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Managing Tropical Forages Field Genebanks at CIAT¹

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Abstract

Two main reasons are identified for maintaining permanent field collections of tropical forages: (1) the inability to produce reliably sufficient and viable seed, and (2) outcrossing or highly heterozygous species where particular genotypes or populations should be conserved. The history, origin and management of tropical forage field collections maintained at CIAT are described. Details are given for each of the collections, i.e., the grasses—*Andropogon*, *Brachiaria* and *Panicum*, and the legumes—*Arachis* and *Leucaena*. Management experiences are given, and research needs for proper management are defined.

Resumen

Dos razones principales existen para mantener colecciones permanentes en el campo de forrajes tropicales: (1) la incapacidad de producir semilla suficiente y viable con seguridad, y (2) especies alógamas o altamente heterocigóticas, en las cuales deben ser conservados algunos genotipos específicos o ciertas poblaciones. Se describe la historia, el origen y el manejo de colecciones de campo de forrajes tropicales mantenidas en CIAT. Se dan detalles para cada colección, i.e., las gramíneas—*Andropogon*, *Brachiaria* y *Panicum*, y las leguminosas—*Arachis* y *Leucaena*. Se discuten las experiencias del manejo y necesidades para futura investigación.

Introduction

The forage germplasm held in the genebank of the Centro Internacional de Agricultura Tropical (CIAT) is mainly stored as seed, which provides a long-term and economical means of storage for most species, that can be dried and behave as orthodox seeds. However, some forage grasses rarely produce seeds and are known as shy seeders; others produce few viable seeds or are sterile hybrids. Several species of fodder trees may have a long juvenile phase before they produce seeds.

The most common alternative to seed storage is to store the plants as living collections in field genebanks. However, maintenance in field genebanks is not secure since the plants are at risk from pests and diseases. It is also costly in terms of management and requires large areas of land to accommodate sufficient numbers of individuals to represent the genetic diversity in the population, especially in large fodder tree species.

The objective of this paper is to give the background for the forages held in permanent field collections at CIAT (Table 1), describe management experiences of these collections, and define research needs for proper management. We also took advantage of this opportunity to document the history of these different collections at CIAT.

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Field collections at CIAT

Field collections of tropical forages were originally established by the former Tropical Pastures Program (TPP) as working collections with the main objective of preliminary characterization of germplasm at Quilichao (Figure 1) for further use in selecting from the available natural variation or incorporating materials into breeding projects. Collections were maintained in the field also for seed increase, especially where this was difficult, as in the grasses. The responsibility for maintaining these 'breeders collections' was initially separated for legumes (E. M. Hutton) and grasses (J. W. Miles) until about 1985. In the mid 1980s, the *Leucaena* collection, grasses at Palmira since 1989, and in 1990 the remainder of these collections were handed over to the CIAT Genetic Resources Unit (GRU), as the GRU took over more and more operations concerning forage genetic resources. The ultimate goal of the field collections will be to reduce them to a minimum as soon as possible, as long as sufficient viable seed is produced or conservation is made possible by other means.

Grasses

All grass field collections at CIAT are periodically cut at least once a year. Maintenance fertilizer is applied every 2 to 5 years. The *Brachiaria* and *Panicum maximum* collections have been burnt every other year to minimize the effects of smut and rust.

Andropogon. A collection of about 90 accessions of *Andropogon* species, with emphasis on *A. gayanus* (= gamba grass), has been maintained under the responsibility of the GRU at CIAT-Palmira (Table 2) since 1989 (CIAT, 1989). *Andropogon* is a predominantly outcrossing grass. The germplasm was acquired equally through donation from African or other institutions, such as the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, and joint collection of CIAT and national agricultural research institutions (NARIs). Little is known about the initial collection strategy, whether plants were collected vegetatively or by seeds, and how many plants were sampled. Accessions have been introduced by seed to CIAT. Plants released from quarantine were vegetatively transplanted to a field plot at Quilichao in 1981 (Division 6-4), where they served for preliminary evaluation of acid soil adaptation and phenology (J. W. Miles, 1983, unpublished data), and for increasing seed for storage and distribution. From Quilichao, they were transferred to a plot (J1 Norte) at Palmira in about 1985, where they suffered heavy contamination. Recuperable accessions were later transferred vegetatively to another plot (P1) at Palmira in 1993. Every accession is maintained by 6 individual plants in two rows. Plants look morphologically quite similar probably because of vegetative propagation. Plot size is 2.00 x 1.50 m², with 4 m distance between plots. Regular cultural practices of these plots consist of hand-weeding and cutting them down to about 20 cm on a 3-monthly interval. So far, seed is being harvested without any isolation measure.

An unpublished proposal (J. W. Miles, 1982) discussed 5 different management schemes for *Andropogon gayanus* (Table 3), however, none of them was specifically put in place. Although they had been mainly propagated vegetatively within CIAT, most important management problems have been both contamination of accessions through outbreeding and mechanical mixture caused by fallen seed, and probably genetic erosion because of the very few individuals representing the accessions. This led probably to some loss of accession integrity. It may be much more adequate to store the diverse genetic combinations of this germplasm in the form of seeds than trying to maintain specific genotypes, which probably have, over the years, unnoticed genetically eroded.

Brachiaria. As of June 1984, only 76 accessions of nine *Brachiaria* species were available in the CIAT gene bank in Colombia. CIAT maintained this small collection at Quilichao (Table 2), which originated from donations of seed samples by several institutions, especially from the National Agriculture Research Station at Kitale, Kenya. The major part of the *Brachiaria* collection at CIAT

originated from the 1984/85 expedition in eastern Africa, which was carried out in collaboration with the International Livestock Centre for Africa (ILCA, now ILRI) and several African institutions, supported by the International Board for Plant Genetic Resources (IBPGR) (Keller-Grein et al., 1995). The collection strategy followed, attempted to maximize the diversity sampled from as many populations and in as diverse environments as possible (G. Keller-Grein, 1996, personal communication). Preferably, *Brachiaria* germplasm was not collected directly from the roadside; when the vegetation was uniform, samples were taken about every 30 to 50 km, otherwise with special attention to changes in altitude or vegetation. Almost exclusively vegetative material was collected from 10 to 15 individuals, which was then transferred to NARI's experimental stations or ILCA headquarters, where they were planted directly in the field or in pots. From those plants, axillary buds were cultured *in vitro* to be introduced, by about 5 to 10 vials per accession representing the maximum of individuals, directly to CIAT from the respective NARIs (Figure 2). Most of the accessions from that collection are duplicated at International Livestock Research Institute (ILRI). In contrast, much of the germplasm received from other institutions was lost in the donors' collections (Keller-Grein et al., 1995). Most *Brachiaria* species are apomicts, but most accessions also show certain percentages of sexuality (Valle and Savidan, 1995), which may lead to some outcrossing and difficulties in maintaining accession integrity.

The field collection of *Brachiaria* was established at Quilichao (Division 6-4) in 1981. Since 1985, new plants were added as 10 individuals in plots (2 x 3 m²) side by side at Quilichao, as they were released from Colombian quarantine. Rows between plots measure 1.5 m. The collection has been used for morphological and isozyme characterization (Keller-Grein et al., 1995), for determining reproductive mode (Valle and Savidan, 1995), spittlebug resistance (Valério et al., 1995), and forage quality (Lascano and Euclides, 1995). Plants also served for distribution as tillers and seed, however, only little good quality seed was produced since establishment. Since 1992, accessions have been also planted at Popayán (Table 2), where good quality seed can be produced. The basic collection, however, is still maintained at Quilichao.

As with the other grasses, the main management problem arises from contamination of plots by both naturalized *Brachiaria* or other grass weeds, and fallen seed from adjacent plots. No isolation measures were taken for sexual accessions, and it is not known to which extent the genetic integrity of these accessions was maintained. For these sexual materials, similar propagation schemes as those discussed for *Andropogon gayanus* (Table 3) may be considered.

***Panicum*.** An important donation to CIAT came as seeds from the National Agriculture Research Station at Kitale, Kenya, in September 1981. It originated from collections mainly in Kenya of the former Trust Fund Forage Collection and Evaluation Project of the Food and Agriculture Organization of the United Nations (FAO), based at the National Agriculture Research Station at Kitale (Ibrahim, 1984). Accessions from viable seed were transplanted to unreplicated field plots, containing a total of 20 plants arranged in two rows each, at Quilichao (Division 6-4; Figure 1). Where fewer seedlings were obtained from the original seed, plots were completed using vegetative propagules from the seedlings that were obtained. The two rows were 50 cm apart and plants were planted at 50 cm within rows, with 1.50 m distance between plots. Transplanting was completed by December 1982, following quarantine inspection of seedlings in the glasshouse, and the collection was maintained at this site until July 1984. Plots were cut to about 20 cm on seven occasions during this period with harvests 10 to 14 weeks apart. Qualitative ratings on agronomically important characters were made, and seed was harvested (J. W. Miles, 1985, unpublished report).

The largest part of the collection of *Panicum* germplasm at CIAT originated from a donation by ORSTOM (Office de la Recherche Scientifique et Technique d'Outre-Mer) in November 1983. It had been collected from 1964 to 1969 in Ivory Coast, Kenya and Tanzania as seed. The most important species is *P. maximum* (= guinea grass), a tall bunch grass, that reproduces predominantly

by apomixis. However, sexual populations were located near Korogwe in Tanzania (Savidan *et al.* 1984). The *Panicum* collection of ORSTOM in Ivory Coast was abandoned (G. Rippstein, 1995, personal communication), after it had been donated as seed to CIAT, EMBRAPA/CNPGC in Brazil, and the Estación Experimental 'Indio Hatuey' in Cuba. While the germplasm was comprehensively characterized morphologically, agronomically, and cytologically in the 1970s by ORSTOM in Ivory Coast (Pernès, 1975; Savidan *et al.*, 1984) and in the 1980s by EMBRAPA/CNPGC in Brazil (Costa *et al.*, 1989), additional accessions collected from East Africa (Ethiopia, Burundi, Rwanda, and Zimbabwe) and maintained at CIAT have not been characterized or evaluated, as yet (Maass *et al.*, n.d.). In Brazil, seed of all apomictic accessions was increased and is maintained in cold, dry conditions both at CNPGC (20% relative humidity, 10 °C) and CENARGEN, while sexual plants are still maintained in the field at Campo Grande. Viability problems occurred in several accessions so that regeneration from seed has been impossible in several cases (L. Jank, 1996, personal communication).

The initial field collection at Quilichao was transferred to another plot in 1984 (Division 5-11), where subsequently new accessions from the ORSTOM collection were added after release from Colombian quarantine. The accessions of this permanent field collection were maintained by 10 plants in 2×2 m² plots at Quilichao since late 1984 until the end of 1995. It had been impossible to produce high quality seed because of smut incidence at Quilichao. Therefore, plots were periodically cut and burnt. The transfer of plants to Popayán did not reduce the smut problem. Recently, only 415 (about 80%) of the accessions could be rescued because many plots at Quilichao had been contaminated. Recuperated plants were potted in the glasshouse at Palmira, where good quality seed is being produced at present.

Hyparrhenia. About 40 accessions of different species of *Hyparrhenia* have been maintained in a field collection at Quilichao (Division 4-5) since the early 1990s, as they were released from Colombian quarantine. *Hyparrhenia* is an outcrossing grass, for which a strategy of germplasm maintenance needs to be defined, similar to that of *Andropogon gayanus*. The collection at Quilichao has essentially served for initial seed increase, although no isolation measures have been applied. For proper maintenance, basic biological knowledge about this genus needs to be generated concerning taxonomic status of species, interspecific crossability and population biology. Maintenance by weeding and periodically cutting back, is carried out as with other grasses.

Other grasses. Until mid 1982, a grass field collection was grown at Palmira in order to maintain accessions that do not set seed, and as a demonstration plot. The latter purpose was not fulfilled, since many of the materials were not representative of the main stream of germplasm of the CIAT TPP (now, Tropical Forages Program), and the former was in doubt due to the proximity of the plots to each other, causing a high percentage of contamination. Materials were therefore transferred and re-arranged in another plot (CIAT, 1983) at Palmira (J1 Norte), where many accessions were lost over the years because of excessive mechanical contamination of the plots. The responsibility for maintaining these plots passed from the TPP to the GRU in 1989.

The recuperated accessions (about 40%) were again vegetatively transferred to a new plot (P1) at Palmira in 1993, where most accessions are now maintained by 6 individual plants in two rows, being the plot size 2.00 x 1.40 m². Some accessions, such as some species of *Pennisetum* and *Tripsacum*, exceed the plot size and they need to be trimmed regularly. Besides cutting the 3-m-ways between plots, maintenance is restricted to weeding the plots and cutting them periodically, about 3-monthly. Contamination of the plots with local grass weeds (e.g., *Sorghum halepense*) is the principal problem for very labour-intensive maintenance, as already pointed out with *Andropogon*. Seed is being collected, where possible, to reduce this field collection to a minimum.

Legumes

Arachis. Accessions of the wild peanut relative, *A. glabrata* (= rhizoma peanut), were introduced to CIAT as rhizomes from an old perennial nursery at Gainesville, Florida, by G. M. Prine in 1976 (Maass and Ocampo, 1995). After maintaining them in a field collection at Palmira (J1) until 1991, some accessions, such as CIAT 9083 and 9097, seem to be different morphologically from the material maintained in the USDA collection (Pittman, personal communication, 1993). Since 1992, new *Arachis* germplasm, especially *A. pintoii*, was introduced largely as stolons, some materials also as seed. These latter accessions originated predominantly from collection by EMBRAPA/CENARGEN, CIAT, and other institutions in Brazil. The collection strategy pursued for wild *Arachis* species was reported by Valls and Pizarro (1994) and Valls et al. (1995). Introduced accessions originated from an unknown number of plant individuals. Most accessions held at CIAT are not only duplicated as seed and/or plants at EMBRAPA/CENARGEN, but also at Texas Agricultural Experiment Station, Stephenville, Texas, USA, from where they are introduced into the United States Department of Agriculture (USDA) collection at Griffin, Georgia (Valls et al., 1994).

Arachis pintoii (= Pinto's peanut) plants were established as 40 individuals and other more difficult species to be propagated vegetatively as at least 10 individuals, in plots (2.0 x 1.2 m²) with a 4:1 sand:soil mixture, side by side, separated by Eternit divisions, at Palmira (N2) since 1993, as they were released from Colombian quarantine inspection. Plants are being used for morphological and isozyme characterization (Maass et al., 1993; Maass and Ocampo, 1995; CIAT, unpublished data), and for initial seed increase for storage and distribution. Stolons have also been distributed in the region.

Most important cultural practices are monthly cuttings to avoid mechanical contamination among accessions. Seed production has been very difficult for several reasons: (1) some species, such as *A. glabrata* and *A. repens* rarely produce seed, and if so, the offspring is morphologically very distinct from the mother plants; (2) the prolific seed producing species *A. pintoii* has been heavily attacked by a burrowing chinch bug (*Cyrtomenus bergii*: Hemiptera) (Kelemu et al., 1994), which can only be controlled by alarming quantities of systemic insecticides. Presently, a study on biological control of the chinch bug is being conducted at CIAT (A. M. Torres, 1996, personal communication).

Leucaena. The collection of *Leucaena* species at CIAT was largely donated by institutions, such as CSIRO, the University of Hawaii, and the International Development Research Centre (IDRC, Canada) collections in Antigua and Belize. A smaller number of materials originated from direct collections by CIAT and national scientists (Lascano et al., 1995). Many donated accessions were originally introduced by more than 200 seeds from an unknown number of trees. The different seed collection strategies of the collectors contributing to the major *Leucaena* germplasm collections were detailed by Hughes et al. (1995). From 29 accessions collected during joint CIAT and NARI missions, two thirds originated from 1 to 3 trees. Originally, *Leucaena* at Palmira was a breeder's collection, from where parents were selected to breed for acid soil tolerance (Hutton and Chen, 1993).

The field collection of *Leucaena* is maintained as an arboretum at CIAT (J1). In the larger, older part, 5 trees per accession were planted unreplicated at 1 m within and 4 m between rows, while in the younger part, there are 3 trees per accession at 2 m within and 4 m between rows. Hardly any maintenance was needed until the trees grew too high to be practical for seed harvesting, and thus were cut down at 1 m above ground in 1985. As some aged trees died, a rotational cutting regime every four years was to be established in 1992, when part of the older collection was cut down again. However, because of budget shortfall, this was omitted. Most important problems in managing the *Leucaena* collection are that many species intercross and thus specific or superior genotypes are not maintained in the form of seed (Hughes et al., 1995). In addition, trees are

maintained in better physiological status under regular cutting, so as to avoid aging and out-competing of weak accessions by the most vigorous ones. Shorter, physiologically younger trees would also not be attractive to big birds (e.g., herons), that can cause considerable damage of the trees.

Research needs

Research needs have been identified in two areas: (1) to help reducing field collections to a minimum and (2) to improve them where they need to exist.

Research in the first area should aim at studying the necessity of maintaining field collections by identifying alternatives for conservation (e.g., seed, *in vitro* or *in situ* conservation). In forages, research on seed physiology (e.g., orthodox/recalcitrant seed) would make a major impact in defining conditions for production and conservation of high quality seed and thus, reduce the need for maintaining field collections. Studies in reproductive biology (e.g., outcrossing rates, sexuality/apomixis) would also help to identify accessions or species that can well be stored as seed.

In the area of improving the management and maintenance of field collections, besides proper and comprehensive characterization of accessions conserved in a field genebank (non-seed producing, specific heterozygous genotypes, or outcrossing populations), it is necessary to study the existing diversity within populations (accessions, provenances) to be maintained. Cultural management practices that may help reduce the risk of contamination (by weeds; by same species in adjacent plots) should be investigated. Finally, protocols need to be established for maintaining an accessions according to its breeding behaviour and the principle objectives for conserving it in the germplasm collection.

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Table 1. Tropical forage germplasm field collections at CIAT (no. of accessions as of 31.10.1995).

Genus	Palmira	Quilichao	Popayán	Total distinct
Legumes				
<i>Arachis</i>	84	-	-	84
<i>Leucaena</i>	163	-	-	163
Other shrub legumes	144	-	-	144
Grasses				
<i>Andropogon</i>	75	-	-	75
<i>Brachiaria</i>	-	435	162	435
<i>Hyparrhenia</i>	-	40	-	40
<i>Panicum</i>	-	500	112	500
Other	76	-	-	76
Grand total	542	975	274	1517

Table 2. Location and edapho-climatic conditions of the CIAT forage germplasm field collection sites.

Site	Latitude	Altitude (masl.)	Temp. (°C)	Annual rainfall (mm)	Dry months (no.) ^a	Soil	
						pH	Type and special characteristics
Palmira	3° 30' N	965	23.8	954	4	8.0	Vertisol, high base saturation, high concentration of soluble salts, no Al
Quilichao	3° 06' N	990	23.8	1799	3-4	4.0	Oxisol, low base saturation, > 80% Al saturation
Popayán	2° 25' N	1730	18.6	2483	2-3	5.0	Andisol (volcanic soil), organic matter 23%, medium-high base saturation, no Al

a. Dry month = <60 mm per month, bimodal rainfall pattern.

SOURCE: Howeler, 1986; CIAT, unpublished data.

Table 3. Proposed alternative propagation options for *Andropogon gayanus* germplasm, in order of decreasing control over the genetic integrity of individual accessions.

Propagation method	Main advantages	Main disadvantages
1. Vegetative propagation	Maintain individual genotypes within accessions	Maintain large number of clones; large and permanent field collections required; distribution of clones complicated by quarantine restrictions
2. Controlled self-pollination	Individual genotypes largely maintained; botanical seed available for distribution	Seed increase by selfing at high cost; unknown ability to produce S_1 hybrids - risk of loss of specific genotypes
3. Controlled interpollination within accessions	Maintain genetic integrity of individual accessions, if on sufficiently large scale	Large population size required to avoid random loss of genes; high costs because of isolation required
4. Uncontrolled intercrossing among accessions, seed bulked within accessions	Genes present in a given accession preserved if seed of enough plants per accession is bulked; greatly reduced cost relative to previous options	After 3 to 4 generations of such propagation, accessions would likely be indistinguishable
5. Uncontrolled intercrossing among accessions, seed bulked over accessions	Least expensive; genes present in a collection preserved if large enough populations are maintained and care is taken to minimize natural selection	Least control over genetic integrity of individual accessions; specific gene combinations would tend to be lost

SOURCE: J.W. Miles, 1982, unpublished data.

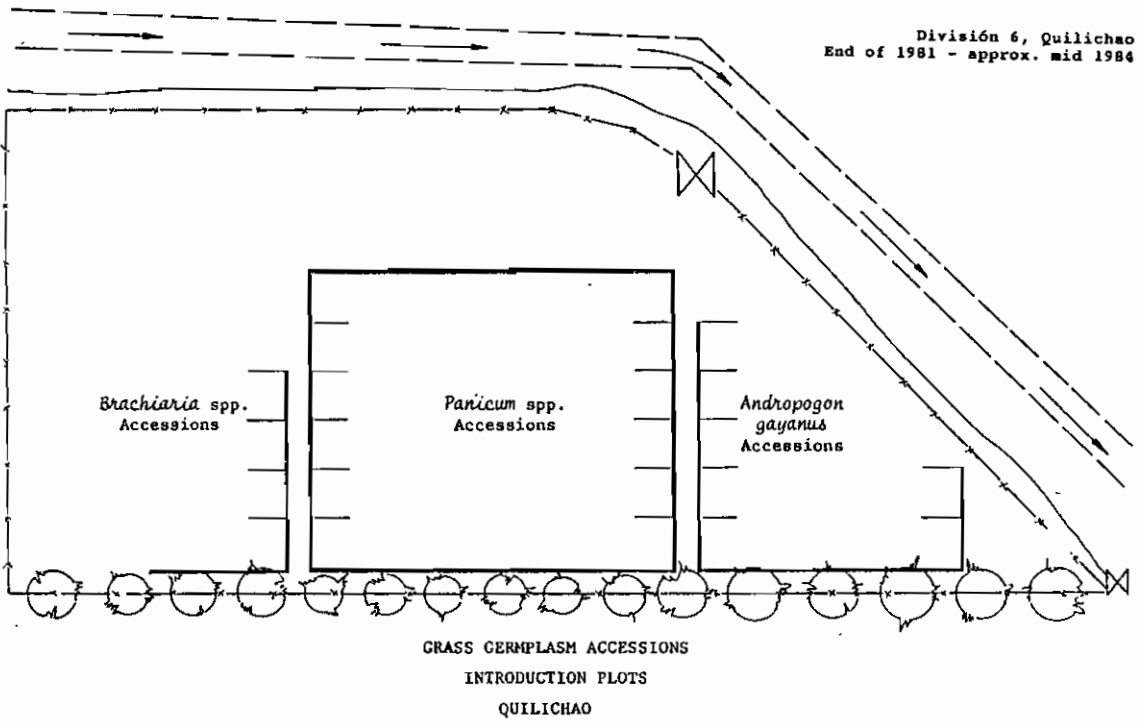


Figure 1. Grass germplasm collections of the CIAT Tropical ^{Pastures} Forages Program at Quilichao (Division 6-4) in the early 1980s (drawing as of March 1982).

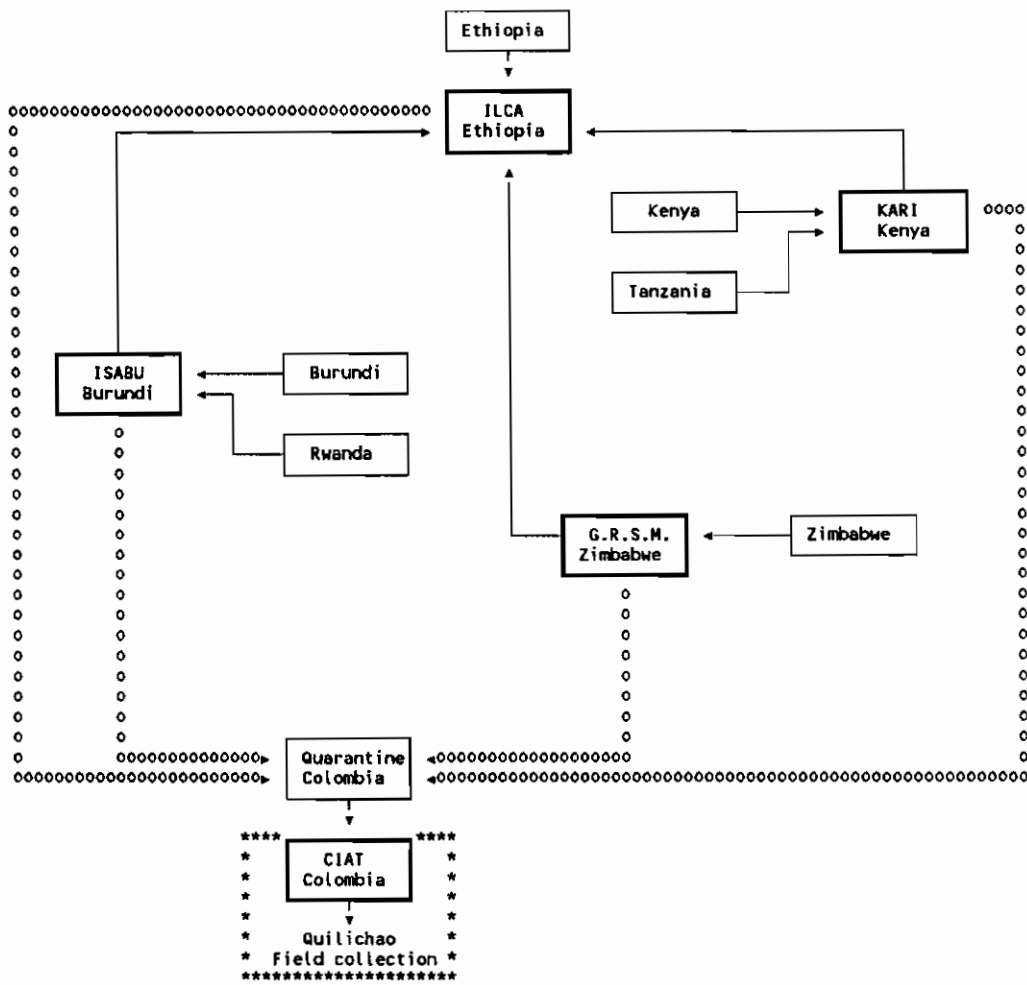


Figure 2. Transfer of *Brachiaria* germplasm from East African countries to CIAT in Colombia. (Single line boxes = country; double line boxes = interim institution, where tissue culture was conducted; —> = germplasm flow as vegetative material; - - -> = germplasm flow *in vitro*; KARI = Kenya Agriculture Research Institute at Muguga; ISABU = Institut des sciences agronomiques du Burundi; G.R.S.M. = Grasslands Research Station Marondera.)