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Establishment of Productive Rangeland with High-Yielding Fodder Shrubs

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Executive Summary:

Rangeland-based production of livestock is a principal source of income for the populations of semi-arid areas of southern Africa. This program aims to facilitate the establishment of improved fodder reserves in areas degraded by overgrazing and poor management a)by introducing promising fodder species and sustainable land use principles. b)by selecting the most promising species and individual shrubs for drylands (on the basis of agronomic and nutritional traits) and c)by making these superior shrubs available through clonal propagation. All aspects of the program have been addressed, at one or the other of the collaborating institutions, during this first year of the program. The program also aims as transferring technologies developed or improved in Israel, promoting development of technologies and expertise in South Africa and collaboration between academia and laypersons in communities where rangeland-based animal husbandry is practiced.

Seed of the target species designated in the research proposal have been introduced to South Africa, seedlings produced and trial sites established. Indigenous species valued by local ranchers have been added to the list of species to be studied and efforts to germinate their seed have begun. Sustainable land use practices, such as alley cropping, water harvesting and the use of water-absorbing polymers are being tested. Selection of promising shrubs at older fodder shrub plantations in Israel and at the younger introduction sites in South Africa has been underway. The work includes evaluation of individual shrubs on the basis of nutritional factors (metabolizable energy yield, gross energy and ash content), agronomic features, palatability and responses to clonal propagation treatments. Methods are being developed for the clonal propagation of target species, by rooting cuttings, micropropagation and somatic embryogenesis.

The Israeli collaborating scientists traveled to South Africa this year for meetings with their South African counterparts, research staff, graduate students and collaborators in the agricultural community. University facilities were visited and a five-day field trip made to the dry Northern Cape, which is a main area of rangeland in the country. Two of the UP group, a technician and a graduate student, both of whom are involved in evaluation of the nutritional value of fodder shrubs, spent two weeks in the Israeli animal nutrition laboratory to learn a key technique for evaluation of metabolizable energy yield in fodder plants. Graduate and undergraduate students at UP and at BGU are participating in the program.

Section I

A) Research Objectives

Rangeland-based production of livestock is a principal source of income for the populations of semi-arid areas of southern Africa. Overgrazing and poor management of rangeland has led to erosion and desertification. This program aims to facilitate the establishment of improved fodder reserves in such areas, composed of high-yielding perennial shrubs and trees, which would aid in establishment of a sound economic base for rural agrarian populations. Specific objectives are to:

1. introduce promising fodder species (shrubs and trees) to target areas in Southern Africa, for performance and palatability trials.
2. introduce and test sustainable land use principles on plots of introduced fodder shrubs.
3. select the most promising species and individual shrubs on the basis of agronomic traits, palatability and nutritional tests, for cultivation on poor soils in areas where rainfall is irregular.
4. develop procedures for large-scale clonal propagation of the selected shrubs: establishing centers in South Africa for fodder shrub propagation and distribution.

Aspects of each of these goals were addressed during this first year of the program.

B) Research Accomplishments

1. introduction of fodder species to target areas in Southern Africa: field trials and demonstration sites with seedlings

Although this program began officially in September 2001, aspects were initiated shortly after submission of the proposal to AID in 1995. Seed of *Atriplex nummularia*, *A. canescens*, *A. halimus* and *Cassia sturtii*, collected at the Omer Introduction Site (Israel), were transferred to the University of Pretoria. Seedlings were produced and were planted out at trial sites in Pretoria, Kathu, Postmasburg Kuruman, Mier, Bloemfontein and Pofadder in the RSA, where their performance was compared with other accessions and other species. Observations conducted over some five seasons have facilitated preliminary evaluations of adaptation, productivity, nutritive value and palatability. Of these (reported

on in the M.Sc. thesis of P.J. Malan), the Pretoria and Pofadder plantings have been used in follow-up studies while the Mier planting, in the duneveld of the Kalahari, is also suited for and will be used in follow-up work on palatability and nutritive value. *A. canescens* cv. Santa Rita seed has been harvested on the Bloemfontein site.

Seedlings of selected *Atriplex* species and *Cassis sturtii* have been produced in Pretoria, at the Hatfield Experimental Farm, for a summer planting at a new site, Klipkoppies, near Kenhardt. This site is on a sheep ranch belonging to Mr. Albert van Niekerk, an enthusiastic collaborating sheep rancher, who has already made provision for soil cultivation, protective fencing and an irrigation system for the establishment phase. Planting will be done in December 2001.

The impact of birds on the Hatfield planting of "elite" selections from the Verschoor and Malan studies has necessitated the erection of bird netting to protect the surviving plants. Although no seed was produced from these plants last season the seed potential for 2001/02 is looking good.

2. *selection and germplasm collection*

In Israel, selection and clonal propagation has focused on *Atripex nummularia*, *A. canescens* and *Cassia Sturtii*. Field trials of *Haloxylon persicum* and *H. aphyllum*, begun in an earlier AID-sponsored framework (CDR TA-MOU-94-CA14-030), have also continued. Selection of shrubs began at the Omer site (described in the research proposal) in February 2001, with monthly observations and measurements continuing through July 2001 and again in October. Cuttings from promising shrubs of each species were brought to the lab and greenhouse for tissue culture work and rooting trials (see *5. Clonal propagation by cuttings and micropropagation*, below). All species had been planted in randomized block design, with a consistent line spacing 1m x 2m. The site is not irrigated or fertilized, and many plants did not survive in the approximately 35 years since planting. Those individuals that did survive hold promise.

Some new vegetative growth was observed on *Atriplex nummularia*, *A. canescens* and *Cassia sturtii* on the first (mid-February) visit, although most of the *A. nummularia* and *A. canescens* and some of the *A. nummularia* also bore flower buds. *C. sturtii* shrubs on the borders of the Cassia plots were flowering and some had immature pods. These were not included in the selection. During the February and March field trips thirteen individual plants were singled out on the basis of survival, form and seasonal foliage production; 3 of A. nummularia, 3 of A. canescens and 7 of C. sturtii (table 1). Measurements of their heights and crown diameters were made on each visit (Table 1.). Measurements taken on the first and second field trips indicated that these promising C. sturtii shrubs averaged 1.62 m high X 1.2 m in diameter; the A. nummularia, 1.70 m high X 2.18 m in diameter and the A. canescens, 0.88 m high X 1.66 m in diameter. Later measurements showed minimal change in diameter and height during spring-summer. Palatability was not evaluated, as the area has been closed to grazing since its establishment.

Table 1. Selected shrubs of *A. nummularia*, *A. canescens* and *C. sturtii*

shrub #	Selection date	species	height, cm	diameter, cm	characteristics
1	Feb 13	A. numm.	185	230-180	erect, broad leaves, thick woody stems
2	Feb 13	A. canesc.	107	188-180	spreading form, small leaves
3	Feb 13	A. numm.	120	183-150	low bush, green foliage
4	Feb 13	A. canesc.	093	185-182	
5	Feb 13	A. canesc.	066	127-135	spreading form
6	Feb 13	A. numm.	205	261-306	erect, woody stems
7	Feb 13	C. Sturtii	162	065-130	flowering bush, green pods
8	Feb 13	C. Sturtii	162	120-100	highly –branched bush
9	Feb 13	C. Sturtii	165	144-110	highly –branched bush
10	Mar. 13	C. Sturtii	161	112-110	highly –branched bush
11	Mar. 13	C. Sturtii	162	095-100	highly –branched bush
12	Mar. 13	C. Sturtii	173	117-106	relatively high
13	Mar. 13	C. Sturtii	170	144-136	tallest in the block

The general appearance of the shrubs at the Omer site was much better on the March field trip than in February, following rains. All showed new growth. *Atriplex nummularia* was at the peak of blooming and *A. canescens* was beginning to flower. One month later, all Cassia shrubs were flowering and bore many green pods. The shrub to shrub variability was far less evident than earlier in the season. *A. canescens* had many new and shoots which were beginning to become woody. Yellow leaves with black spots were indicative of a fungal

infection on shrubs #2 and #5 (*A. canescens*). A. nummularia shrubs were already past their peak, with yellowish leaves and bearing maturing seeds.

During subsequent field trips (June 12, July 15 and October 17), in the dry summer months, all shrubs appeared dryer than before and were covered in dust. Measurements of height and diameter indicated that there was no new growth after June.

On the mid-October field trip it was observed that there had been an unauthorized grazing event. Droppings indicated that this had been done by sheep. *A. canescens* and *A. nummularia* shrubs were heavily grazed, with only the hard woody stems remaining. *C. Sturtii* had not been touched, apparently because of the concentration of the foliage and soft young shoots at the crown, which was less accessible to the sheep.

In South Africa, differences between individual plants with respect to palatability to sheep were recorded in early browsing trials carried out on the Hatfield Experimental Farm, near the campus of UP in Pretoria, confirming reports in the literature and verifying the potential benefits of selection and vegetative propagation. More in-depth studies of the nutritive value of several of the more promising spp. from these earlier trials are being carried out in the UP Department of Animal Science, while the relative palatability of these spp. from different sites is being evaluated in the U.P. Department of Range and Forage Science.

A nummularia cv. GrootFontein was selected 15–20 years ago by Gerhard de Kock at the Grootfontein Agricultural College at Middleburg, Eastern Cape Province. We have selected superior (elite) descendents of this cultivar, growing at the Hatfield Experimental Farm (see I), to be used for seed production and to provide material for vegetative propagation.

Evaluations have been conducted at the "Lovedale" site of edible material production (Venter), quality of edible material (Sparks) and relative palatability (Venter) of the various species and cultivars that have survived.

A decision was taken in the summer of 2000-01, to include indigenous species in our selection spectrum and for "plantation" plantings and indigenous range restoration, rather

than to focus only on exotic species. This was largely the result of on site observations of the flora and information collected from collaborators in the Northern Cape, during a week-long field trip by Rethman, Van Niekerk and Birnbaum. The species initially identified were Shepherd's Tree (*Boscia*), which is widely adapted in the Northern Cape and Northern Provinces, and Gannabos (*Salsola*), which is one of the more palatable components of Nama Karoo.

The germination of Boscia seeds, which we had collected this past season, has proven to be most difficult and the species is being short listed as "recalcitrant", requiring detailed study of its seed production, seed quality and establishment techniques, as well as the possible use of vegetative (cuttings and tissue culture) propagation techniques. With respect to Salsola we hope to access farmer-harvested seed from the Nama Karoo area and collect indigenous knowledge (from local land users) about how to establish this species, before conducting phytotron and field studies to improve/ensure good seed production and seedling establishment.

It has also been decided to broaden the range of indigenous species to include *Tripteris sinuatum*, *Eriocephalus africanus*, *Sutherlandia fructesens* and *Tetragonia calycina*. These are adapted to the target area, are highly rated in terms of both ecological and nutritional value and, most importantly, we have been able to access seed (self collected and purchased). This should improve our chances of obtaining results in the relatively short contract period.

3. testing of sustainable land use principles on plots of introduced fodder shrubs in South Africa

Existing work on alley cropping with *Leucaena* at the Hatfield Experimental Farm of UP has been expanded, to study the effect of water harvesting using contour bands on block plantings of spp. such as *A. canescens*, and *C. sturtii*. Preliminary water harvesting techniques were applied to "bare patches" in the Karoo range on "Lovedale", a ranch in the Northern Cape.

Greenhouse studies were initiated to test the use of water absorbent polymers in the rooting zone (see also Verschoor & Rethman). The initial trial was inconclusive, unfortunately, as overzealous labour, concerned by the apparent stress treatments (implicit in such work), over-watered the trial. This will be repeated.

4. analyses of plant material (Israel: training in Israel: transfer of methods to South Africa)

The Hohenheim gastest, a system for gas analysis for estimating metabolizable energy yield of forage was set up at the BIDR animal physiology lab. Leaves and shoots from mature plants of *Atriplex nummularia* and A. *canescens* and *Cassia sturtii*, selected in *2. selection and germplasm collection*, above, were analyzed by bomb calorimetry for gross energy and ash content. *In vitro* metabolizable energy value of the samples are being determined by the Hohenheim gastest method.

Ms. Elize Ferreira, a technician in the UP Nutrition Laboratory and Ms. Annelize van der Baan, a UP graduate student, spent two weeks in Israel learning the analytical methods for metabolizable energy yield (see Section II, D. Collaboration, Travel, Training and Publications, below) which will be applied in South Africa. Facilities are now being organized at UP for this purpose.

5. clonal propagation by cuttings and micropropagation

Efforts toward development of methods (cuttings and tissue culture) for clonal propagation of *A. nummularia* and *A. canescens* were begun at the start of this collaboration. These were conducted by an M.Sc. student from UP, working first at BIRD and later at UP. The work in Israel targeted preliminary selections of 25 year old shrubs at the Omer fodder shrub introduction site, where the average annual rainfall is 120 mm. Grazing had not been permitted at this site, so information on palatability was lacking. The criteria applied for selection were survival and green biomass [shoot and leaf] production. In South Africa, selection at the Hatfield site, where average annual rainfall is 650mm., was based on leaf retention after the preceding cold winter; recovery

after grazing, growth form suitable for grazing and preferential grazing by sheep for six seasons prior to the selection date.

Cuttings trial (Israel)

Cuttings were taken in the field, transported in plastic bags and processed in the laboratory (60 km away) on the same day, in order to prevent drying or loss of viability. Half of the cuttings were of the current year's growth only (semi-woody) and some had woody growth from the previous season. 27 cuttings were taken in February, 2001: 3 cuttings from each of the three shrubs selected of each species. The approximate length of each was 15-16 cm. Before inserting into moist substrate (50 % potting mix [coconut husk fiber and polystyrene beads] and 50 % local loess soil), leaves were stripped from the basal 5-8 cm. of each. The basal end of each cutting was dipped into water and then into "Hormoril T-8", a commercially available rooting powder containing 0.80% IBA and 5% TBZ in a talcum powder base. Finally, cuttings were inserted to a depth of approximately 6 cm into moistened substrate in 10 cm diameter pots. By the end of one month, 8 of the 9 cuttings of *A. nummularia* and 4 of the 9 cuttings *A. canescens had rooted*. 7 of the *A. nummularia* and 3 of *A. canescens* were semi-woody, indicating that semi-woody cuttings of the current year's growth root better than older material. Rooting trials conducted later in the summer months gave poor results with a very low rate of rooting. Rainfall in the Negev is limited to the winter period (essentially December-February). In mid-summer plants are under stress, not producing new shoot growth or foliage, and perhaps not able to produce roots. Growth resumes at the beginning of winter, which might be the best season for rooting cuttings.

Attempts to root cuttings of *Cassia Sturtii* taken in February and March failed. After some time in pots, leaves and stems of *Cassia* cuttings became brown and were covered with fungal spores. Attempts will be made this year to root cuttings of *C. Sturtii* shrubs growing on the BIDR Sede Boqer campus, which appear to be in better condition than those in Omer and free of heavy contamination.

"*In vitro*" procedures - Two *in vitro* approaches have been taken, conventional micropropagation, aimed at mass propagation via production and rooting of microshoots, and somatic embryogenesis.

Micropropagation (Israel and South Africa)

The media for each of the stages of micropropagation, initiation, multiplication, shoot extension and rooting used by Malan, were based on the Murashige and Skoog (MS) formulation (Murashige and Skoog, 1962). Initiation was carried out on phytohormone-free medium. Various combinations of benzylaminopurine (BA) and naphthaleneacetic acid (NAA) were included in the media for subsequent stages. While results in trials conducted at various times and with plant material of different origins were inconsistent, there were successes in micropropagation and acclimatization. This work served as the basis for current studies, which are aimed at establishing routine protocols for the species discussed above and other fodder species.

Tissue cultures were initiated in Israel from material taken from the field grown shrubs at Omer, described above, and in South Africa from shrubs grown in a greenhouse. Culture initiation was done on the same dates as the visits to the field, from material collected from the selected shrubs.

Shoots, approximately 25-30 cm. in length and including the stem tip, were prepared for disinfestation by removing dry leaves and stems and cutting each into two parts. They were washed in dilute Palmolive dishwashing detergent for 5 min. on a magnetic stirrer, followed by washing in running tap water for 30-4 0 min. They were then stirred in 5 % benlate for 5 min. on a magnetic stirrer and transferred to 20% commercial bleach solution (0.6% NaOCl) containing2-3 drops of Tween 20 (a wetting agent) per 100 ml for 15 min. Once in NaOCl the shoots were moved to a laminar flow hood, where they were rinsed three times with sterile distilled water and left in the final rinse water until explants were cultured.

Explants. consisting of terminal and axillary buds with or without a section of the subtending stem. 0.4-0.8 cm in length. were taken aseptically and inoculated onto hormone-free full-strength Murashige and Skoog basal medium (Murashige and Skoog. 1962). containing nicotinic acid (0.005 g/l), pyrydoxine HCl (0.005 g/l), thiamine HCl (0.005 g/l). myo-inositol (0.1 g/l), sucrose (30 g/l) and gelrite (2.4 g/l) (Malan. 2000). The pH of the medium was adjusted to 5.8 before autoclaving Cultures were held under cool white fluorescent lamps (50 $\mu mol\ m^{-2}\ s^{-1}$) in a 16/8 hour photoperiod at 23-25°C. By the end of two weeks of culture (the initiation phase) 85-90 % of the explants of both Atriplex species remained free of visible contamination. Only 10% of the Cassia explants survived the initiation phase without displaying contamination. Previous work (Malan and Birnbaum) had shown that the rates of success in disinfestation were higher with material of greenhouse origin then that of field origin.

In earlier. preliminary work. it had been found that shoot multiplication was best without phytohormonal additions for *A. nummularia*, with 0.20 mg/l BA and 0.50 mg/l NAA for *A. canescens* and with 0.20 mg/l BA and 0.05 mg/l NAA for *A. halimus*. In the current work. apparently clean cultures of *A. nummularia* and *A. canescens* were transferred to MS medium as described for culture initiation. supplemented with 0.20 mg/l BA and 0.05 mg/l NAA.

The date in Table 2 indicates that MS medium supplemented with BA and NAA at these concentrations is suitable, if not optimized, for shoot multiplication and callus formation. 18 days after transfer of A. nummularia to this medium, 92 % of the cultures of plant #3 and 72 % of the cultures of plant #6 had formed callus and shoots.

A. canescens developed more slowly. showing the first appearance of shoots 23 days after transfer from the initiation stage medium. There was a high rate of shoot formation in this medium and in some test tubes rooting occurred. The shoot height varied between 0.6 - 1.10 cm.. leaves were small and thick. and 2-4 buds formed in each test tube. The cultures of this species did not grow well and appeared to be weak and less viable than

those of *A. nummularia*. They did not form callus on MS medium supplemented with 0.20 mg/l BA and 0.05 mg/l NAA or on hormone-free MS (table 2).

Table 2. Responses of *A.nummularia* and *A.canescens* cultures grown on MS medium + 0.20mg/l BA and 0.05mg/l NAA

Plant #	Species	Cultures per plant	Callus	Shoots	Roots
1	*A. numm.*	25	-	+	±
3	*A. numm.*	27	++	++	-
6	*A. numm.*	29	++	++	-
4	*A. canesc.*	11	-	++	+
5	*A. canesc.*	11	-	+	+
2	*A. canesc.*	11	-	+	+

Key to the table:

code	Callus, dimensions	Shoots per explant	Roots per explant
-	none	none	none
±			occasional formation of
+	0.7cm ø x 0.9cm high	1-2	1
++	1.0cm ø, x 1.2cm high	3-4	2-3
+++	>1.0cm ø x >1.2cm high	>4	>3

The data also demonstrates plant to plant variability within each species in terms of *in vitro* responses, supporting the plan to follow up selection in the field with selection *in vitro*, for individuals that may be propagated clonally most efficiently.

The callus of *A. nummularia* was friable and light-yellow in color. In some test tubes calli occupied more than half of the medium surface. In addition to callus, between 2 and 8 buds were observed in each test tube. Shoots and buds that formed on the callus were reddish-green with small thick leaves and appeared to be vitrified (watery, wet thick leaves). Vitrification in Atriplex cultures, also observed by Malan (2000), might have several causes; among them a high concentration of mineral salts in the medium and low concentration of gelling agent (low osmotic pressure). *A. nummularia* cultured on MS

basal without hormones generally formed only one shoot per culture and 2-3 buds. Some cultures formed roots. Callus did not occur.

In addition to the initiation trial of *C. sturtii* on hormone-free MS medium containing nicotinic acid (0.005 g/l), pyrydoxine HCl (0.005 g/l), thiamine HCl (0.005 g/l), myo-inositol (0.1 g/l) ("basal vitamins", Malan 2000), sucrose (30 g/l) and gelrite (2.4 g/l) (Malan, 2000), explants were cultured on 3 other media formulations (Table 3), MS salts with Morel vitamins (Morel, 1950), MS salts with half-strength Morel vitamins and Woody Plant Medium including vitamins and amino acids (WPM) (Lloyd and McCown, 1981).

Table 3. Survival of *Cassia sturtii* cultures initiated on different media.

Nutrient medium and components	**cultures initiated**	**% surviving**
MS + basal vitamins	36	27
MS + Morel vitamins	36	22
MS + 50% Morel vitamins	36	14
WPM	36	69

The most suitable of these was WPM, on which survival in the initiation stage was 69% . On all other media the percentage of survival was significantly lower and after some time "*in vitro*" leaflets fell, browning occurred and growth was inhibited. Browning also occurred on WPM, but much later that in other media, and leaflets remained attached longer.

There was no pronounced growth of *Cassia* cultures. The next steps in this work will be aimed to identify an appropriate mineral salt concentration and concentrations of phytohormones that will elimination of formation of phenols, and promote survival, multiplication and development of Cassia.

The medium formulation for Haloxylon shoot multiplication, which has proven best thus far, is also based on the MS basal medium and is supplemented with the Morel vitamin formulation, 0.01 mg/l NAA and 0.05 mg/l BA. In future efforts we will attempt to improve upon this and develop the Haloxylon micropropagation system further.

Somatic embryogenesis

Efforts, begun in a previous AID program (CDR TA-MOU-94-CA14-030) have continued toward development of a system for clonal propagation of *Haloxylon aphylum* (Minkw.) Iljin through somatic embryogenesis.

Somatic embryos may be generated indirectly through callus. The most suitable media for callus induction from hypocotyls of *H. aphyllum* has proven to be BRP medium with 1mg/l 2,4-D and 0.1 mg/l BAP or with 2 mg/l 2,4-D and 1 mg/l BAP (media 1 and 2 in Figure 1). We were not successful, however, in induction of somatic embryos from this callus on a variety of basal media and combinations of phytohormones and additives, although embryogenic-like callus was generated (Aida E. Ramirez M.Sc. thesis, Studies Toward Induction of Somatic Embryogenesis in Haloxylon aphyllum *(Minkw.) Iljin*, 2000).

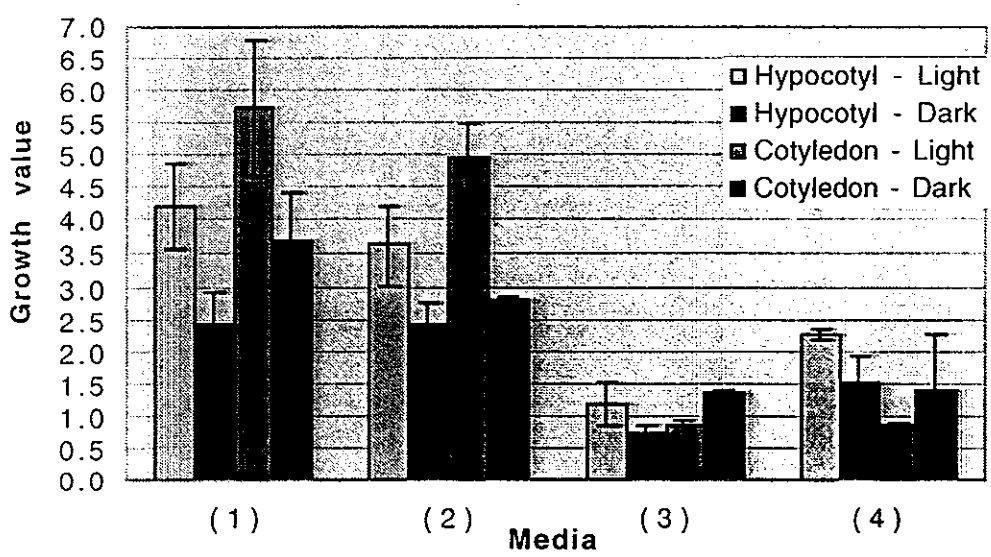


Figure 1: GV (growth value) of callus cultures initiated from hypocotyls and cotyledons of *Haloxylon aphyllum* after three months of cultivation in total darkness or under 16h illumination/8h darkness, in 4 media formulations.

(1) BRP medium + 1 mg/l 2,4-D and 0.1 mg/l BAP: culture initiated on the same medium prior to transfer to this.

(2) BRP medium + 2 mg/l 2,4-D and 1 mg/l BAP: culture initiated on the same medium prior to transfer to this.

(3) BRP, hormone free media; culture initiated on medium (1) prior to transfer to this.

(4) BRP, hormone free media; culture initiated on medium (2) prior to transfer to this.

Callus was initiated from cotyledons and hypocotyls of *H. aphyllum* on media determined in our previous work to be the most suitable for callus induction. Contrary to previous results, we found that calli initiated from cotyledons was more friable and appeared to be more promising for continued development into embryogenic callus. Also, the growth rate of calli initiated from cotyledons cultivated on media supplemented with 2 mg/l 2,4-D in combination with 1 mg/l BA or with 1 mg/l 2,4-D in combination with 0,1 mg/l BA was higher, under illumination as well as in darkness (figure 1). A "growth value" for callus, GV, representing the change in mass from time 0 to the end of the culture period, was calculated from fresh weight as the ratio of fresh weight at time "t" (after 4 weeks of cultivation) to fresh weight of the inoculum (time 0) (formula 1).

Formula 1. $\mathbf{GV = (m_t - m_0)/m_0}$

GV	-	Growth Value
m_t	-	fresh weight of biomass in time t [g]
m_0	-	fresh weight of biomass in time 0 [g]

The average inoculum weight per Petri dish was 0,84 ± 0,18 g for calli initiated from hypocotyls and 1,13 ± 0,17 g for calli initiated from cotyledons. Efforts at induction of somatic embryos from these calli are in process.

Somatic embryogenesis may also occur directly from cultured explants. Growth regulators in the medium have been reported to play a major role in stimulating this process (Williams and Maheswaran, 1986). Mechanical stress such as wound or excision treatment (Choi and Soh, 1996a) or chemical stress such as osmoticum or heavy metals have also been effective for induction of somatic embryos on media without supplemental growth regulators (Linossier et al, 1997; Choi et al. 1998). Investigation of the possibility of generating direct somatic embryogenesis of *H. aphyllum* was also begun this year. In our previous work (Aida E. Ramirez and E. H. Birnbaum. . Induction And Characterization of Callus In *Haloxylon aphyllum* (MINKW.) ILJIN; Proc. 4th International Symposium On In Vitro Culture And Horticultural Breeding, International Society For Horticultural Science, Commission Biotechnology, Tampere, Finland, July 2-7, 2000) the combinations of auxins and cytokinins which were tested failed to generate direct somatic embryogenesis, therefore an alternative approach has been taken. Following the hypothesis that endogenous phytohormone levels might prevent somatic

embryogenesis (Choi et al. 1997b; Choi and Soh, 1996b), we have attempted to induce somatic embryogenesis by inactivating this control mechanism. Plasmodesmata are generally believed to play a role in integrative cell-cell communication. Disruption of this communication by plasmolytic pretreatment is thought to inactivate the developmental control imparted by endogenous phytohormone levels. Choi and Soh (1997a) reported that plasmolyzing pretreatment broke plasmodesmatal connections between cells of cotyledon explants of *Panax ginseng* and that the frequency of direct single embryo formation was enhanced. Similar effects were reported in carrot embryogenic cells (Wetherell, 1984).

A plasmolysis pre-treatment was applied to cotyledons and hypocotyls derived from seedlings that had germinated on MS medium without growth regulators. (The germination time was 3 weeks). Plasmolysis was induced by immersing the tissues in a solution of 1.0 M sucrose for 1, 3, 6, 12 and 24 hours. At the end of this period the explants were deplasmolysized by decreasing the molarity of the solution stepwise at 5 min. intervals by dilution with water, until a final sucrose molarity of 0.1 was attained. Following deplasmolysis, the explants were cultured for 8 weeks in Petri dishes containing 30 ml of BRP medium (Burnouf-R'vich and Paupardin, 1985) containing 0.1 M sucrose. At the end of the culture period, microscopic observation did not reveal disruption of plasmodesmatal connections between cells. Small amounts of callus were observed along cut edges of the explants, but formation of somatic embryos did not ensue. The lack of an embryogenic response might be due to ineffectiveness of the plasmolysis pretreatment because of low sucrose concentration or short duration of the treatment or because the hypocotyls and cotyledons do not have predetermined embryogenic potential. For example, in carrot embryogenic cells, treatment with 1.0 M sucrose for 45 min was sufficient to plasmolyze and to severe the plasmodesmata (Wetherell, 1984), while Choi and Soh (1997a) reported obvious plasmolysis in ginseng cotyledons when treated with 1.0 M sucrose for 24 hours. However, plasmolysis of oat coleoptile cells with 2.0 M mannitol for 24 hours did not result in breakage of a majority of the plasmodesmata (Drake et al. 1978). More definitive information might be attained through histological observations, which have not as yet been carried out.

Atriplex nummularia

The effects of illumination, primary explants and growth regulators were determined on initiation cultures of *A. nummularia*. Leaf discs, 5 mm in diameter, and adventitious buds were cultivated on half strength MS medium supplemented with 2,4-D, IAA, IBA, Kinetin, BAP or GA at different concentrations (Table 4). Media was changed each 4 weeks. The culture room was maintained at 23 ± 1 °C with a 16-h photoperiod under 50 μmol m^{-2} s^{-1} cool-white fluorescent illumination or total darkness.

At the end of 4 weeks, callus formation was induced from adventitious buds of *A. nummularia* on the media containing either 2,4-D , IAA or BAP. Callus initiation was more frequent on adventitious buds growing under illumination than in the darkness (Table 4). At the end of a second 4-week period of culture under illumination, callus also appeared in cultures initiated from adventitious buds on MS media containing 1 mg/l IBA and 10 mg/l IBA . More callus formed on MS medium with 10 mg/l IBA than with 1 mg/l IBA. Shoot formation (approx. 6 mm long with purple pigmentation) occurred at the end of the second 4-week period on medium supplemented with 0.5 mg/l IAA. On the basis of these results, combinations of promising growth regulators are now being tested.

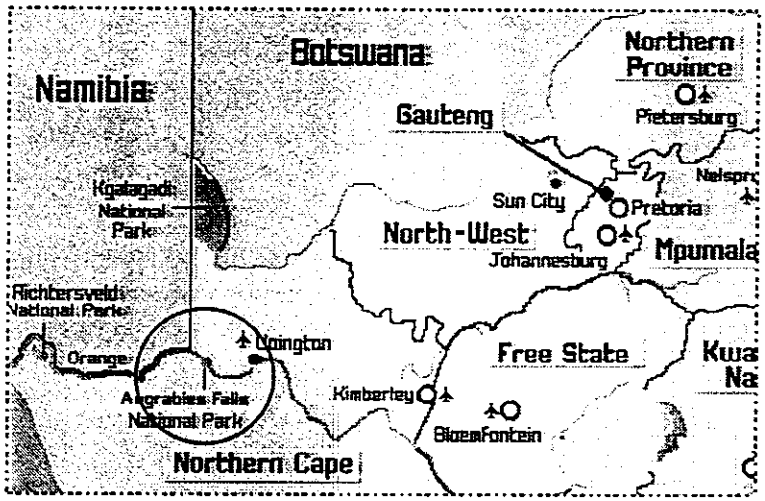
Table 4. Responses of primary explants of *Atriplex nummularia* to concentrations of individual growth regulators after the initial 4 weeks of cultivation (- = no callus formation; + = beginning of callus formation; ++ = callus of 1-3 mm diameter; +++ = callus of 4-5 mm in diameter).

Growth regulator	Callus formation on Leaf discs		Callus formation on Adventitious buds
	Illumination (16 h)	Dark	Illumination (16 h)
hormone-free	+	++	–
0.1 mg/l 2,4-D	–	–	+++
0.5 mg/l 2,4-D	–	–	+++
1.0 mg/l 2,4-D	–	–	+++
0.1 mg/l IAA	–	–	++
0.5 mg/l IAA	–	–	++
1.0 mg/l IAA	–	–	+
1.0 mg/l IBA	–	–	+
5.0 mg/l IBA	–	–	–
10 mg/l IBA	–	–	++
0.1 mg/l Kin	–	–	–
0.5 mg/l Kin	–	–	–
1.0 mg/l Kin	–	–	–
1.0 mg/l BAP	–	++	++
5.0 mg/l BAP	–	++	++
10 mg/l BAP	–	–	++

C) Scientific Impact of Collaboration

Each of the collaborating scientists has been active in his respective area of responsibility, in both research and training, and each has collaborated with the others. Work on selection and clonal propagation has been pursued by Birnbaum in Israel and Rethman in South Africa while Degen and Van Niekerk have worked in their respective countries on evaluation of nutritional value of the shrubs included in the plan of work (Degen) and identification of additional promising fodder species (Van Niekerk). Graduate students and postdoctoral fellows have been working on aspects of the program at both Universities (see Section II, D Collaboration, Travel, Training and Publications, below).

Birnbaum and Degen visited South Africa in February 2001, visiting the University of Pretoria facilities for animal husbandry, animal physiology, plant cultivation and plant propagation and meeting with faculty, technical staff and graduate students involved in the program. Following preliminary sessions at the University campus, Degen remained in the Pretoria area to have in depth meetings with students (Annelize an der Baar, Lindeque du toit, Jaco Hattingh and Francois Sparks) and technical staff involved in animal husbandry and nutritional evaluations and to view rearing facilities, grazing areas and analytical facilities. He also visited the Irene Research station and met with researchers working on sheep and goat and cattle. Birnbaum, Rethman and van Niekerk traveled to the Northern Cape - Kalahari Desert area (near the borders of South Africa with Botswana and Namibia; see map, right), to visit trial sites and grazing areas and to meet with collaborators in the local communities. With respect to both disciplines, the meetings were fruitful, leading to better understanding, inclusion of additional species in the selection program, additional training actions and better communication with members of communities in trial areas (details in other sections of this report).



D) Description of Project Impact

It is too early in the project to claim impact through implementation of results. However, communication between the collaborating scientists has been fruitful in further defining objectives and broadening the targets of the program. There has definitely been positive impact on relations with the Bushmen (represented by agricultural extension agent Mr. Desmond Smith) and the Afrikaaner community (Mr. Albert van Niekerk, rancher) involved in animal husbandry in the Northern Cape.

E) Strengthening of Developing Country Institutions

Ms. Elize Ferreira Ms. Annelize van der Baan came to to Degen's lab at BIDR in May 2001, combining this travel with a visit to a laboratory in Wageningen, Holland, where similar work is being conducted. Support for this action came in part from this AID program. Changes in the budget requested to facilitate this are presented in Section II, Budget, below. Following their visits, facilities were prepared and supplies acquired at UP to evaluate metabolizable energy yield of plant material according to the methods used by Degen. After returning to UP, Ms. van der Baan began experiments on the nutritive value of drought tolerant species and is currently half way to completion.

The only significant institutional constraint that we face is related to the flow of funds. The research is managed financially by a subdivision of UP, which does not have prior experience with AID CDR programs. There as been a problem in the flow of funds to UP, addressed in Section II, budget, below. We hope AID will release additional, overdue, funds shortly, so that UP will continue to allow expenditures in this framework.

F) Future Work

The work is, in general, following the schedule presented in the proposal.

a. Shrub selection in Israel of is underway, targeting *Atripex nummularia*, *A. canescens* and *Cassia sturtii*. Efforts are concentrated at the Omer site, with attention to *C. sturtii* and *Haloxylon* species on the grounds of the BIDR as well. These will continue into the coming year.
b. Selection in South Africa continues at field trials of the shrubs listed in the original proposal and new species have been added to the list for study and consideration. Shrubs at field trials in South Africa, established before the start of this program at 8 locations are being followed. Seedlings have been prepared for planting in December 2001 at Klipkoppies, near Kenhardt. More seedlings of these *Atriplex* and *Cassia* species will be produced for autumn plantings at five of the trial locations. The planted area at "Klipkoppies" (Mr. Albert van Niekerk) will be expanded. An area will be established at Mier, where we have the co-operation of the local extension officer, Mr. Desmond Smith, at "Lovedale", near Pofadder (in co-operation with Mr.

Willem van Niekerk) and in the winter rainfall area of Namaqualand, on the farms "Pendoornhoek" and "Olienfontein", in co-operation with Mr Koos Beukes.

c. Water-harvesting measures have been applied in field trials and soil ameliorants in potting mixes in South Africa. Alley cropping trials include *Leucaena*, *A. canescens*, and *C. sturtii*. Preliminary water harvesting techniques were applied to "bare patches" in the in the Northern Cape. In the future the field trials will be extended to revegetate bare patches, use bare patches as runoff areas, use small run-on areas for the establishment of nuclear plantings (which hypothetically will then spread out), apply mini-catchments in conjunction with mulches (to start or accelerate the ecological process of range improvement). In this context, we plan to test exotics, such as Australian Acacia (in the Sahel) and Atriplex (in Texas), which have been used to improve the micro-climate on micro-sites, to make them better suited for the establishment of desirable indigenous species. These efforts will continue to be addressed during the full duration of the project.

d. A system for estimating metabolizable energy yield of forage was set up at BIDR and leaves and shoots from mature shrubs have been analyzed for gross energy and ash content. *In vitro* metabolizable energy value of the samples are being determined by the Hohenheim gastest method. A technician and a graduate student from UP have been trained at BIDR in these methods and have set up facilities to implement them at UP. Intake, digestibility and palatability trials will be carried out on these plants at both institutions during the duration of the program.

e. Development of clonal propagation systems by tissue culture (micropropagation and somatic embryogenesis) and cuttings has been underway and will continue through at least the next two years at BIDR and is planned to begin in the coming year at UP, where it will be addressed through the duration of the project. Trials to correlate ease of rooting with season or stage of vegetative activity will continue. Efforts to root Cassia sturtii, which has thus far been recalcitrant, will receive further attention. The expression of plant to plant variability in vitro, viewed as a major criterion for "secondary selection" and observed in cultures of atriplex, will be pursued. Attempts will be made to eliminate the vitrification observed in cultures of *Atriplex* through use of half strength media and/or use of greater amounts of gelling agent and will explore

the effects of various concentrations and types of phytohormones on each species. With respect to all species for which *in vitro* propagation procedures are being developed, clonal selection at a secondary level, based on performance in tissue culture and in the greenhouse, will be made as the work progresses, in both countries. Characteristics that will be selected for will include responses of culture initiation, rates of axillary branching, rooting, amenability to direct or indirect somatic embryogenesis, and success in acclimatization.

f. Birnbaum and Degen visited UP this year, as planned. Rethman and van Niekerk are scheduled to visit BIDR in the coming year. An unscheduled, but important, visit by two members of the UP team, working on evaluation of plant nutritional value, was made to BIDR (this will be addressed in Section II, budget).

More extended training of a UP graduate student at BIDR has been delayed due to changes in personnel at UP and concerns for security under the current situation in the Middle East. Possible solutions to this problem and alternatives are being considered.

Section II

A) Managerial Issues

Describe any project managerial issues that have arisen since the last Management Report.

Turnover of personnel at the technical and trainee (graduate student) levels at UP since submission of the research proposal and current concerns for personal security by candidates have resulted in delays in the planned training in Israel. For this reason the decision was taken to give two people short-duration training in the analytical methods used in the animal physiology laboratory in May, 2001. An appropriate candidate is being sought for the training in clonal propagation techniques. Also, the alternative of an additional, more extended, visit to UP by Birnbaum, to work intensively with the tissue culture technician and a graduate student, is being considered.

B) Budget

Changes in the budget are requested in order (a)to accommodate difficulties in enlisting a student to spend 10 months in Israel for training in clonal propagation and (b)to cover costs of two trainees in the area of nutritional value evaluation. We request to delay the scheduled training in clonal propagation and associated expenditure until year 2 or 3. We (Rethman and Van Niekerk) request to cover the travel ($1,600) and per diem ($1,507) costs of two UP personnel who visited the BIDR animal nutrition lab in May 2001, by reducing sums in other budget lines in the year 1 and year 2 budgets, as follows:

line	Year	original budget, $	requested action	requested revised allocation
	1			
a.	trainee*, 10 months	-8,500	delay to Year 2	
b.	short-term trainees, 2		per diem expenses	1,507
c.	field technician	2,400	-650, to line b.	1,750
d.	**Travel & Transportation**	6,172	-457, to line b.	5,715
e.	Materials & Supplies	4,000	-400, to line b.	3,600
	2			
f.	trainee*, 10 months		from year 1	8,500
g.	field technician	2,400	-400, to line h.	2,000
h.	**Travel & Transportation**	8,772	plus 1600 for Int. travel	10,372
i.	**Equip., repair & maintenance**	3,600	-800, to line h.	2,800
j.	**Materials & Supplies**	1,600	-400, to line h.	1,200

Funds have not been transferred to UP since the original advance of $7,500. This is largely due to late submission of financial reports; however, in order to assure continuity in the program, we request that a transfer of outstanding funds be processed as soon as possible. The financial management of this program at UP is by Research Enterprises, a subdivision of the university, which has no previous experience with US AID CDR programs. Our request for this consideration stems from the following e-mail was received earlier this month:

Date: Wed, 14 Nov 2001 21:22:34 +0200
From: Annatjie Fourie <annatjie@postino.up.ac.za>
X-Accept-Language: en
MIME-Version: 1.0
To: Birnbaum Elliott <elliott@bgumail.bgu.ac.il>
Subject: Funds

Dr Birnbaum

Due to the fact that we have overspend on the work that we are doing for you we have requested $12394.37 on the 31 May 2001 of which we have still not received any funds. The only funds we have received is the $7500. RE at UP cannot fund this project for too long and we must now please ask you to transfer funds to the account of RE at UP urgently, details below:

RE at UP
ABSA Bank Limited
Lynnwood Road
Pretoria
South Africa
Account no.: 540492034

Any further questions please mail Annetjie or Prof WA van Niekerk.

We thank you.

Regards
Annetjie

C) Special Concerns

none

D) Collaboration, Travel, Training and Publications

1. Consultation in South Africa, Birnbaum and Degen, February - March, 2001
   a. meetings at UP of Rethman, Van Niekerk Degen and Birnbaum with UP personnel and graduate students
   b. in depth meetings of Degen to with students (Annelize an der Baar, Lindeque du toit, Jaco Hattingh and Francois Sparks) and technical staff involved in animal husbandry and nutritional evaluations
   c. visit of Degen to analytical and rearing facilities and grazing areas in Pretoria environs and to the Irene Research station.
   d. travel of Birnbaum, Rethman and van Niekerk to the Northern Cape - Kalahari Desert area, to trial sites and grazing areas

e. meetings with collaborators in the Northern Cape: bushman agricultural extension agent, Mr. Desmond Smith and Afrikaaner community leader, Mr. Albert van Niekerk.

The meetings in Pretoria and in the Northern Cape were fruitful, leading to better understanding, inclusion of additional species in the selection program, additional training actions and better communication with members of communities in trial areas. During our tour of rangeland in the Northern Cape, it was concluded, through field observations and consultation with sheep ranchers, that it would be justified to add indigenous species that are important in the natural feed base to the list of target species, for further study. They are Boscia albitrunca (shepherd's tree) and three species of Salsola. Seed of Boscia albitrunca and Salsola spp. have been collected from Kenhardt and Pofadder, RSA, for use in propagation.

2. Training and technology transfer

a. Two UP personnel, Ms. E. Ferreira, a Nutrition Laboratory technician and Ms. A. van der Baan, a graduate student, spent two weeks in A. Degen's BIDR laboratory learning the analytical methods for metabolizable energy yield which will be applied in South Africa.

b. Ms. Annalize van der Baan began experiments on the nutritive value of drought tolerant species and is currently half way to completion.

c. Lindeque du Toit, working towards an MSc Agric concentrating on the energy supplementation of oldman saltbush, is close to completing his experimental phase.

d. Chris Keen, a student, has conducting initial studies on germination of Cassia and use of polymers for water retention.

e. Danie Venter a student, initiated *Atriplex* seedling production, Boscia germination studies, palatability studies on shrubs at "Lovedale"; development of "Klipkoppies" site. Danie has also networked with members of the "Arid Zone Forum" which held a meeting at Calitzdorp in the Little Karoo and has made a presentation on his program to an Arid Zone Revegetation Workshop in Western Australia.

f. Andrei Korchagin (Kazakhstan) is working on selection, micropropagation and rooting of cuttings of *Atriplex* and *Cassia* at BIDR.

g. Dr. Lenka Langhansova, a postdoctoral fellow (Czech Republic) is working on somatic embryogenesis of *Haloxylon aphyllum* and *Atriplex nummularia* at BIDR.

h. Ms. Annemarie Liebenberg, tissue culture technician at UP also networked at Calitzdorp "Arid Zone Forum" to stimulate her participation in work on drought tolerant shrubs; has already done a lot of work with Paul Malan and Elliott Birnbaum on tissue culture and cuttings).

i. Undergraduate students: two women students conducted 4th year projects, under the supervision of graduate students Chris Keen and Danie Venter.

j. Willem van Niekerk will make presentations to South African Society of Animal Science and the Australian Society of Animal Production in May and June of 2002.

3. A visit by Rethman and Van Niekerk to Israel is planned for 2002.

4. Two more graduate students will join the program at UP in the near future.

- Ms Trove Willcock, graduating with a Animal/Pasture Science major, will concentrate on propagation of fodder shrubs, with particular reference to indigenous species. The work would include seed production, seed treatment, germination studies, cuttings and tissue culture. She would be a candidate for working at Sede Boker in the future.
- Mr Pieter Vermaak, has already completed his course work for an MSc. In 2002 he will work on the degradability and fermentation of different components of fodder shrubs.

E) Request for American Embassy Tel Aviv or A.I.D. Actions

Please see request to accelerate the transfer of funds to UP and for changes in allocations to budget lines, above.