

— SHORT COMMUNICATION —

**USE OF A PREPARED CELLULASE SOLUTION FOR SCREENING FORAGE
GRASS GERMPLASM FOR DIGESTIBILITY**

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(Received 14 September 1993; accepted 18 December 1993)

ABSTRACT

A reliable rapid and inexpensive laboratory procedure is needed to screen native African forage grass species for forage quality. This study determined the potential of using a prepared cellulase solution to assess *in vitro* dry matter solubility of 22 native Kenyan forage grasses and 18 native warm season grasses from Missouri, USA. A prepared cellulase solution was used for screening the grass species for digestibility using two procedures. One procedure involved digesting grass samples in prepared cellulase solution without any pre-treatment (CSD), and the other procedure used an acid pepsin pre-treatment prior to digestion in the prepared cellulase solution (APCS). The CSD procedure in comparison to APCS generally underestimated *in vitro* dry matter solubility by a range of up to 23%. The variations were highly species-dependent. Although the APCS procedure was more time consuming, it made it possible to identify a group of species resolvable into high (42.6–61.4%), medium (36–40%), and low (29–34%) digestibility. With intensified efforts it is possible for one person to obtain *in vitro* dry matter solubility estimates on up to 200 samples in 30 days with minimal expense. Results of this investigation points to the need for further research on the potential of the two digestibility procedures for rationalising numbers in germplasm collection for initial screening.

Key Words: Cellulase solution, forage grass germplasm, screening

RÉSUMÉ

Une technique de labo sure, rapide et abordable pour le criblage de la qualité des espèces africaines d'herbes fourragères est indispensable. Cette étude a établi la possibilité d'utiliser une solution de cellulase pour l'évaluation *in vitro* de la solubilité de la matière sèche de 22 essences fourragères rencontrées au Kenya et de 18 autres provenant des zone chaudes du Missouri, USA. Une solution de cellulase été utilisée pour cribler la digestibilité des différentes espèces au moyen de deux procédés. Une première méthode consiste à faire digérer les échantillons dans une solution de cellulase préparée au préalable sans aucun traitement antérieur (CSD); la seconde méthode passe par un prétraitement avec de la pepsine acide avant l'application de la solution (APCS). Les résultats estimatifs de digestibilité obtenus suivant la méthode du

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CSD, sont en général, plus près de 23% inférieurs à ceux de l'APCS. Les variations étaient hautement dépendantes de l'espèce. Le procédé du APCS bien que long a permis l'identification de 3 groupes d'espèces que l'on peut qualifier: à haute digestibilité (42,6 à 61,4%), moyen (36,0 à 40%) et faible (29,0 à 34,0%). En intensifiant les efforts, il est possible d'estimer à peu de frais la solubilité in vitro de la matière sèche de près de 200 échantillons en 30 jours. Les résultats de ce travail démontrent la nécessité de faire plus de recherches sur les potentialités éventuelles des deux méthodes de digestibilité pour la rationalisation du criblage des collections de germplasm.

Mots Clés: Solution de cellulase, espèces fourragères germplasm, criblage

INTRODUCTION

Eastern Africa has rich biodiversity of native genetic resources reported to have forage potential (Pratt and Gwynne, 1977). It is widely recognised that forage genetic resources in contemporary African agriculture play a major role in the interface between agriculture and livestock production. This is either through crop rotation, intercropping and/or mixed farming (Tohill *et al.*, 1990). The species serve mostly as natural pastures from which livestock primarily obtain feed. The pastures are, however, of low nutritive quality (ILCA, 1984). Besides productivity, two traits: digestibility and intake are considered important to further improve animal performance (Bughrara *et al.*, 1991).

Members of grass species in the genera *Brachiaria*, *Cenchrus*, *Urochloa*, *Sorghum*, *Chloris*, *Panicum*, *Paspalum*, *Cynodon*, *Pennisetum*, *Setaria*, among others, have much more commercial importance in the new areas of introduction outside Africa itself than the centre of origin. Some of the challenges possibly associated with their limited usefulness in Africa is the sheer number of species available (700–800 species from 42 genera) (Pratt and Gwynne, 1977). It has thus been impractical to evaluate most of these especially individually, for lack of suitable screening tools. Yet such screening is necessary for opening up germplasm for use by plant breeders. Frankel and Brown (1986) have suggested that this can, however, be achieved by structuring germplasm collections into broadly represented "core collection" and a 'reserve collection'. This is a new look at a large size of germplasm collection embodied in the current thinking for rationalising numbers for use. With this in mind, a preliminary study was undertaken with unselected and randomly sampled warm-season grass species, both from Kenya and Missouri, USA, to demonstrate the potential for

using a cellulase digestion technique in initial germplasm screening.

MATERIAL AND METHODS

Twenty-two grass species were randomly sampled from a large fallow open natural grazing field around the International Centre for Insect Physiology and Ecology at Duduville—Nairobi, Kenya. For comparison, 18 warm season naturally growing grass species were similarly sampled from the Agronomy Research Centre of the University of Missouri—Columbia, USA. Kenyan sampling was undertaken in the first week of July 1992, when most species had ripened, with drying inflorescences. Agronomy Research Centre collections from Missouri were obtained in the last week of August 1992, when plants, although with mature inflorescences, were still moderately green.

Harvests were taken at both locations by cutting the plants 5 cm above the soil surface in more than two separate non-overlapping hills (replicates). Plant material was dried for 72 hr at 50°C and ground to pass through a 1-mm screen using a Udy mill.

The type VII purified cellulase (Sigma Chemical Co., St. Louis, MO, USA), of concentration 8 g L⁻¹ with a reported activity of 5-g unit per mg solid, was used in the CSD method. Ten mL of prepared cellulase solution from 8 g L⁻¹ of citrate buffer solution was added to each duplicate set of 20 x 200 mm screw cap culture tubes each containing 200 mg of herbage. The capped culture tubes were placed in the incubator basket (50°C) and rotated by inversion end over end at 8 rpm for 5 hr, after which samples were centrifuged for 15 min at 2000 rpm. The supernatant was carefully pipetted out, another freshly prepared 10 mL of prepared cellulase solution added, and samples digested for an additional 20 hr.

TABLE 1. Digestibility of warm-grasses grown in Missouri (M) and Kenya (K) resolved in three empirical APCS quality backgrounds

Species names	Country of collection & code	APCS minus CSD= difference (%) ^a
A. The 'high' APCS quality background		
1. <i>Panicum</i> SPB	M49	18.0
2. <i>Pennisetum americanum</i>	M50	16.0
3. Giant foxtail (<i>Setaria faberi</i>)	M52	18.0
4. Yellow foxtail (<i>Setaria</i> sp.)	M45	13.0
5. Crabgrass (<i>Digitaria</i>)	M46	15.0
6. <i>Sorghum bicolor</i> (short variety)	M48	10.0
7. <i>Sorghum halepense</i>	M55	13.0
8. <i>Digitaria</i> sp.	K13	23.0
9. <i>Sorghum</i> sp.	K25	15.0
10. <i>Driochloa</i> sp.	K1	19.0
11. <i>Panicum dichotomiflorum</i>	M51	13.0
B. The 'moderate' APCS quality background		
12. <i>Dicanthium</i> sp.	K12	16.0
13. Barnyard grass (<i>Echnichloa</i>)	M57	15.0
14. <i>Andropogon scoparium</i>	M37	12.0
15. <i>Eragrostis</i> sp.	M54	12.0
16. <i>Eragrostis</i> sp.	K22	15.0
17. <i>Setaria</i> sp.	K19	15.0
18. <i>Paspalum</i> sp.	M53	12.0
19. <i>Panicum</i> (ex. Hinksen Creek)	M47	17.0
20. <i>Eragrostis</i> sp.	K11	10.0
21. <i>Echnichloa</i> sp.	K18	16.0
22. <i>Dactyloctenium</i> sp.	K5	16.0
23. <i>Tripsacum dactyloides</i>	M14	12.0
24. <i>Andropogon</i> sp.	M56	12.0
25. <i>Cenchrus</i> sp.	K6	20.0
26. <i>Pennisetum</i> sp.	K10	11.0
27. <i>Panicum virgatum</i>	M14	9.0
28. <i>Sorghastrum nutans</i>	M28	0.0
29. <i>Andropogon gerardi</i>	M31	9.0
30. <i>Panicum</i> sp.	K21	12.0
31. <i>Imperata</i> sp.	K8	12.0
C. The 'low' APCS quality background		
32. <i>Hyperhenia</i> sp.	K17	10.0
33. <i>Bouteloa</i> sp.	K9	9.0
34. <i>Sporobolus</i>	K23	9.0
35. <i>Polygonum</i> sp.	K20	12.0
36. <i>Cymbopogon</i> sp.	K16	12.0
37. <i>Sporobolus</i> sp.	K4	14.0
38. <i>Aristida</i> sp.	K26	12.0
39. <i>Eragrostis</i>	K24	12.0
40. <i>Digitaria</i> sp.	K15	5.0

^aThe difference is the improvement of dry matter solubility estimation by the APCS over the CSD procedure.

^bHigh (42.6–61.4%), medium (36–40%), and low (29–34%) digestibility.

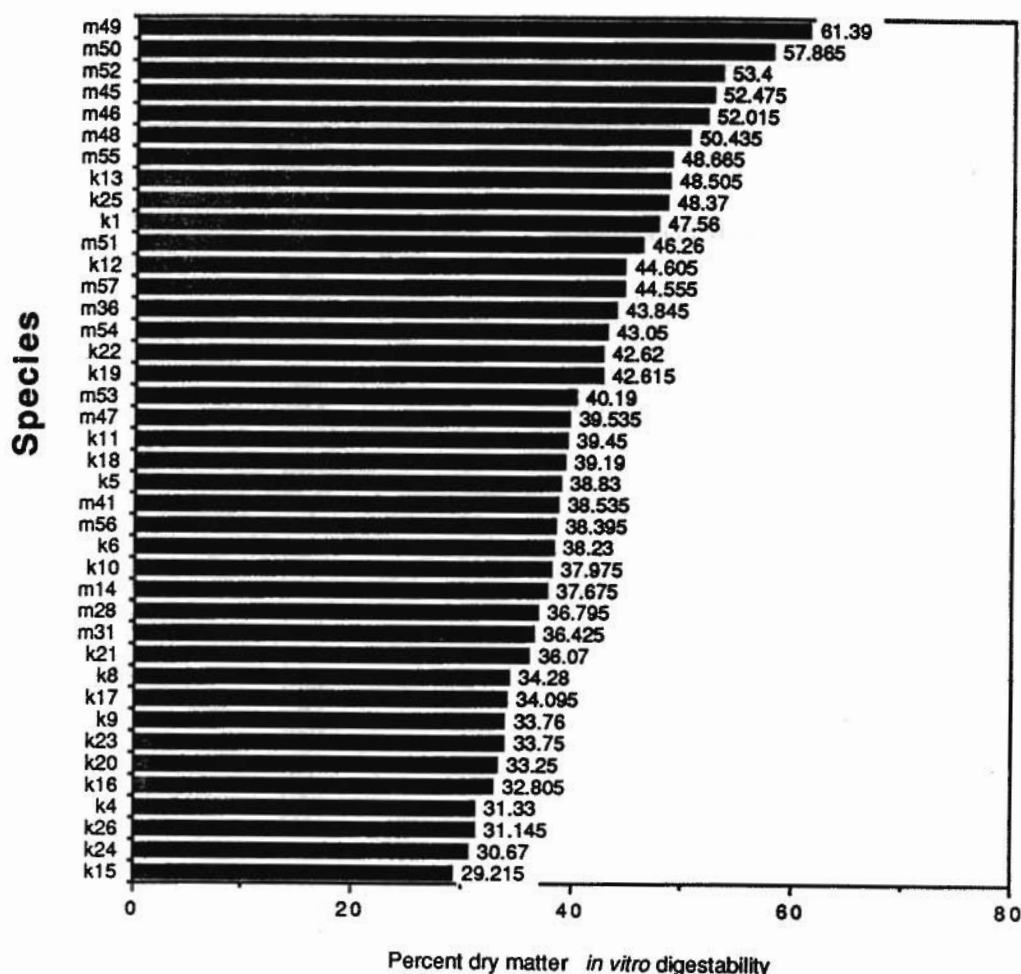


Figure 1. Comparison of acid-pepsin cellulase dry matter solubility of some Kenyan (k) vs. Missouri (m) grass species.

In the APCS method, two grams pepsin (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in 1 L of 0.125 N HCL. Twenty mL of the acid-pepsin solution was added to each culture tube and incubated for 48 hr as above. The tubes were centrifuged and the supernatants removed as previously described. Herbage residues were washed three times in hot water before a further 20 hr digestion in a cellulase solution. In both CSD and APCS procedures, indigestible residue was collected individually on Whatman 541 filter paper, washed three times in water (90–100°C) and twice with acetone. The herbage residue was oven-dried at 100°C for 36 hr, and dry matter digestibility calculated as the percentage of original dry weight of the herbage sample. Where duplicate

estimates differed by more than a 3% margin, they were re-analysed.

Attempts were also made to read the 40-sample wet digestion data into the near-infra red spectrophotometer (NIRS) as a blind assay, to assess if a computer programme would possibly provide a good NIRS omnibus interspecific calibration for spectral prediction equation for digestibility of the warm-season based grass germplasm. This failed.

RESULTS AND DISCUSSION

Dry matter solubility (DMS) was underestimated by the CSD protocol and ranged from 0 in M28 (in Indian grass) to 23% in K13 (*Digitaria* sp.) (Table

1). Species specific variations in CSD underestimations were highly apparent and were attributable to possible quantitative species differences in cell wall composition. In M28, no improvement was apparent with the acid-pepsin pretreatment. Bughrara and Sleper (1986) reported that the CSD procedure of forage consistently underestimated *in vivo* DMD by approximately 12 percentage units. The underestimation by CSD procedure in this study was upto 23% in *Digitaria* species (Table 1). Bughara and Sleper (1986) proposed that in which case, the CSD procedure can be used in plant breeding where large numbers of samples need to be analysed in a short time with minimal expense. On the basis of data in Figure 1, it would appear, however, that the APCS procedure might instead be more appropriate when interspecific ranking of samples is of interest in vastly divergent grass species. A casual perusal of the APCS ranking as elicited in Figure 1 makes it possible to identify a block of individuals, for example, as resolved into the high, moderate, and low APCS quality backgrounds (Table 1). The low APCS quality background for example, would likely be considered for gene introgression for other useful sources of productivity-promoting gene assemblies.

Although 10 of the Duduville collections (K21, K8, K17, K9, K23, K20, K16, K4, K26, K24 and K15) showed the lowest APCS digestibility ranking in the range of 34–29%, it should be noted that both the Duduville and Agronomy Research collections were harvested under two different growth conditions (temperate and tropical). This ranking is thus unlikely to be repeatable but it does demonstrate the application of this procedure to assessment of variation in digestibility in a range of grass species. It was possible to run 80 samples in 10 days single-handedly, suggesting that over time, enormous amount of screening work can be covered across several seasons.

The failure to calibrate the NIRS suggests that wet chemistry data on herbage samples must be obtained under a uniform set of conditions. Interspecific spectral equations may also be difficult to achieve but should that be possible, the NIRS procedure could be particularly useful in large number germplasm screening. Samples can be analysed in a short period of time and multiple analyses can be obtained simultaneously with the

NIRS instrumentation when properly calibrated (Bughrara *et al.*, 1991).

Results of this study point to the need for further research on the potential of the above procedures of rationalizing numbers in germplasm collections. It is considered that these procedures have value in opening up the African germplasm for use by African plant breeders.

ACKNOWLEDGMENTS

This study was conducted when the first author was a visiting scholar at the University of Missouri, Columbia, USA. Funding for the visit to Professor Sleper's laboratory was sponsored by the International Board of Plant Genetic Resources (IBPGR) to whom the authors are grateful. Dr. Margaret Magai did specimen identification from photo specimens and her contribution is greatly appreciated.

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