Genetic Resources Communication

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#### SUMMARY

Fifty-one accessions of African *Trifolium* species were evaluated in single nursery rows at Grafton Agricultural Research and Advisory Station in 1978, 1981 and 1985. Of these species, accessions of the annuals *T. tembense*, *T. mattirolianum*, *T. rueppellianum* and *T. usambarense*, and the perennials *T. semipilosum* and *T. burchellianum*, appear to be worthy of further investigation. There are early flowering accessions of these species which are productive and set large quantities of seed. Regeneration from seed was not investigated, however field observations suggest regeneration does not often occur. All species had high levels of hard seed. Rhizobium specificity and disease resistance, particularly to viruses, also need to be considered before further recommendations can be made.

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#### Introduction

The genus *Trifolium* contains 40 species which are native to the eastern, central and west African highlands and parts of southern Africa ( $10^{\circ}N$  to  $34^{\circ}S$ ). They occur mostly at altitudes above 500m and up to 4200m. The majority of potentially useful species, except *T. semipilosum*, are adapted to moist situations with habitats such as riverbanks and swamp edges. For example, *T. africanum* and *T. burchellianum* have been described in lightly grazed, moist cool, high altitude sites (Jones et al., 1974). Agronomic testing of certain species of indigenous clovers of Africa was attempted in Australia as early as 1936. Little progress was made as seed lots were very small and effective nodulation could not be obtained (Norris and Mannetje, 1964). There had been continuing introductions into the Australian collection but little work with the species until recently.

A total of 199 accessions representing 20 *Trifolium* species of African origin were grown at the NSW Agriculture Research and Advisory Station at Grafton in 1978 (see Appendix 1 in wilson and Bowman, 1993). Of these, 51 accessions (4 perennial species and 8 annual species) were grown again in 1981 and 1985. These 51 accessions were selected as the highest seed yielders within particular species from the 1978 study. A commercial cultivar of the perennial *T. semipilosum* (cultivar Safari), which had been selected and released for permanent pastures in Australia in 1974 (Mackay, 1973), was used as a control accession for the perennials. It was hoped that there may have been other lines of *T. semipilosum* or other perennial species of *Trifolium* that would have better seed production than Safari. The annual accessions were investigated with a view to finding a ley legume or a suitable high quality species for intensive pastures.

The north coast of NSW lies between a latitude of 28°S and 32°S and extends to the escarpment of the Great Dividing Range. The climate varies from subtropical to warm temperate. Rainfall is high (800 to 1800mm) and falls mainly in the summer and autumn (64% summer dominant at Grafton). Short duration frosts are experienced throughout the region.

#### Methods

In 1978, 199 accessions of African clovers were grown. Fiftyone selections (based on seed yield within a species) were grown again in 1981 and 1985. In 1978 the seeds were germinated in flat trays in a potting mixture consisting of 0.75m<sup>3</sup> fumigated sand: 0.25m<sup>3</sup> peat moss: 2700g Dolomite: 1500g Superphosphate: 1200g UF 38 (a slow release N fertiliser): 700g lime: 800g Gypsum: 360g Potassium sulphate. These trays were placed in the glasshouse on 11.4.78 and seedlings were transplanted to nursery rows on 11.5.78. This mix was successful until the stage were the plants which did not nodulate ran out of nitrogen. In 1981 and 1985 seedlings were raised as individuals in trays of commercial "Quick pots" from 17.4.81 and 10.5.85 respectively. The seedlings were sown into nursery rows a month later in both years. Seedlings were inoculated with "best bet" non-

commercial mixes of Rhizobium strains (Table 2).

Single seedlings were transplanted into single 5m rows in the nursery area at Grafton Research and Advisory Station. These rows were 1m apart and 50 seedlings were transplanted per row. Irrigation was applied to establish the seedlings but they then survived under rainfed conditions. Nitrogen was added in the field to rows of plants which did not nodulate. The rows were hand weeded.

The soil at this site is a sandy clay loam, pH (water) 4.8, Phosphorous (Colwell) 90 ppm. This soil has a hardsetting surface when dry. The climatic data for this site is given in Table 1.

The plants were observed for pest and disease damage, flowering and seeding dates were recorded and seed was harvested and weighed at the end of each season. In 1985 the seed which was collected was germination tested in 1987 after storage at 5°C and 60% for the percentage of hard seed. Observations were also made on plant vigour, morphology and phenology.

#### **Results and Discussion**

As expected the perennial species had much lower seed yields than the annuals. However within the groups themselves there were large differences. The *T. africanum* accessions were the latest flowering and lowest yielding of the perennials, with little difference in seed yields between the accessions. *T. polystachyum* also had consistently low seed yields although it did not flower as late as *T. africanum*. *T. burchellianum* was also late flowering but the seed yields of two of the accessions were slightly more than 100 kg/ha. *T. semipilosum* was the highest yielder of the perennials. The selected line, Safari, produced more than three times the yield of the unselected CPI 57436, which flowered almost 40 days later (Table 3). Some accessions of *T. burchellianum* failed to nodulate with the available inoculants and this could have reduced growth and seed yield.

Of the annuals, *T. rueppellianum* accessions were the most consistent high seed yielders, with some producing an average of more than 700 kg/ha of seed. There appeared to be two groups of *T. rueppellianum* accessions, those that flowered at 90 to 100 days after sowing and those that flowered at 60 to 80 days after sowing. Those that flowered later tended to yield slightly higher although not consistently. *T. tembense* accessions were also high seed yielders although there was more variation. *T. tembense* tended to flower slightly earlier than *T. rueppellianum*. Four accessions of *T. tembense* had consistently low yields (CPI 24977.1, CPI 24977.2, CPI 24977.3 and CPI 24977.4). As all these have the same general CPI number it is likely they came from the same parent line and are different selections or generations of that line.

T. masaiense, T. mattirolianum and T. usambarense were the lowest yielders of the true perennials, with consistent yields of over 400 kg/ha of seed (Table 3). T. lugardii and T. steudneri were both high yielders. Although they are classified as annuals they tended to act like perennials in this environment. All species had high hard seed levels except for T. usambarense. It may be possible to select in other accessions of T. usambarense for

higher levels of hard seed.

Higher seed yields were produced in 1981 and 1985 than 1978. There was good late summer/autumn rain in both years just after sowing which may have assisted early growth of the plants. 1978 actually had greater spring rainfall (Table 1) than the other years but this did not appear to compensate for the slow early growth. Small (1968) demonstrated that flowering of South African clovers was stimulated by low night temperatures. Temperatures in winter in Grafton were down to 13°C which may have assisted flowering and hence seed yield.

Flowering of these accessions occurred from late winter to mid-summer at Grafton. Of the perennials, *T. africanum* flowered in October, *T. burchellianum* flowered from September to October, *T. polystachyum* in September and *T. semipilosum* and *T. usambarense* were the earliest in August. Of the annuals, *T. baccarinii*, *T. lugardii* and *T. usambarense* flowered in August, *T. masaiense* and *T. mattirolianum* in September, *T. rueppellianum* in July/August, *T. steudneri* in July and *T. tembense* from June through to August. There is room for selection for earlier flowering types in both *T. rueppellianum* and *T. tembense*.

From this nursery evaluation work at Grafton it was known that large quantities of seed were set by many of these accessions in this environment. However it was not known whether regeneration would occur, although seedling germination was noted in some cases from dropped seed in the nursery (Table 4). Seed of some accessions was sent to agronomists in other regions to sow in field areas and observe for regeneration. T. tembense (CPI 24978 and CPI 20746), T. mattirolianum (CPI 37936) with T. rueppellianum (CPI 10021) were sown in May 1990 at Tatham, near Casino at 3 kg/ha. T. tembense (CPI 20746) established, flowered on 1.8.90 and seeded by 29.8.90 but no regeneration was observed in 1991. T. rueppellianum (CPI 10021) flowered but did not set seed (B. Clark, pers. comm.). At Duck Creek, near Alstonville, T. rueppellianum (CPI 21155 and CPI 25346), T. tembense (CPI 25349 and CPI 10026), T. usambarense (CPI 33185) and T. mattirolianum (CPI 37936) were sown on 1.3.90. Flowering and seeding were only noted for T. tembense accessions but no regeneration was recorded (F. Biddle, pers. comm.). The same six accessions were sown in March 1990 at three sites at Samford, in Queensland. The T. tembense lines were the earliest to flower but all lines set seed at each site. However plants were not observed in October 1992 at any of the sites (R.M. Jones, pers. comm.). Hence although all lines established well and set seed at the various sites, no regeneration was observed. This was only a superficial look at regeneration and further work is required to investigate the conditions that break hard seed and trigger germination in these species.

African *Trifolium* species have no affinity with rhizobia of European clovers (Norris, 1959). Specific rhizobia still need to be found for some of these lines before their full potential will be realised. Norris and Mannetje (1964) confirmed that these *Trifolium* species are highly specific in their rhizobial requirements. Apart from strain specificity between species, there are also differences between varieties and in some cases between different accessions of the same species. General inoculations can not yet be made for all these species and accessions.

#### Conclusions

Neither of the most promising perennials, *T. burchellianum* and *T. semipilosum*, persists well in nursery rows yet when heavily grazed these two species compared favourably with *T. repens* in a moist sandy soil in the Upper Clarence Valley, near Grafton (Garden, 1977); there are many examples of persistent, grazed swards of *T. semipilosum* (Cameron et al., 1989). These two perennials are worth further investigation, particularly for situations where white clover (*T. repens*) is not persistent or productive. They are reputed to have greater tolerance of low pH and strong tap root development (Jones et al., 1974). Their productivity and persistence under grazing needs to be studied further. *T. africanum* appears to be too late in maturity to be useful on the NSW north coast.

The most promising annual species are *T. mattirolianum*, *T. rueppellianum*, *T. tembense* and *T. usambarense* all of which are productive, with early maturing accessions and produce large quantities of seed. These warrant more widespread evaluation under grazing and investigation into their ability to regenerate. They should be particularly considered on seasonally waterlogged soils and as components of a pasture ley.

### Acknowledgments

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Table 1. Monthly rainfall (RF: mm) and average temperature (T: °C) at Grafton Research Station for 1978, 1981 and 1985.

Year		Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total	
1978	RF	130.2	70.2	172.2	72.2	40.4	23.0	19.8	32.8	108.2	86.4	16.6	86.6	858.6	
	Т	24.9	25.2	23.7	20.4	17.3	13.9	13.4	13.7	16.3	18.1	21.3	23.5		
1981	RF	17.2	234.4	27.2	296.4	132.2	16.6	37.0	15.8	5.4	72.4	148.8	102.6	1106.0	
	Т	24.9	25.0	22.6	20.5	16.5	13.7	13.2	13.6	18.7	18.8	20.3	23.9		
1985	RF	56.2	166.5	169.7	114.4	145.4	23.6	155.2	25.5	19.3	143.7	48.2	144.1	1211.8	
	Т	25.2	23.2	22.7	19.9	17.2	13.2	13.5	14.1	17.5	19.4	21.5	23.8		

## Table 2. Characteristics of the African Trifolium species sown at Grafton in 1978, 1981 and 1985.

<u>Key:</u> A = annual, P = perennial, BGA = effected by Blue green aphid, V = effected by a virus or powdery mildew, Rhizobium = non-commercial "best bet" rhizobium strains, Origin = the general region from which that species came.

Species	Accession Number	Origin	Annual or Perennial	Pest or Disease	Rhizobium
T.africanum	CPI 26602.1	South Africa	Perennial		Mix
T.africanum	CPI 26622.2	South Africa	Perennial		Mix
T.africanum	CPI 26626.1	South Africa	Perennial		Mix
T.africanum	CPI 26629.1	South Africa	Perennial		Mix
T.africanum	CPI 26629.2	South Africa	Perennial		Mix
T.baccarinii	CPI 25343	Nigeria	Annual		CB 771
T.baccarinii	CPI 36208	Belgian Congo	Annual		CB 771
T.burchellianum	CPI 24132	East Africa	Perennial	V	CB 727
T.burchellianum	CPI 32718	East Africa	Perennial	V	CB 727
T.burchellianum	CPI 60388		Perennial	v	CB 727
T.lugardii	CPI 37934	Kenya	Annual	BGA	Mix
T.masaiense	CPI 25373	East Africa	Annual		CB 770
T.mattirolianum	CPI 37936	Ethiopia	Annual	v	NA176
T.polystachyum	CPI 33176	-	Perennial		Mix
T.rueppellianum	CPI 20744	-	Annual	BGA,V	CB 758
T.rueppellianum	CPI 21155	Kenya	Annual	BGA,V	CB 758
T.rueppellianum	CPI 25346	Kenya	Annual	BGA,V	CB 758
T.rueppellianum	CPI 25346.3	Kenya	Annual	v	CB 758
T.rueppellianum	CPI 25375.4	Mbulu	Annual	v	CB 758
T.rueppellianum	CPI 27217	Kenya	Annual	BGA,V	CB 758
T.rueppellianum	CPI 33177	East Africa	Annual	BGA,V	CB 758
T.rueppellianum	CPI 33178	East Africa	Annual	BGA,V	CB 758

Species	Accession Number	Origin	Annual or Perennial	Pest or Disease	Rhizobium
T.rueppellianum	CPI 33180	Kitale	Annual	BGA,V	CB 758
T.rueppellianum	CPI 37936	Kenya	Annual	BGA,V	CB 758
T.rueppellianum	CPI 37936.1	Kenya	Annual	v	CB 758
T.rueppellianum	CPI 37942	Kitale	Annual	BGA,V	CB 758
T.rueppellianum	CPI 48538	Ethiopia	Annual	BGA,V	CB 758
T.rueppellianum	CPI 58016	Kenya	Annual	BGA,V	CB 758
T.semipilosum	Safari	Kenya	Perennial	v	CB 782
T.semipilosum	CPI 57436	-	Perennial	v	CB 782
T.steudneri	CPI 25348	Kenya	Annual		CB 714
T.steudneri	CPI 37947	Kenya	Annual		CB 714
T.tembense	CPI 20746	-	Annual	v	CB 771
T.tembense	CPI 24977.1	Molo	Annual	V	CB 771
T.tembense	CPI 24977.2	Molo	Annual	V	CB 771
T.tembense	CPI 24977.3	Molo	Annual	v	CB 771
T.tembense	CPI 24977.4	Molo	Annual	v	CB 771
T.tembense	CPI 24978	Kenya	Annual	V	CB 771
T.tembense	CPI 24978.1	Kenya	Annual	V	CB 771
T.tembense	CPI 24978.2	Kenya	Annual	V	CB 771
T.tembense	CPI 24978.3	Kenya	Annual	V	CB 771
T.tembense	CPI 33184	Lumbwa	Annual	v	CB 771
T.tembense	CPI 25349	Kenya	Annual	v	CB 771
T.tembense	CPI 25376	Bashai	Annual	v	CB 771
T.tembense	CPI 58010	Kenya	Annual	v	CB 771
T.tembense	CPI 100026	Ethiopia	Annual	v	CB 771
T.usambarense	CPI 22165	Kenya	Annual	v	CB 771
T.usambarense	CPI 25350	East Africa	Annual	V	CB 771
T.usambarense	CPI 25377	-	Annual	v	CB 771
T.usambarense	CPI 33185	East Africa	Annual	V	CB 771
T.usambarense	CPI 33186	East Africa	Annual	V	CB 771

Table 3. Seed harvest times and seed yields of African *Trifolium* accessions in nursery rows: Sowing dates were 11.4.78, 17.4.81 and 10.5.85. Seed harvested in 1985 was tested for hardseed content in October 1987 after storing at 5°C and 60% RH.

Key: FF = Date of first flower, SH = Date of seed harvest, Y = seed yield of individual sowings (kg/ha), MDF = Mean days to first flower, MDS = Mean days from first flower to seed harvest, MY = Mean seed yield, %HS = % Hard seed, %D = % Dead seed, \* = contains *T. repens* seed.

Species & Accession Number	FF 1978	SH 1978	Y	FF 1981	SH 1981	Y	FF 1985	SH 1985	SY	MDF	MDS	MY	% HS
T.a.fricanum CPI 26602.1	3.10	28.11	15	30.9	16.11	96	29.9	11.11	13	160	48	41	75%
T.a.fricanum CPI 26622.2	3.10	28.11	29	16.9	16.11	134	28.9	11.11	4	155	53	56	33% 56%D
T.a.fricanum CPI 26626.1	3.10	28.11	7	30.9	16.11	89	29.9	11.11	11	160	48	36	68%
T.africanum CPI 26629.1	3.10	28.11	20	28.9	16.11	162	23.9	11.11	40	157	48	74	-
T.africanum CPI 26629.2	19.9	28.11	32	26.9	16.11	-	23.9	11.11	9	153	56	20	58% 36%D
T.baccarinii CPI 25343	7.8	28.9	125	29.7	4.9	248	31.7	30.9	89	91	50	154	-
T.baccarinii CPI 36208	11.8	6.10	42	14.8	3.9	388	16.8	8.10	471	103	43	300	-
T.burchellianum CPI 24132	22.9	28.11	168	11.9	19.10	18	16.9	25.11	248	136	58	145	73%
T.burchellianum CPI 32718	2.10	28.11	115	16.9	28.10	18	26.9	25.11	218	144	53	117	72%
T.burchellianum CPI 60388	16.11	-	388	12.8	6.10	-	28.9	11.11	1	149	49	-	85%
T.lugardii CPI 37934	7.8.	-	424	26.8	28.9	668	8.8	8.10	716	103	47	603	99%
T.masaiense CPI 25373	13.7	14.11	692*	20.7	7.9	480	5.7	24.10	299	102	94	490	74%
T.mattirolianum CPI 37936	13.7	28.9	365	1.7	28.8	416	1.7	30.9	656	94	75	479	76%
T.polystachyum CPI 33176	6.9	13.11	14	4.8	14.9	130	12.8	23.10	43	117	60	62	47%
T.rueppellianum CPI 20774	14.8	28.9	56	31.7	4.9	1165	5.8	8.10	1030	106	48	754	97%
T.rueppellianum CPI 21155	2.8	6.10	144	20.7	4.9	1016	12.7	8.10	862	90	56	674	84%
T.rueppellianum CPI 25346	14.8	28.9	137	29.7	4.9	1388	23.7	8.10	1036	101	43	854	98%
T.rueppellianum CPI 25346.3	11.8	28.9	308	4.8	9.9	942	5.8	8.10	1032	106	49	761	98%

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Species & Accession Number	FF 1978	SH 1978	Y	FF 1981	SH 1981	Y	FF 1985	SH 1985	SY	MDF	MDS	MY	% Н
T.rueppellianum CPI 25375.4	13.7	20.9	142	2.7	3.9	900	5.7	8.10	428	75	76	490	93%
I'.rueppellianum CPI 2 <b>72</b> 17	12.7	19.9	183	2.7	28.8	828	11.7	30.9	939	77	69	650	89%
T. <b>r</b> ueppellianum CPI 33177	2.8	19.9	90	2.7	28.8	941	5.7	30.9	427	72	64	486	82%
T.rueppellianum CPI 33178	2.8	26.9	163	11.7	3.9	880	11.7	30.9	1100	77	63	714	83%
T.rueppellianum CPI 33180	2.8	26.9	134	6.7	28.8	917	11.7	30.9	848	75	63	633	88%
T.rueppellianum CPI 37936	17.7	26.9	263	10.8	18.9	1033	5.7	30.9	988	89	63	761	92%
T.rueppellianum CPI 37936.1	2.8	13.10	218	17.7	3.9	952	15.7	8.10	976	80	68	715	92%
T <b>.ru</b> eppellianum CPI 37942	18.8	6.10	168	29.7	4.9	519	25.7	8.10	242	93	54	310	79%
T.rueppellianum CPI 48538	13.7	26.9	93	17.7	28.8	845	17.6	30.9	700	64	74	546	80%
T.rueppellianum CPI 58016	12.7	14.9	154	7.7	28.8	740	12.7	24.9	379	69	63	424	97%
T.semipilosum Safari	2.8	15.11	123	10.7	21.9	856	16.7	7.11	280	58	97	420	69%
T.semipilosum CPI 57436	2.8	15.11	129	28.7	8.10	226	24.8	9.11	33	97	85	129	28% 65%
T.steudneri CPI 25348	17.7	19.9	174	20.7	28.8	676	18.7	16.9	886	87	54	579	97%
T.steudneri CPI 37947	17.7	14.9	180	10.8	14.9	870	18.7	16.9	1136	74	51	729	95%
T.tembense CPI 20746	30.6	29.8	420	22.6	28.8	490	1.7	27.8	663	66	61	524	96%
T:tembense CPI 24977.1	12.7	7.9	23	20.7	28.8	28	8.7	16.9	146	82	55	66	97%
T.tembense CPI 24977.2	12.7	7.9	36	23.7	3.9	77	5.7	17.9	222	82	57	112	97%
T.tembense CPI 24977.3	12.7	29.8	36	10.7	28.8	27	5.7	16.9	143	77	67	69	95%
T.tembense CPI 24977.4	12.7	7.9	48	11.7	3.9	40	27.6	16.9	120	75	45	69	989
T.tembense CPI 24978	12.7	1.9	63	12.8	4.9	674	5.7	2.9	1033	88	47	590	94%
T.tembense CPI 24978.1	2.8	1.9	27	6.7	4.9	898	5.7	9.9	832	83	62	586	89%
T.tembense CPI 24978.2	2.8	29.8	84	6.7	4.9	888	8.7	9.9	911	84	50	628	97%
T.tembense CPI 24978.3	2.8	1.9	26	10.7	28.8	27	5.7	9.9	88 <b>7</b>	85	48	313	949

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Species & Accession Number	FF 1978	SH 1978	Y	FF 1981	SH 1981	Y	FF 1985	SH 1985	SY	MDF	MDS	MY	% HS
T.tembense CPI 33184	2.8	1.9	108	19.8	11.9	408	11.7	9.9	1129	100	38	548	95%
T.tembense CPI 25349	30.6	1.9	140	29.6	24.8	807	24.6	27.8	1438	66	61	795	81%
T.tembense CPI 23576	30.6	1.9	160	1.7	3.9	779	1.7	28.8	640	69	62	526	84%
T.tembense CPI 58010	30.6	1.9	219	22.6	4.9	527	20.6	27.8	742	62	68	496	94%
T.usambarense CPI 22165	2.8	19.9	41	13.7	12.8	461	1.7	24.9	812	84	54	438	38%
T.usambarense CPI 25350	11.8	26.9	44	17.7	28.8	532	5.7	24.9	856	90	56	477	63%
T.usambarense CPI 25377	10.6	14.9	41	10.6	12.8	386	24.6	24.9	484	53	84	304	11%
T.usambarense CPI 33185	2.8	19.9	41	17.7	28.8	619	1.7	24.9	816	85	58	492	41%
T.usambarense CPI 33186	2.8	19.9	109	17.7	12.8	488	8.7	24.9	874	88	51	490	49%

Table 4. Agronomic characteristics of African *Trifolium* accessions. Note: Maturity range comments have been made from growing out a number of accessions in other years besides those described in this paper.

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Species	Noted weakness (e.g.	Comments
species	disease susceptibility)	Comments
T.africanum	Late flowering, poor seeding ability	Can root from nodes
T.baccarinii		Can root from nodes
T.burchellianum		
T.lugardii		Annual but acted as a perennial, hardy
T.masaiense		
T.mattirolianum	Seed heads susceptible to grazing	
T.polystachyum		Seedling germination noted
T.rueppellianum	Seed heads susceptible to grazing, seed shattering	Seedling germination noted
T.semipilosum		
T.steudneri		Annual but acted as a perennial, hardy
T.tembense	Seed heads susceptible to grazing	Can root from nodes
T.usambarense		Seedling germination noted, responded to fertility and moisture

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