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Co-cultivation of a Kalahari Desert Truffle and Watermelon for Commercial Purposes

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Executive Summary

This project was aimed at assessing the possibility of cultivating the Kalahari Desert truffle *Kalaharituber pfeilii* (formerly *Terfezia pfeilii*) as the mycorrhitic symbiont of local watermelon (*Citrullus lanatus*) varieties cultivated in the Kalahari.

Out of the four years allotted to this project, one was wasted due managerial problems reported at the time. During the three remaining years we have worked out the best inoculation system and irrigation regime as well as adopted a cheap soil sterilization method. Several indications that truffle inoculation was helpful to the watermelon seedlings were evident (truffle-inoculated plants were established faster, looked healthier, and produced higher watermelon yields). Mycorrhiza status of seedlings was checked periodically both under a microscope and by molecular analysis of fungi in root samples. However, only once was a definite (but faint) truffle mycorrhiza verified by our molecular technique. **No truffles were ever found in our plots.** Some of the set backs encountered are detailed below.

Truffles **were** found in a mixed crop traditional field elsewhere, where the fight against Acacia saplings re-growth never stopped for the 5 years of this plot's existence. Also, our *in vitro* success in inoculating Kalahari watermelon seedlings was achieved using isolated mycelia – as opposed to dry fruit bodies (spores) used for our plots. These facts lead us to conclude that although watermelon may support truffle mycorrhiza, it is not a primary host, and possibly does not enhance fast enough spore germination, resulting in low or late inoculation. We assume that the existence of mycorrhized Acacia roots in the soil early in the season before commercial plots are being sown - enables early watermelon root inoculation leading to faster accumulation of the critical mycelial mass necessary for fruit body formation. Thus, as such, truffles are not annual. The time taken up by spore germination and mycelia establishment is critical, and for successful cultivation mycorrhized roots have to be in the soil before watermelon sowing.

A follow up project using our conclusion is called for.

Section I: Technical Progress.

A. Research Objectives

The discovery of watermelon as a *bona-fide* symbiont of *Kalaharituber pfeilii*, (former *Terfezia pfeilii)* the Kalahari Desert truffle, led us to propose the present study aimed at co-cultivation of *K. pfeilii* and watermelon in watermelon fields in Namibia. *K. pfeilii* fruit bodies abound in the Kalahari when appropriate rainfall conditions prevail (rainfall higher than 200-250 mm per year, well distributed throughout the rainy season). In wet years, tons of wild truffles are shipped out of the country during the fruiting season and sold for good prices in South Africa and Germany. Other markets are opening up, thus generating a cash income for the gatherers – the most remote desert dwellers. However, during the seasons of 2002, 2003 and 2004, very small amounts of truffles – or none at all – were collected, due to the erratic rainfall. This demonstrates the dependence of yields on the weather and serves to emphasize the importance of this program.

Three different domesticated watermelon varieties are cultivated by Kalahari farmers; all belong to the species *Citrullus lanatus (vulgaris)*. One produces pale red (pink) fruits for fresh consumption (called by locals Magapu). The other two are oval in shape and greenish white when ripe. The larger of the two, known locally as Makatane or Tsama and is used for cooking. **This is the watermelon we used in our experiments** (see also below). The smaller melon, Makatse, is used as a feed and a source of water for stock animals.

Over 50% of the households in the Omaheke region (where experiments are conducted) are poverty stricken. The region has the lowest national rates of adult literacy and school enrolment. An improvement in the livelihoods of the residents will have to be based on increasing benefits from the exploitation of natural resources. The truffle-watermelon project was in line with the need for diversification and livelihood improvement.

B. Research Accomplishments

As a rule 3-4 months prior to watermelon sowing 2-3 sub-plots of 4x4 m at the Ben-Hur Center were well wetted and covered with transparent 0.1-mm thick plastic sheets, for solarization (use of solar heat for sterilization, *see* Katan et al., 1987) of the soil (Fig 1).

Figure 1. Preparation of the experimental plot. Solarized subplots were kept under plastic sheets for three months prior to onset of the experiment.

The plastic sheets were removed just before onset of the experiment (seeding and inoculation of all plots). At seeding – one solarized and one non-solarized plots were left either non-inoculated (first two seasons) or inoculated with cooked truffle slurry (this last season).

A low-pressure drip irrigation system was set up, and a water line was drawn from the Ben-Hur fresh water reservoir to be used as necessary for irrigation. (details concerning this last season irrigation regime may be found below).

A slurry of water soaked ground dry truffles was the chosen method for inoculation. Crushed dry truffles were found difficult to evenly spread, and were less effective for plant well being.

The watermelon plants were kept strictly within each plot by pruning any branch growing out of the plot border, so as to force the roots to remain in the vicinity of truffle spores and mycelia.

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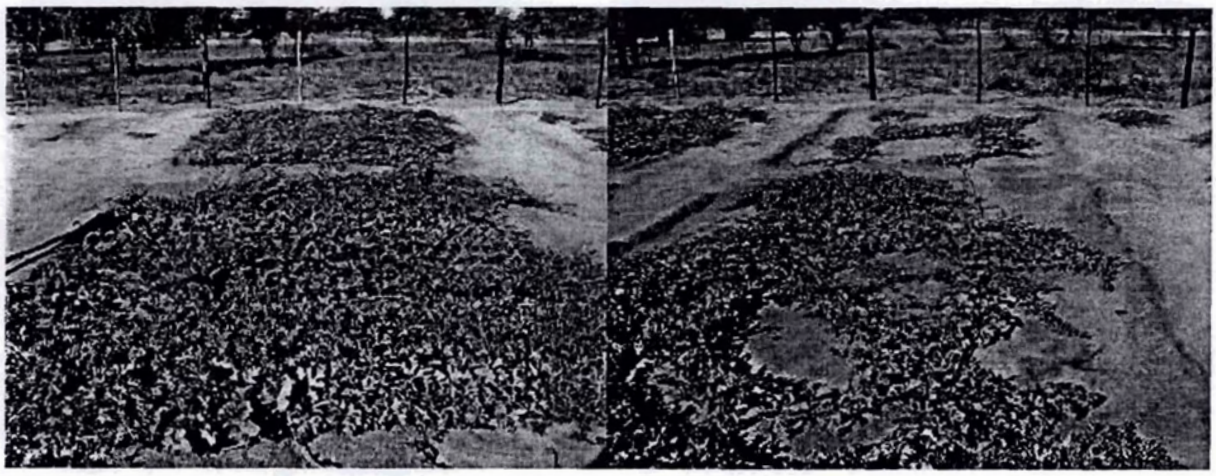
Generally plants in solarized plots seemed to fare better indicating successful elimination of pathogenic bacteria, small animals and fungi. Plants in non-inoculated plots did not establish well, needed to be reseeded, and those in the solarized bed were even worse (fig. 2), leading us to believe we succeeded in establishing truffle inoculated plants. Since only one root sample from one plot turned out to be slightly inoculated (Fig 3), we surmised that the truffle slurry might have served as a fertilizer. Therefore, at the last season we inoculated all plots: all but two with the usual slurry, and two (controls) with cooked slurry. Again, plants in the control plot fared much worse, as indicated by their yield (Please see below).

Figure 2. Control and inoculated plots.

A. Inoculated (At the top, solarized plots) **B. Control (non-inoculated)**



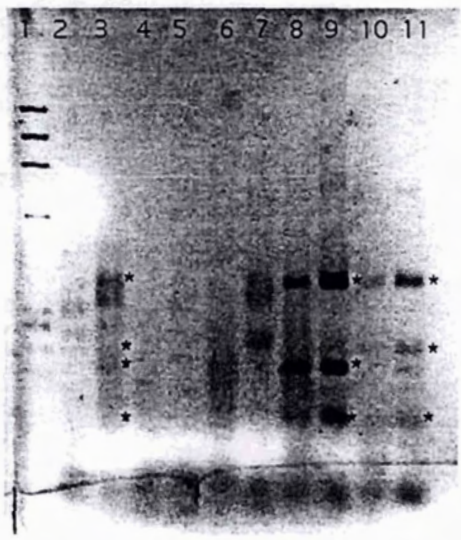


Figure 3. A PCR-RFLP profile of the ITS region of watermelon root DNA samples.
* indicate truffle size fragments both in root sample and positive controls.

Lane1. Size marker
Lane3. The single positive sample
Lanes8-11 pfeilii mycelia from the lab
(positive controls)

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Suspecting that either watermelon may only serve as a secondary host, or that the Tsama variety was not suitable inoculation inoculated by the truffle - we planned:

1. To use perennial Acacias identified in our lab as natural hosts (unpublished data) as inoculum carriers. To this end two areas outside the fenced trial plot were also fenced off. These included, one area surrounding Acacia Hebeclada (Fig. 4) and one surrounding Acacia Erioloba. These two additional plots and acacia saplings within the trial plot were inoculated with truffle slurry (collected during the 2005 truffle season) in late July 2005, to enable truffle spores to germinate as soon as the first rains come, thus infecting the Acacia roots before the watermelon season.

Figure 4. Fenced off and inoculated *Accacia hebeclada* area

2. To test whether the Magapu variety (likely to have been the originally identified watermelon hos, Kagan-Zur et al. 1999) is a better host candidate than the Tsama. These two watermelon varieties were supposed to be compared. However, commercial watermelon varieties, **unlikely** to be susceptible to truffle mycorrhiza (Kagan-Zur & Roth- Bejerano, unpublished results) were used by the Namibian team by mistake, instead of the Magapu. During VKZ's visit - Magapu seeds were added to the beds of the foreign varieties about three weeks later than the initial sowing. Instructions were left with the Namibian team to nurse these new seedlings, and cut off any foreign

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watermelon restricting their development. These instructions were not followed, possibly because it was difficult to distinguish between the two. However, since the magapu seeds were sown rather late, they never developed well, and never set fruits. **Thus we failed to test the possibility that magapu was a better host than Tsama.**

Beds were laid out as in the Table. 1

Table 2. Experimental plot map, November-December 2005

9 Inoculated with wet slurry Seed : Tsama	***10*** Inoculated with wet slurry Seed : Tsama
8 Inoculated with wet slurry Seed : Tsama	***7*** Inoculated with wet slurry Seed : Tsama

4 Inoculated with wet slurry Seed : Crimson Sweet + Magapu	***3*** **Solarised.** Inoculated with wet slurry. Seed: Crimson Sweet + Magapu
5 Inoculated with wet slurry Seed : Tsama	***2*** **Solarised.** Inoculated with wet slurry. Seed : Tsama
6 Inoculated with cooked slurry Seed : Charleston Grey + Magapu	***1*** **Solarised.** Inoculated with cooked slurry. Seed : Charleston Grey + Magapu

Acacia Hebeclada Inoculated with wet slurry Seed : Magapu	**Acacia Eriloba** Inoculated with wet slurry Seed : Magapu

Since we suspected that over irrigation might have caused germinated truffle spores to suffocate, we decided to inoculate twice: Once at the time of sowing, repeating the procedure used before; the second three weeks later during VKZ's visit, at the beginning of December, when germination and establishment of the watermelon seedlings were complete, and irrigation could be reduced (see below). Two plots, one solarized and one non-solarized were inoculated twice with a cooked slurry, at both inoculation times (1+6 Table 2) to serve as non-inoculated controls.

The initial irrigation regime was 30 min twice a day for three weeks, to enable watermelon germination and establishment. This was reduced to 30 min once a day, only in the evenings, as of the beginning of December, to enable better aeration of the soil for the benefit of germinating truffle spores, and reduced again to 30 min twice a week when fruit-set began. Instructions were left not to irrigate if rains were adequate (leaving moist soil at a depth of 20-30 cm at least), and were followed correctly.

Irrigation pipes were removed from beds 9+10 (Table 1). These beds served as inoculated controls for natural conditions, receiving only rainwater during the season. However, the 2006 season was rainy (Table 2) and irrigation was mostly unnecessary. Truffles were collected all over the Kalahari (as opposed to the first 3 years of the project), but not in our plots!

Table 2. Rain data for the 2006 season.

Nov 05	61	mm
Dec 05	15.1	mm
Jan 06	149	mm
Feb 06	186.5	mm
Mar 06	134.4	mm
Apr 06	45.5	mm
May 06	2.5	mm
Total	594	mm

The two fenced off acacia sites were inoculated again along with seeding the magapu around trees and saplings, in early December. These watermelon plants never took off properly, never set fruits and their roots were never well mycorrhized by any kind of mycorrhizal fungi.

The inoculation of Acacias done in July and meant for spores to be available for germination as soon as the first rains occur, seems to have worked: Acacia young fresh roots seemed do show initial truffle mycorrhiza in December. No photographs are available, as there is no camera-equipped microscope at the Ben-Hur. However mycorrhiza level was too low to be detected by our molecular technique, and probably was too low to help the watermelon seedlings. At the

end of the season specific search for Acacia young roots failed to find any good ones. The ones we did collect did not show any good mycorrhization, and molecular analysis did not reveal a truffle profile. **Thus we failed to study the ability of truffle mycorrhized Acacia roots to serve as inoculum donor to the truffles.**

Watermelon yields were checked again, (Table 3) and, again, control plots had produced the lowest yield, despite their being inoculated by truffles (COOKED), which may indicate that the benefit to watermelon seedlings from truffle inoculation was not due to fertilization. What does the live truffle slurry contribute, if no truffle mycorrhiza can be identified is a mystery at the moment. The low yields of this season indicate the necessity for soil fertilization, or for a mixed crop practice.

Table 3. Watermelon yields 2006 season

Plot No.	No. of fruits	Total weight (Kg)	Average weight/fruit (g)
(Control) 1	18	**4.0**	220
2	42	7.5	180
3	57	10.4	180
4	11	10.3	935
5	43	10.0	230
(Control 6)	14	**3.0**	215
7	80	24.5	305
8	80	19	240
No irrigation 9	64	23.5	365
No irrigation10	64	24.6	380

C. Scientific Impact of Collaboration

The initial plot establishment described here for this year, including cleaning, checking and setting up the drip irrigation system, as well as seeding and initial inoculation was done exclusively by the Namibian team. VKZ came only during the second inoculation period and again for summing up the project, in May. No new managerial skills were offered at this point.

CRIAA SA-DC continues to provide regular feedback on the activities of the project to the Indigenous Plant Task Team (IPTT), and hopes to find new funding to continue the project on its own.

D. Description of Project Impact.

There are no applicable results.

E. Strengthening of Developing-Country Institutions.

A microscope, an electrical heating plate, perishable laboratory equipment and chemicals needed for mycorrhiza assessment were purchased for the project and are kept at the Ben-Hur Center for use as necessary. Low-pressure drip irrigation equipment purchased in Israel was installed in the plot. Mr. Mbazo Motlhaping was trained during all the visits of Israeli scientists to Namibia in the use of all equipment (scientific or agricultural) as well as procedures involved in this project, and has become quite proficient.

F. Future Work

No future work is planned for this project. This is the final report.

Section II

A. Managerial Issues

Although the fields were visited weekly by Mr. Mbazo Motlhaping, who lives close-by, and who undertook this mission - it was still not perfectly smooth as seen above. Written instructions were left with Mr. Mothlapeng, in early December 2005 and additional instructions sent by E-mail to Mr. Cole. In addition, a new person (Piet Phillander, a local resident) was identified to carry out the weeding and irrigation activities, as the previous person was found to be unreliable. This new person was trained regarding the activities that we needed to be carried out. Prof. Kagan-Zur was unable to undertake a trip to Namibia twice within a one month

period during the teaching semester. It was mutually decided that the Namibian team should be capable of initial establishment of the plot, and that VKZ should be present at the more crucial step of the second inoculation. This seems to have been a mistake in view of the use of wrong watermelon seeds.

B. Budget

During this year the sum of $1846 was transferred from Travel to Materials, as VKZ traveled on her university travel budget. The sum of $1469 was transferred from Salaries to Materials in the Israeli budget: Since the Israeli team managed to operate mainly with their tenured, university paid staff, to leave more funds for the research. The transfers were approved by the AID-CDR authorities.

C. Special Concerns

None are applicable to this project.

D. Collaboration, Travel, Training and Publications

No publications have resulted from this project. Issues pertaining to training and travel were discussed in the relevant section of this Report, especially in the following sections: Section I C. Scientific impact of collaboration; Section I E. Strengthening of developing country institutions; and Section II A. Managerial issues.

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

None at this stage. Thank you for your offer.

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