affected than that of leaves by an increasing acidity and this verifies earlier work with other species (Yan *et al.* 1992). It is known that low pH may inhibit plant growth directly (Andrew 1976, Mahler and McDole 1987, Shubert *et al.* 1990a, Wilkinson and Duncan 1989), probably by adverse effects at the root plasmalemma level, and also restricting net H^+ release by H^+ ATPase activity, and thus limiting dry matter production during vegetative plant growth (Van Beusichem 1982, Shubert *et al.* 1990b). In acid conditions, more energy is needed to extrude protons into the soil (Nowotny *et al.* 1998). The necessary ATP for this process might be synthesized via the glycolytic pathway. The increase in invertase activity in plants grown at pH 4.0 and 3.0 suggest increased demand of substrate for respiration. Compared with control, in plants exposed to pH 4.0 we also observed a higher activity of GK, PGI and PK, three enzymes with an important role in the regulation of glycolysis (Podestà and Plaxton 1994). The higher activities of these glycolytic enzymes could explain the increase in invertase activity and also indicate an increased glucose turnover and herewith an increased ATP production. In plants exposed to pH 3.0 we observed the highest activities of GK and PGI both in roots and leaves of kikuyu, but a lack of activity of PK, the last enzyme of glycolytic path, suggesting a block to glucose turnover, a decreased ATP synthesis and, consequently, a reduction in amino acids and biomass production, leading to slow growth.

Furthermore, the increase in PEPCK content suggests that in, acid stress condition, this enzyme may create an alternative path to supply pyruvate which could be used in respiratory processes (Wingler *et al.* 1999). However it is not, itself, capable of making up for the lack of reducing power. The G6PDH, the primary regulated enzyme of OPPP, provides $NADPH^+$ for biosynthetic reactions. The decreased G6PDH content, in kikuyu grass grown at pH 3.0, would thus cause a further lack of carbon skeleton and reducing power necessary to metabolic processes. The observed increase in MDH content, in leaves of kikuyu when the pH decreased, suggested a possible role of replenishment in reducing power, in stressed plants with disturbed metabolism; on the contrary, the decreased MDH content in roots grown at pH 3.0 may be due to direct exposure of roots to the high H^+ concentration, which causing intracellular pH variations, could interfere on some metabolic processes (Shankar *et al.* 2001). In short, these findings suggest that kikuyu is a grass tolerant to acidity, showing no differences in nutritive properties and biomass production between control plant, and those grown at pH values greater than 4.0. The acidity resistance of kikuyu can be

explained by the capacity of this grass to withstand the decrease of G6PDH and PK, important enzymes involved in carbohydrate metabolism. This suggests the possible utilization of kikuyu grass, with established fodder utility, in acid soils, where the survival of other species is markedly reduced.

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