FORAGE BREEDING IN TAIWAN
— Review —

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Summary

Collection, evaluation and preservation of tropical forage grasses, napier grass breeding, tissue culture of pangola grass and alfalfa selection were conducted in Taiwan. The results showed that some species such as pangola grass (*Digitaria decumbens*) with good performance and wide adaptability were selected and released. Forage yield and quality of napier grass have been improved and have good results in animal performance. Embryogenic callus cultures from young inflorescences and stem segments of pangola grass could provide an alternative method for rapid propagation and improvement. Three better varieties of alfalfa were selected. They maintain satisfactory stands for two or three years on well-drained sand loam and loam soil, and used only as annual crop in flat area and acid soil However, more studies in forage breeding for acid soil are required to maximise the forage quality and animal production. Thus, animal fed with forages of high quality and a few grains supplement will be the future achivement in livestock industry.

(**Key Words**: Forage, Breeding, Cytogenetics)

Introduction

Forage crops are characterized by a great deal of genetic sources. Thus new varieties might be produced with little or no breeding. Many forage species are propagated by seeds but some of them are preserved and reproduced vegetatively. This allows special breeding methods to be applied, such as polycross and its variants (Frandsen and Frandsen, 1950). The apomixis, such as that observed in *Cenchrus ciliaris* (Burson et al., 1984), would allow one to fix any desired heterozygous gene combination and to maintain new heterozygous varieties with valuable agronomical traits, disease resistence and drough tolerance, etc. Intergenetic crossing are received more attention (Burton and Powell, 1968). However, seed setting of their progenies tends to be low. Cytoplasmic male sterility of some species has been available for some years and been used in F1 hybrids (Powell and Burton, 1966; Wit, 1974). The production of commercial hybrids with good performance remains a challenge to the breeder. The use of cell and tissue culture for identification and selection of useful variants at cellular level has also become one of the most powerful tools in modern plant breeding (Vasil, 1984, 1987). There are two major forage crops in Taiwan. One is napier grass (*Pennisetum purpureum*) and the other one is pangola grass (*Digitaria decumbens*). Vegetative propagation is the only practical means of establishing napier and pangola grasses. Even when it is mechanized, vegetative propagation invariably involves more labors and higher costs than that sowed by seeds and it might be an deterrent to set up grass establishment programmes in developing countries. These grasses comprise only one or a few genotypes and are more prone to serious disease problems. Pangola grass has been severely attacked by Pangola stunt virus in Central America and by rust in Australia and Taiwan. Napier grass has also become liable to fungus attack and nematode in recent years. The main activity of forage breeding in Taiwan Livestock Research Institute (TLRI) are includes germplasm collection, evaluation and preservation, napier grass breeding, pangola grass cell and tissue cultures, and alfalfa selection.

Collection, Evaluation and Preservation of Germplasm

Current breeding work is in urgent need of

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gene pools from which new genes can be introduced into existing cultigens in order to improve yield stability and resistance to disease and adverse conditions. Many species of grasses and legumes have been collected from foreign countries or around Taiwan and evaluated in the field for yield, quality, winter hardiness, seasonal production, regrowth characteristics and heading date, etc. Some species with good performance and wide adaptability to local environment were selected and recommended to farmers to grow, namely, napier grass, (*Pennisetum purpureum*), pangola grass (*Digitaria decumbens*), South African pigenon grass (*Setaria sphacelata*), guinea grass (*Panicum maximum*), kikuyu (*Pennisetum clandestinum*), Italian ryegrass (*Lolium multiflorum*), oat (*Avena sativa*) and red oat (*A. byzantina*), berseem clover (*Trifolium alexandrinum*), intortum clover (*Desmodium intortum*), and siratro (*Macroptilium atropurpureus*). Native species of grass and legume collected from low and high elevations in Taiwan are also good sources of germplasm. Some grass and legume species have potential to be developed as forages or turfs in the future, namely, *Polypogon fugax, Bromus catharticus, Paspalum dilatatum, Cynodon datylon,* Dichanthium, Digitaria, Panicum, Alysicarpus, Desmodium and Arachis, etc. At present, there are 329 species of grasses and 80 species of legumes preserved in the germplasm plots at TLRI. Collection and selection of tropical forage species for acid soil will very important in the future.

Napier Grass Breeding

Napier grass is one of the most important forage grasses in Taiwan. It is grown widely from flat area to 1000m high above sea level. It has high herbage yields, strong competition and persistence. However, the herbage quality is fair. The hairy leaves and sheaths make hand cut difficulty. Vegetative cuttings are mostly used for propagation as the seed are very small and shedding readily. The breeding program have been conducted since 1978 to improve napier grass by crossing with related species such as pearl millet(*P. americanum*), which have hairless and dwarf genes (Cheng and Lo, 1984, 1986, 1987, 1988a, 1988b, 1990). The breeding is concerned directly or indirectly with forage yield and its seasonal distribution, nutritive value, seed production, resistance to adverse weather condition, persistency and conversion to meat, milk and fibre production. The breeding scheme is shown in figure I. The results are summarized as follows:

1. Strain 7262 with very few hairs on leaf, late flowering and erect bunch type, was obtained from open-pollinated population of CV. A146 A148, A149 and produced 20% more dry matter than CV. A146 which is cultivar in Taiwan(table 1). It is also good for management by mechine from planting to harvest. A dwarf hybrid strain 7301 with smooth leaf, produced above 50% more

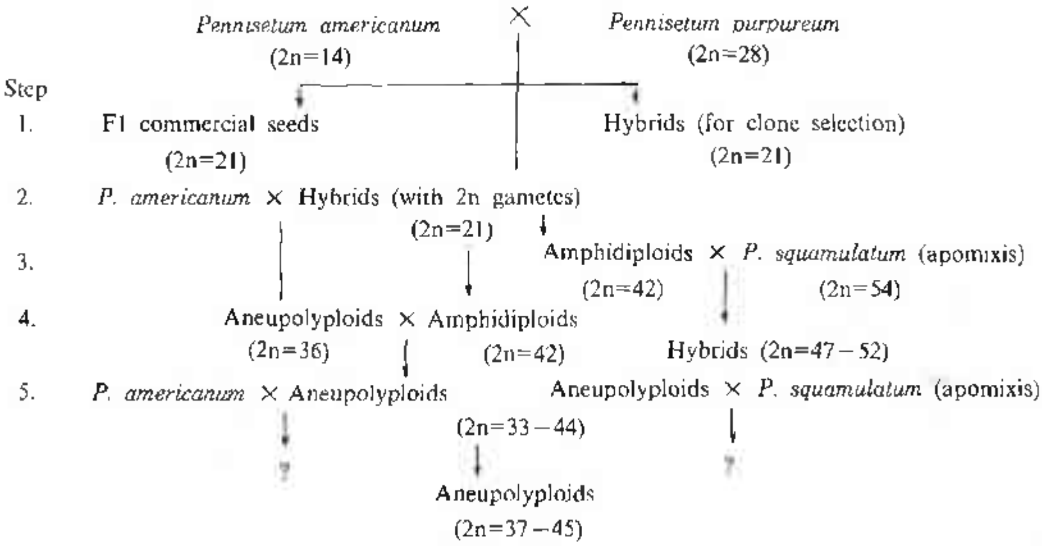

Figure I. Breeding scheme of napier grass and related species.

leaves than CV.A146 (table 2, 3), were obtained from hybrids of pearl millet crossed with napier grass. Dairy cows fed with this grass produces more milk than they were feed with pangola hay (table 4). Good results were also obtained when it was grazed by goat.

2. The hybrid seeds can be produced by crossing between cytoplasmic male sterile pearl millet and napier grass (step 1, figure 1). Some combination with better yields were selected (table

TABLE 1. AGRONOMIC TRAITS AND FORAGE YIELDS OF A146 AND STRAIN 7262*

Strain	Plant heigh (cm)	Leaf no. (no./plant)	Tiller (no./clone)	Leaf v.s stem (dry wt.)	Fresh wt. (MT/ha)	Dry wt.
7262	189.2[a]**	9.5[a]	22.7[b]	0.84[a]	231.8[a]	40.5[a]
A146	187.6[a]	9.0[a]	26.6[a]	0.80[a]	190.2[b]	33.5[b]

*Means of six locations.
**Means with the same letter in the same clomnn are not significantly different at 5% level.

TABLE 2. CHEMICAL COMPOSITION AND DIGESTIBILITY OF DRY MATTER OF A146 AND DWARF NAPIER 7301

Strain		Leaf v.s Stem	Crude protein	Crude fiber	P	K	Ca	Mg	ADF	Digesti-bility
			(………				%			………)
A146	Leaf	1.31	16.8	23.7	0.24	1.94	0.41	0.21	45.2	68.4
	Stem		11.1	25.4	0.15	3.64	0.21	0.26		
7301	Leaf	2.14	19.8	21.3	0.28	1.95	0.53	0.38	38.0	73.2
	Stem		14.9	23.4	0.25	3.64	0.33	0.37		

TABLE 3. YIELDS AND CHEMICAL COMPOSITION OF SIX TROPICAL FORAGE GRASSES

Strain		Fresh wt.	Dry wt.	Crude protein	Crude fiber	P	K	Ca
		(MT/ha)		(………		%		………)
Pangola	A24	98.6	19.7	9.0	26.1	0.2	1.5	0.49
	A254	101.8	20.9	9.2	25.3	0.24	1.2	0.62
Pigeon	A232	115.6	16.9	9.9	27.8	0.21	1.58	0.24
Guinea		135.7	24.2	10.0	26.7	0.30	2.14	0.89
Napier	A146	319.5	51.8	11.4	24.9	0.35	2.18	0.37
	7301 (Dwarf)	262.9	39.2	17.4	23.9	0.53	3.57	0.44

TABLE 4. MILK PRODUCTION AND QUALITY OF DAIRY COWS FED WITH DWARF NAPIER GRASS AND PANGOLA HAY

Forage	Uptake	Milk	Lactose	Fat	Protein
	(kg/day)		(………	%	………)
Dwarf napier (7301)	31.3	21.5	4.68	3.30	3.08
Pangola hay	6.8	19.5	4.64	3.36	3.05

5). The cost of hybrid seed production and some technical problems were required to be solved in the furture.

3. The results showed that forage quality and yield of napier grass were improvement. The hybrid strains are triploids, 2n=21 and propagated vegetatively. Thus, a fertile hybrid would offer opportunities for seed production and further improvement through hybridization. Hybrid plants which produced 2n pollen were selected (step 2 , figure 1). Most progenies of these hybrids were hexaploids, amphidiploids, 2n=42 (step 3, figure 1). Some amphidiploids produced good seeds were better than their triploid parents in yield and agronomic characteristics (table 6). It showed that the amphidiploids were allopolyploids and had the potential to produce higher yield or quality, and would be a useful gene sources for further improvement of napier grass.

4. Aneupolyploids were obtained by backcrossing hybrid pennisetum with pearl millet (step 2, figure 1) or by crossing aneupolyploids with amphidiploids (step 4, figure 2). The somatic chromosomes of these aneupolyploids ranged from 34 to 44. All aneupolyploids were perennial and have great variations in agronomic characteristics. Some partial fertile aneupolyploids with dwarf and late flowering would be useful for future germplasm transfer. A high degree of incompatibility was found when aneupolyploid was crossed with pearl millet or napier grass (step 5, figure 1). Hybrids could be obtained crossing between aneupolyploid and *P. squamulatum* or amphidiploids (step 3, 4, figure 1). The result showed that it was possible to transfer frost resistance and apomixis genes from *P. squamulatum* to cultivar. Based on the results obtained, it showed that the new strains produced by various cross breeding methods might be used either directly for commercial production or as materials for

TABLE 5. YIELD AND AGRONOMIC TRAITS OF NAPIER GRASS AND HYBRID LINES

Line	Plant height (cm)	Tiller (no./clone)	Fresh wt. (MT/ha)	Dry wt (MT/ha)
Tift 23DA × A146	210.0^{b*}	47.2^{a}	298.2^{a}	47.7^{bc}
Tift 23DA × A148	219.3^{ab}	49.6^{a}	333.5^{ab}	51.4^{a}
Tift 23DA × A149	212.0^{b}	46.1^{a}	296.2^{b}	45.0^{c}
Tift 23A × A146	231.5^{a}	41.6^{ab}	330.4^{ab}	49.8^{ab}
Tift 23A × A148	223.0^{a}	46.8^{a}	299.4^{b}	47.3^{bc}
Tift 23A × A149	229.0^{a}	40.5^{ab}	350.2^{a}	52.8^{a}
A146	215.6^{ab}	35.9^{b}	295.4^{b}	48.4^{b}

*Mean with the same letter in the same column are not significantly different at 5% level.

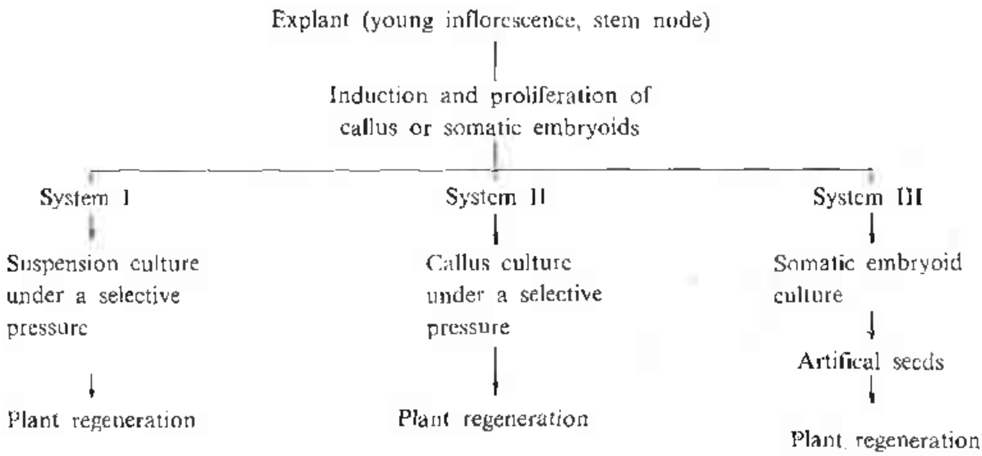

Figure 2. The culture scheme of pangola grass.

TABLE 6. YIELD AND AGRONOMIC TRAITS OF HYBRID STRAINS OF NAPIER GRASSES

Strain	Plant height (cm)	Leaf no. (no./plant)	Tiller (no./clone)	Leaf vs. stem	Fresh wt. (MT/ha)	Dry wt. (MT/ha)
7262	180.9^{a*}	6.4^{abc}	23.0^{ef}	0.9^{d}	457.4^{a}	59.5^{a}
7301	89.9^{h}	6.9^{a}	44.7^{b}	1.9^{b}	257.1^{d}	31.8^{d}
7336	149.6^{de}	6.3^{bcd}	28.2^{de}	1.0^{d}	367.5^{b}	50.3^{b}
7339	157.3^{cd}	6.2^{bcd}	32.8^{cd}	0.9^{d}	348.9^{b}	44.2^{c}
7380	105.5^{g}	5.2^{ef}	59.7^{a}	0.9^{d}	251.3^{de}	32.5^{d}
7404	152.6^{cd}	5.7^{def}	18.5^{f}	1.0^{d}	223.5^{d}	27.2^{e}
7407	171.2^{b}	6.8^{ab}	24.9^{ef}	0.5^{e}	219.9^{e}	27.1^{e}
7413	107.2^{g}	5.4^{ef}	37.6^{bc}	2.6^{a}	355.5^{b}	47.8^{bc}
7418	144.9^{e}	5.8^{cde}	34.1^{cd}	1.0^{d}	318.2^{c}	40.3^{c}
7419	125.9^{f}	5.1^{f}	38.0^{bc}	1.3^{c}	251.4^{de}	30.6^{de}

#. Growth period was from Oct. 3, 1987 to May 3, 1989.

@. Strains 7262, 7301, 7336, 7339, 7380 are triploids, and, strains 7404 and 7407 are aneuployploids, and strains 7413, 7418 and 7419 are amphidiploids, respectively.

* Means with the same letters in the same column are not significantly different at 5% level.

germplasm improvement to transfer desired traits among species or genus. These materials might be used for researches of cytogenetics, tissue culture and gene location, too.

Tissue Culture of Pangola Grass

The potential used of plant cell and tissue culture techniques in the improvement of cereals and grasses have been enumerated and discussed extensively (Chandler and Vasil, 1984; Vasil, 1981, 1984). Already some varieties and lines of major food crops which have been bred using these techniques are being released to farmers. Pangola grass was identified male sterile and did not set any seeds (Cheng, 1986). Therefore, further improvement through conventional breeding techniques is difficult, or where the efficiency of conventional methods is inadequate. Novel techniques such as biotechnology are needed to apply in breeding of pangola grass. Tissue culture of pangola grass is part of the biotechnology program at TLRI. The whole scheme is shown in figure 2. Objectives of the work were to :

- increase genetic variability and induce novel variability
- breed for high quality and disease resistance, especially rust
- select for tolerance to soil stress, such as salt tolerance
- propagated by using cell culture techniques for application in artifical seed production

Several findings and promising results have been achieved. Young inflorescences, stem node and leaf were used as sources of explants. These materials not only provide ample areas of intercalary meristematic tissues for rapid initation of callus, but also simplified the procedures of inocula disinfection. Embryogenic cultures were obtained from young inflorescences, young stem nodes, and young leaf with 2, 4-D (2, 4-dichlorophenoxy acetic acid) as an auxin source. The frequency of embryogenic callus formation was varied among different genotypes. The plantlets were regenerated via callus or somatic embryogenesis and had normal complement of chromosomes (Cheng, 1984; Cheng and Lo, 1987). The results showed that embryogenic callus cultures from young inflorescences and stem segments could provide an alternative method for rapid propagation and improvement of pangloa grass. Callus of pangola grass lost its regenerative potential easily by long-term culture and subculture. However, callus cultured in liquid medium containing 0.5% NaCl maintained regeneration potential for up more than 250 days. Difference was observed among genotype in callus induction on NaCl medium and plant regeneration from the NaCl tolerant calli. Callus induction was dramatically decreased with increased levels of

NaCl in all cultivars. Plant regeneration from *non-selected* calli was very low, but, unexpectedly, regeneration was increased with increased NaCl level (0 to 1%). Regeneration of plants from embryogenic callus cultures was highly efficient and rapid in pangola grass. One thousand plants could be produced from a young inflorescence explant within six months and transplanted to grow in soil. These results were particularly significant and useful for species that is propagated by cuttings such as pangola and napier grasses. Somatic embryogenesis has great potential to biology as well as agriculture since it provides an important tool for the study and analysis of molecular and biochemical events. Somatic embryoids can be subject to any subsequent manipulation such as scaled-up embryogenesis or artifical seed production or to establish pathogen-free planting stock, genetic crop improvement, and for germplasm storage and dissemination (Datta and Potrykus, 1989; Fuji et al., 1987; Vasil, 1981).

Performance of Alfalfa Varieties in Taiwan

Alfalfa (*Medicago sativa* L.) is a high-quality, perennial forage legume which produces excellent hay. It grows well on well drained soil without hardpans and does not tolerate a water table near the soil surface except for very short period. Flooding quickly destories alfalfa stand. Alfalfa is especially sensitive to acid soil and grows poorly in these conditions unless soil is limed and fertilized. Thirty varieties introduced from foreign countries were evaluated in different soils and climate conditions in Taiwan. The soil properties at different locations are listed in table 7. Three better varieties were selected from each location. There were PNR577, Florida 77 and Moapa 66. Dry matter yields from 1985-1987 at four locations were presented in table 8. The dry matter yields were maintained more than 20

TABLE 7. SOIL PROPERTIES OF FOUR DIFFERENT LOCATIONS

Location	pH	O.M.	T.N.	A.P	A.K	Ex. Ca	Ex. Mg	Ex. Al	Texture
		(……… %	………)	(………………	………	mg/kg	………	………)	(USDA)
Hsinhua	5.4	1.8	0.09	96	155	648	169.2	335	L
Pingtung	8.2	2.1		10.5	68	5500	5200	tr.	SL
Hwalian	7.9	2.1	0.12	28.0	115.0				L
Changhua	7.5	2.5	0.17	18.3	20.5				SL

TABLE 8. DRY MATTER YIELD OF ALFALFA CULTIVARS AT FOUR DIFFERENT LOCATIONS

Location	Cultivar	First year	Second year	Third year
		(………………	(MT/ha)	………………)
Changhua	Florida 77	22.4	30.6	17.2
	PNR 577	27.5	33.6	21.0
	African	16.8	19.6	14.8
Hwalian	Florida 77	16.9	20.0	14.4
	PNR 577	17.0	19.8	16.6
	African	13.5	14.8	10.6
Hsinhua	Florida 77	18.6		
	PNR 577	18.4		
	African	15.5		
Pingtung	Florida 77	24.2	29.7	20.1
	Moapa 69	27.6	25.0	13.8
	African	25.4	26.1	12.7

mt/ha in Changhua and Pingtung areas. At Hsinhua, it could be used only as annual crop in flat area and acid soil. Therefore, alfalfa stands to maintain a productive capacity were depended on soil type, climate, fertilization and management. It was possible to maintain satisfactory stands for two or three years on well-drained sand loam and loam soil. It did not persist in pooly drained and acid soil. Aluminum toxicity might be a problem during flooding with low pH soil. The reason that alfalfa is damaged by flooding is little known. High temperature during flooding strongly influenced the relative effect of flooding duration on regrowth rate of alfalfa tops. Flooding young plants for just 2 days at soil temperature above 30°C reduced the growth rate at 50% of the level that it was grown without flooding (Thompson and Fick, 1981). Selection of alfalfa for acid soil tolerance is a good approach to overcome this problem. Alfalfa selected in acid soil produced higher yield than that selected in limed soil, when it was tested in topsoil with a broad range of pH and fertility conditions (Bouton and Summer, 1983; Bouton et al., 1986). Selection of well-drained soil site is very important for alfalfa establishment, persistence and production before real resistant varieties are released.

Literature Cited

Bouton, J. H. and M. E. Summer. 1983. Alfalfa, *Medicago sativa* L., in highly weathered, acid soil V. Field performance of alfalfa selected for acid tolerance. Plant Soil. 74:436.

Bouton, J. H., M. E. Summer, J. E. Hammel and H. Shahandeh. 1986. Yield of an alfalfa germplasm selected for acid soil tolerance when growth in soils with modified subsoils. Crop Sci. 26:334-336.

Burson, B. L., P. W. Voigt and E. C. Bashaw. 1984. Approaches to breeding apomictic grass. pp 14-17. In Proc. 40th Southern Pasture and Forage Crop improvement Conf., Batson Rounge, Louisiana.

Burton, G. W. and J. B. Powell. 1968. Pearl millet breeding and cytogenetics. Adv. Agro. 20:48-89.

Chandler, S. F. and I. K. Vasil. 1984. Optimization of plant regeneration from long term embryogenic callus cultures of *Pennisetum purpureum* (Napier grass). J. Plant Physiol. 117:147-156.

Cheng, Y. K. 1984. Tissue culture of *Digitaria decumbens* Stent. I. Callus induction and plant regeneration from young inflorescence of pangola grass. Taiwan Livestock Res. 17(1):55-67.

Cheng, Y. K. 1984. Breeding of napier grass/pearl millet hybrid in Taiwan. FFTC Book Series. 25:194-203.

Cheng, Y. K. 1986. Cytological and histological investigation in male sterile anthers of pangola grass. Taiwan Livestock Res. 19(2):99-114.

Cheng, Y. K. and K. T. Lo. 1987. Somatic embryogenesis and plant regeneration from stem segment cultures of pangola grass. Taiwan Livestock Res. 20(2):69-79.

Cheng, Y. K. and K. T. Lo. 1987. The cytological mechanism of 2n pollen formation in hybrid napier grass. Taiwan Livestock Res. 20(2):91-103.

Cheng, Y. K. and K. T. Lo. 1988a. Histological and cytological mechanism of 2n pollen formation in hybrid napier grass. Taiwan Livestock Res. 21(2): 37-46.

Cheng, Y. K. and K. T. Lo. 1988b. Cytology and agronomic characteristics of amphidiploids in hybrid pennisetum. Taiwan Livestock Res. 21(2):63-76.

Cheng, Y. K. and K. T. Lo. 1990. Cytogenetics and agronomic characteristics of aneuploid in hybrid pennisetum. J. Agr. Assoc. China. 23(1):75-86.

Datta, S. K. and I. Potrykus. 1989. Artifical seeds in barely: encapsulation of microspore-derived embryos. Thero. Appl. Genet. 77:820-824.

Frandsen, H. N. and K. J. Frandsen. 1950. The polycross method. A mass hybridization method in the breeding of cross pollinated plants. Plant Breeding 20:39. (Abstr.)

Fujii, J. A., D. T. Slade, K. Redenbaugh and K. A. Walker. 1987. Artifical seeds for plant propagation. TIBTECH. 4:335-339.

Powell, H. B. and G. W. Bruton. 1966. A suggested commerical method of producing an interspecific hybrid forage in Pennisetum. Crop Sci. 6:378-379.

Thompson, T. E. and G. W. Fick. 1981. Growth response of alfalfa to duration of soil flooding and to temperature. Agro. J. 73:329-332.

Vasil, I. K. 1981. Plant cell culture and somatic cell genetics of cereals and grasses. Pl. Mol. Biol. Newslett. 2:9.

Vasil, I. K. 1984. Biotechnology in the improvement of forage crops. Proceedings of the XV. International Grassland Congress. pp. 45-48.

Vasil, I. K. 1987. Developing cell and tissue culture systems for the improvement of cereal and grass crop. J. Plant Physiol. 128:193-218.

Wit, F. 1974. Cytoplasmic male sterility in ryegrasses (Lolium spp) detected after intergeneric hybridization. Euphytica. 23:31-38.