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ARC-Vegetable, Industrial and Medicinal Plants Newsletter

Newsletter of the Vegetable, Industrial and Medicinal Plants, campus in the Crop Sciences Programme of the Agricultural Research Council (ARC)

ARC international collaboration on DIVAGRI pilot and demonstration sites in Botswana and Namibia

Compiled by Ian du Plooy, Aart-Jan Verschoor, Althea Grundling, Mariette Truter & Hintsa Araya

Background

The ARC team is part of a consortium of 20 European and African R&D organisations that drive the DIVAGRI (Revenue **div**ersification pathways in Africa through bio-based and circular **agri**cultural innovations) project, aimed at adapting seven biotechnologies to African conditions, utilising local resources optimally, turning waste into use, thereby creating business opportunities and reducing costs. The project is implemented in Ghana, Namibia, Botswana, Mozambique and South Africa. The technologies aim to produce clean water with a desalination greenhouse and artificial wetland; use waste to produce energy and fertiliser (biogas digester and biochar kiln); improve production (through subsurface irrigation and intercropping) and add value to agricultural products (bio-refinery). The technologies will be introduced at pilot sites and subsequently to farmers at demo sites in the five countries involved. The aim is to co-create and adapt these technologies.

In addition, the international team explores business opportunities and value chains for the communities participating. The ARC is responsible for ensuring sustainable project implementation at pilot and demo sites in the five participating countries, assessing livelihoods, resources, and how livelihoods are affected by the introduction of the innovations. The project uses a trans-disciplinary, participatory approach, involving end-users and partners in the co-development of solutions and business models. The ARC team, in particular, is responsible for establishing and empowering “Communities of Practice” (CoP’s) at the participating demo sites, establishing a baseline farmer profile and monitoring progress towards evaluation (Agrimetrics). The ARC-VIMP is responsible for piloting all seven technologies at the Roodeplaat farm and also leading the implementation of ethnobotanical intercropping technologies at all pilot and demo sites (South Africa, Namibia, Ghana, Botswana and Mozambique) (Fig. 1).

The DIVAGRI work plan provides for physical engagements with each country’s project team and site representatives. Dr Du Plooy accompanied Lothar Vigelahn from WISMAR University to the pilot sites in South Africa and Botswana on 7-10 May, 2023. Dr Verschoor, Dr Du Plooy and Dr Araya attended the second DIVAGRI consortium meeting in Namibia (14-20 May 2023). The meeting with 20 international partners, focused on progress reporting, engaging with partners to plan key project activities, and collectively evaluating pilot site establishment and monitoring, with accompanied collective planning. In addition, data collection at the Ovitoto (Namibia) demo site took place.

ARC-Roodeplaat pilot site (7 May 2023), South Africa

The pilot site installed four of the seven technologies (ethnobotanical intercropping, SLECI (self-regulating, low energy, clay-based irrigation), solar desalination greenhouse and biorefinery, with the Biochar, Biogas and artificial wetland still in the process of installation (Fig 2). The intercropping is combined with SLECI technology (Moringa intercropped with leafy vegetables/leguminous crops). A standard drip, subsurface drip and SLECI irrigation system

Figure 1. The different ecoregions where the bio-based technologies will be implemented at project pilot sites and farmer field demo sites in Namibia, South Africa, Botswana, Ghana and Mozambique. (Map: Mr H Weepener).

Figure 2. Technologies installed at the ARC, Roodeplaat pilot site in South AFrica.

were installed to compare the efficiency of irrigation systems in conjunction with intercropping. Black soldier flies (BSF), and oyster mushrooms are included as part of the bio-refinery.

Buan experimental farm (P1a) – Gaborone, Botswana

The pilot site installed the SLECI and alternative irrigation (drip) trial (Fig 3). However, the trial layout was not done in such a way as to enable an analysis of the results (no replications, randomization, and treatments), with only three rows of maize planted under SLECI and drip irrigation, respectively. Soil moisture measuring equipment was installed, and the water usage data for the two systems was collected. The pilot site leader was made aware of the above observations on the site. A Biochar Kiln was made by a service provider in Gaborone, but it was different from the one for Impala and was also not functioning well (leaking water and to be modified). The intercropping trial is established as per the trial plant layout provided by the ARC-VIMP. The other technologies are still in the planning stage and will be installed within the next two months (multifunctional constructed wetland and biogas digester).

Figure 3. Technologies installed at the Buan experimental farm in Botswane.

NARDI/ Impala (P1b)- Francistown, Botswana

The multifunctional constructed wetland is installed, but it is still to be connected to the outflow from nearby houses. The biochar kiln was built by Page Engineering, located in Broadhurst Industrial, Gaborone, delivered on-site and seems to be working well (Fig 4). The ARC is currently negotiating to build the same kiln by the above service provider for the Roodeplaat pilot site. They do have a small Black soldier fly technology installed and are currently multiplying the larvae. The other technologies are still in the planning stage and will be installed within the next two months (mushroom farming and biogas digester).

Figure 4. Technologies installed at the Impala pilot site in Botswane.

NARDI/Mmamanaka (P1c), Botswana

During the visit to this site, there was a farm walkabout with more than 150 farmers and several high-level officials (MP, NARDI-CEO) in attendance. The farm is well maintained, with several research projects running (Fig 5). However, regarding the DIVAGRI projects, only the ethnobotanical intercropping trial was established, while the other technologies are still in the planning stage and will be installed within the next two months (wetland and biogas digester, mushrooms, etc.). Both Mr Lothar Vigelahn and Dr Ian du Plooy presented on the site visits in a closure meeting at BUAN, with feedback to the project site leaders and management of BUAN and NARDI.

Figure 5. Farmers visit of the pilot site at NARDI/ Mmamanaka in Botswane.

Consortium meeting and Windhoek pilot site visit, Namibia (P2a)

The second DIVAGRI consortium meeting was held from 15 to 19 May 2023 at Windhoek, Namibia. Twenty international partners attended the meeting, which focused on reporting progress, collaborating with partners to plan important project activities, and jointly reviewing pilot site establishment and monitoring, accompanied by collaborative planning. The conference provided an overview of project accomplishments, completed deliverables, and work due in 2023. A report on pilot site installation and implementation progress was presented, and practical solutions to concerns were explored. The pilot site data monitoring system and associated worksheets were introduced and discussed, and team members reminded each other of upcoming project obligations.

Figure 6. The second DIVAGRI consortium meeting at Windhoek, Namibia.

The consortium members visited pilot and demo sites at Ombe, Humulus, and the Hotel School, led by the Namibia University of Science and Technology (NUST) (Fig 6). The visit included visiting the biotechnologies, including ethnobotanical intercropping, multifunctional constructed wetlands, mobile biochar kiln, SLECI self-regulating, low energy, and clay-based Irrigation system, solar desalination greenhouse, integrated biorefinery and solar-enhanced biogas, and representatives were interviewed about the process and its restrictions.

Figure 7. DIVAGRI consortium members visited pilot and demo sites at Ombe, Humulus, and the Hotel School in Namibia.

Baseline monitoring and sustainability indicator surveys were conducted with 12 Ovitoto Community of Practice farmers on the final day. Individual representatives from Ghana, Botswana, and Namibia assisted with data collection for the ARC mission. Overall, the consortium meeting provided an opportunity to market the ARC's capacity in transdisciplinary R&D project management and implementation. It showcased the organisation's ability to conduct scientific M&E and to provide services in international project implementation. The delegation engaged partners in the consortium and shared information on the ARC's scope and achievements.

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Potato tuber moth vs *Tuta absoluta*

Compiled by Dr Diedrich Visser

For visual comparisons, this article must be read in conjunction with the accompanying poster.

Background

Two of the most destructive vegetable pests in South Africa are the potato tuber moth (*Phthorimaea operculella*) and the tomato leafminer (*Tuta absoluta*). Although the latter pest is more commonly known as Tuta, the species has recently been moved to the same genus as the potato tuber moth, i.e. *Phthorimaea* (we retain the genus *Tuta* and common name Tuta here to prevent confusion). Both these pests are leaf mining caterpillars of two very similar micro-moths, and are often mistaken for each other, both in the damage they cause as well as in identifications of the moths and caterpillars. In this article we highlight the most common differences between the two species, to aid farmers in identifications and therefore correct implementation of control strategies.

Moths

The moths of both species are small (<10 mm), and difficult to see in crop fields under normal pest pressure. They hide during daytime and are only encountered when the foliage of plants is disturbed. However, Tuta is known to occur in such huge numbers that “clouds of mingling moths” may sometimes be noticed in fields, even during daytime. For the untrained eye the difference between the moths of these two species is only evident in their size; tuber moth is slightly larger than the moths of Tuta (approximately 8 mm vs 6 mm).

Caterpillars

The caterpillars (larvae) of both species are leafminers. They are seldom found outside their blotch-like leaf mines and therefore are seldom seen. Leaf mines must be dissected or “broken open” to inspect the caterpillars for identifications. Most of the leaf mines will be empty, however, except under high pest pressure. The larvae of the tuber moth is greenish brown to pinkish and about 10 mm in length. Tuta larvae are more greenish and approximately 8 mm in length. A diagnostic feature of the larvae is the thin black line on the prothoracic shield (dorsally behind the head) of Tuta, while the tuber moth larva has a broader dark brown to black shield.

Host plants

Both species attack plants in the Solanaceae family, e.g. potato and tomato. However, the potato tuber moth prefers potato, while Tuta prefers tomato. A mixture of both these species will always be found in potato and tomato fields. In tomato fields Tuta will always be the more abundant and damaging species. The occurrence of the two species in potato fields is, however, more complicated. Under certain conditions, which is currently unclear, Tuta numbers may be much higher than that of the tuber moth (see “damage” and “monitoring” paragraphs below for more information).

Damage

Under optimum conditions the potato tuber moth can be devastating to potato production. Early in the season, while tubers are still forming under the ground, the tuber moth larvae multiply on the foliage, mostly unnoticed. During the second half of the season numbers increase exponentially, but usually never reach high enough numbers to cause foliage dieback. However, under certain conditions, which are currently not completely understood, foliage dieback may be caused by the mining tuber moth larvae. Yield loss is usually due to infested tubers at harvest time. Tubers are infested mainly during the senescence period when first instar larvae move down cracks in the soil to reach tubers under the ground. In tomato fields the tuber moth is perceived as only a minor pest.

Tuta is not known to attack potato tubers under the ground. The damage potential of this species lies in its ability to reproduce at a very high rate, and occurring in much higher numbers compared to the potato tuber moth. In potato fields, however, their high reproduction rate is mostly suppressed in comparison to their occurrence on tomatoes. This may be due to host preferences. However, under certain conditions, which is not completely understood, Tuta may occur in potato fields in such high numbers that the mining larvae may cause foliage dieback. The damage is therefore indirect, causing lower yields. In tomato production, however, Tuta can completely destroy a field in a short time, by destroying the foliage as well as developing tomato fruit.

Monitoring

For both species, commercial pheromone lures and traps are available to monitor moth numbers. Only male moths are caught in the traps, but because the sex ratio is approximately 1:1 for both species, comparative catches over a fixed time span (usually one week) can be used as an estimation of abundance of a specific species. However, because the lure formulations are species specific, and because the effectiveness of the formulations may differ between the two species, counts and analyses must be interpreted carefully when tuber moth and Tuta pheromone catches are compared. In general, we have found that the Tuta pheromone lures are much more potent, and that more Tuta moths are caught in traps compared to tuber moths in the same fields.

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Potato tuber moth vs *Tuta absoluta*

Comparison of life stages and damage

Potato tuber moth adult (8 mm in length)

Tuta absoluta adult (6 mm in length)

Potato tuber moth larva (10 mm in length)

Tuta absoluta larva (8 mm in length)

Potato tuber moth larval damage to tubers

Tuta absoluta larval damage to tomato fruit

Potato tuber moth leaf mine

Tuta absoluta leaf mine

Author: Diedrich Visser, ARC-Vegetables, Industrial and Medicinal Plants (ARC-VIMP)
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Cassava propagation

Compiled by Dr Amelework Assefa

Introduction

Cassava is, after maize, the second most important food security crop grown by more than 40% of the African population. Drought and increases in temperature are the major stress factors that pose a serious threat to world food security, especially in the changing climate scenario. South Africa is one of the countries affected by climate change and has experienced severe drought and floods in recent years. The ability of cassava to withstand difficult growing conditions such as extreme drought, heat, and soil acidity makes it a suitable crop for climate-smart agriculture. Cassava is also listed as the fourth most important source of starch, after maize, wheat and potato worldwide. Cassava supplied more than 8 million tons of industrial starch to the global market and the global demand for cassava starch is projected to be over 10 million tons by 2024. Hence, designing sustainable agro-ecology based solutions and grasping the industrial potential of cassava will improve the livelihoods of small-scale farmers and facilitate the adoption of climate-smart agriculture. The ARC and its partners have identified cassava as a food security crop and as an alternative source of starch for the starch industry to replace imports. Cassava has also been endorsed as one of the climate risk mitigation strategies for managing food price inflation resulting from competing user interests in major staple food crops such as maize, wheat, and potato. Knowledge on propagation and cultivation practices is critical to integrate cassava into the current production systems in South Africa.

Propagation via botanical seed

Cassava propagation using seed is essential only for genetic and breeding studies. The seed is used to create variation by combining selected parental genotypes and to produce segregating generations. Cassava being an outcrossing species, causes any genotype to be extremely heterozygous and propagation using botanical seed may result in segregation of a wide range of phenotypes. Cassava requires a seed dormancy stage of 3-6 months to ripen before planting and the seeds should be stored at ambient temperature. The seeds should be planted in a greenhouse for germination and the seedlings should be transplanted to the field when they are about 20-25 cm tall [1]. Cassava propagation through botanical seed is constrained by low and uneven germination. This may be attributed either to environmental factors such as light and temperature or to the inherent seed dormancy, or a combination of the two. However, seed germination can be hastened either by mechanical scarification of the seed coat, or exposing the seeds to a dry heat treatment at 60ºC for 14 days or to a cold treatment at 4ºC for 24 hrs [2]. Light negatively affects both seed germination and the speed of germination [3]. Plants derived from seed produce fewer and smaller tubers than those from stem cuttings.

Figure 1. Cassava botanical seeds.

Propagation via stem cuttings

Cassava can be propagated by stem cuttings derived from a mature plant. Undamaged and healthy stems should be selected from 10-12 month-old plants for fast and maximum stem sprouting. Stem cuttings (stakes) obtained from the middle part of the stem have better sprouting and good plant establishment than those derived from the lower and upper part of the stem. The stems can be stored for 2 – 5 days in the shade after they have been harvested (Fig. 2) and the distal ends should be covered with moist soil. Stems should not be stored for more than two weeks to obtain at least 80% sprouting. The stems should be treated with maximum care as not to damage the nodes that may result in the loss of the stakes. Depending on the plant height, from one plant, 5-10 stakes of 25 cm in length with 5-7 nodes can be obtained (Fig. 3a). Mature stems should be carefully cut with sharp tools to avoid damage, the stakes should not be splintered, and the bark should not be chipped off. To prevent the spread of pests and diseases, the stakes should be treated with agrochemicals. The implements used to cut the stem should also be cleaned with chemicals before and after use [4].

Rapid multiplication of cassava stem cuttings

On average, a mature cassava plant produces 10 stakes of 25 cm in length over a period of 12 months. As a result of

Figure 2. Cassava stakes

the low multiplication rate of cassava, commercial propagation through stem cuttings is challenging [5]. The International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria has developed a rapid multiplication technology using mini-stem cuttings varying in length depending on the stem portion from which the cuttings are taken [6]. A 5 cm stake with 1 - 2 nodes from the hardwood portion, a 10 cm stake with 4 - 6 nodes from the semi-mature portion, and a 20 cm stake with 6 - 10 nodes from the tip portion of the stem (Figs 3b, c, d) should be used. The 5 cm stake with 1 - 2 nodes taken from the hard wood was found to be a better option than the standard 25 cm stake with 5 - 7 nodes. Mini-cuttings taken from the hardwood can be harvested early at 9-12 months after transplanting and the starch content is comparable to those obtained from the standard cuttings. A single plant can produce about 50 mini-stakes, compared to the normal 25 cm cuttings that produce only 10 stakes per plant.

Propagation via tissue culture in-vitro plantlets

Cassava stem cuttings are perishable, bulky, requires considerable storage space and are difficult to transport. Tissue culture techniques have been utilized for rapid clonal propagation, regeneration, multiplication and *ex-situ* conservation of germplasm [7]. It is possible to produce large numbers of plantlets in a small space. From one nodal explant, 16 000 to 17 000 plantlets with four nodes can be produced *in vitro* per year [8]. Well-rooted cassava plantlets of 4-5 cm in length were found to be successful for hardening off and had the maximum success rate (91%) for establishment in sterilized vermiculite [8]. However, the multiplication and

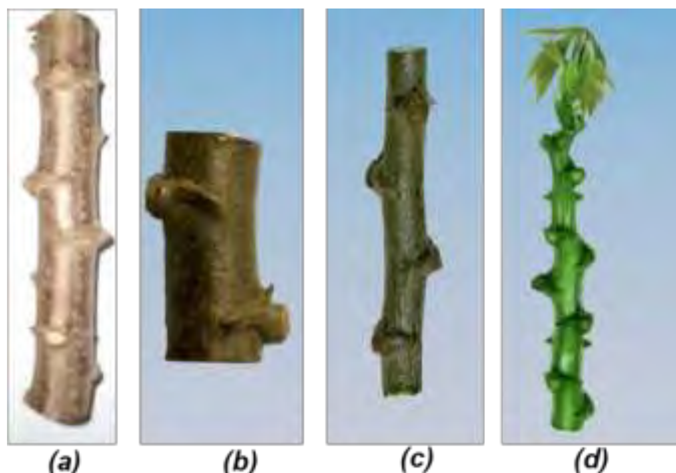

Figure 3. Cassava stem cuttings (stakes). (a) Standard 25 cm cutting with 5 - 7 nodes, (b) 5 cm cutting with 1 - 2 nodes from the hardwood portion, (c) 10 cm cutting with 4 - 6 nodes from semi-mature portion and (d) 20 cm cutting with 6 - 10 nodes from the tip portion the stem [6]

hardening off process takes a long time (70 – 90 days). It was reported that root development from acclimatized plantlets were slow and weak compared to stem cuttings. Recently, a liquid-hydroponic system to induce rapid rooting in cassava was developed at the International Centre for Tropical Agriculture (CIAT), Cali, Colombia [9]. This method can also be used to acclimatize *in vitro* cassava plantlets to *ex vitro* conditions with a high survival rate.

Establishment of propagation nursery

Asexual propagation using stem cuttings is often associated with an accumulation and spread of several pathogens leading to a decline and degeneration of improved varieties [10]. With vegetatively propagated crops, diseases and pests can build up over several generations of propagation and, hence, there is a high risk of spreading diseases that can result in severe crop losses. Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the major diseases of cassava in central, eastern and southern Africa [11]. Diseases are carried in infected plants and transmitted to healthy plants by insect vectors, such as whiteflies (*Bemisia tabaci*).

Establishment of propagation nurseries is crucial to ensure a sustainable supply of disease-free cassava stakes. These nurseries can be established either at research stations or on farmer fields that are isolated from other cassava fields at a distance of about 150 - 200m to reduce the chance of insects carrying plant diseases into the nurseries. The nurseries should be easily accessible to farmers for sourcing planting materials and for training purposes. In areas where tissue culture laboratories are available, it is advisable to start the nursery with *in vitro* plantlets. Once you have planted the nursery, inspect the site for diseases and insect pests every week, rouge out diseased plants and apply pesticides to avoid disease spread within the nursery. For subsequent planting material multiplications, select cassava planting materials from healthy plants that show no leaf discolouration or chlorosis, no shoot tip dieback, no dark or white fungal patches or streaks on the stem and no rotten spots. The turnover time to replace cuttings by *in vitro* plantlets should be three to five years. From a hectare of land at a spacing of 50 cm x 50 cm, it is possible to produce about 40 000 plants. If 10 stakes are obtained from one plant on average, 400 000 stakes can be harvested and that will be sufficient to plant 40 hectares of land.

The ARC aims to develop a nursery system for cassava to support the expanding commercial cassava industry in South Africa because import substitution for starch will support local entrepreneurship and income generation.

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On-farm mycorrhiza production

Compiled by Elsie Cruywagen

Most plant roots are colonized by a group of mutualistic soil fungi called mycorrhizal fungi. The interaction between AMF and plants typically results in a mutually advantageous symbiosis; these benefits may be physiological, nutritional, ecological, or any combination of these. The association is referred to as an "arbuscular mycorrhizal association," where "arbuscular" is Latin for "tree-like," and denotes the interaction of specialized fungal structures with the contents of cortical root cells, and "mycorrhizal" denotes the interaction between the fungus (myco) and the root (rhizo).

Mycorrhizal fungi are of extreme importance in environments with limited water and nutrients as it enables the plants to access more water and nutrients than plants without mycorrhizal mutualists. The hyphae of the fungus outside the root acts as an extension of the root system for the uptake of mineral nutrients, especially immobile nutrients such as P, Cu, and Zn, which are transported back to the root, where the fungus releases them for uptake by the root cells (Douds et al. 2005). Mycorrhizae have also been credited with increasing a plant's disease resistance, improving a plant's ability to grow under drought conditions, and improving soil structure.

Commercial mycorrhizae formulations are available, but they are generally too expensive for small-scale farmers to use. This project, funded by the Water Research Commission, aims to create an on-farm mycorrhizal production system for small-scale farmers by using locally adapted mycorrhizae to create mycorrhizal inoculum.

Starter soil was collected from an undisturbed area on the ARC-VIMP farm. The vegetation was cleared away and while digging down to a depth of about 25 cm, the soil and as many fine roots as possible were collected. A 'trap-pot' system was used to multiply inoculum. Both pot (inside a greenhouse – Fig. 1A) and trench trap systems (outside – Fig. 1B) were evaluated. Several large (5 litres) plastic pots/basins were used for the pot system, while a trench (150cm x 50cm to a depth of 50cm) was dug into the ground and lined with a plastic sheet. The plastic was perforated to allow for drainage. The containers were filled with a mixture of red topsoil (purchased) and compost at a ratio of 5:1 and the starter soil was added at approximately 5% v/v to each container and mixed with the top 15 cm of soil. Four to five bahiagrass (*Paspalum notatum*) seedlings were planted into each pot and up to 30 were planted in the trenches. Maintenance consisted of watering and weeding as needed. Plants were grown for at least three months for inoculum production.

Ten days before using the inoculum, watering was stopped. This killed the plants and tricked the fungus into producing reproductive spores. After 10 days, the inoculum was prepared by cutting off the tops of the grass and pulling up the roots of the bait plants, which were chopped into roughly 1 cm pieces and then mixed back into the soil from the trap-pot or trough. This mixture of roots and soil served as the inoculum.

Approximately 1 kg of soil from the trap pits as well as the trap pots, were collected for counting of sporocarps. Soil was also collected from the ARC-VIMP rain shelter and open field sites where the trials with the produced AMF will be conducted. AMF spores from the soil samples (100 g) were extracted by wet sieving with 500, 425, 250, 106, 45 and 38 µm mesh sizes. The soil from each of the sieves was washed into separate Falcon tubes, centrifuged for 3 min, and then the water was poured off. This was followed by sucrose density centrifugation with 50% sucrose; whereafter sporocarps would be in the sucrose fraction. Sporocarps were washed under running water on a 38 µm sieve to remove all sugar, where after it was washed into 50 ml

Figure 1. Bahiagrass (*Paspalum notatum*) grown in A.) trap pots in the greenhouse and B.) trap trench outside.

Falcon tubes. Sporocarps obtained from the sieves were counted using a stereo microscope at 8x magnification.

Table 1 gives the number of sporocarps recovered by wet sieving from each of the veld soil, topsoil (used in trap pots and pits), rain shelter and open field where the trials will be planted, as well as the trap pits and pots after three months and finally the inoculum produced. The veld soil, which was collected to serve as starter inoculum, had the highest number of sporocarps, followed by the greenhouse trap pots and then the trap pits. The lowest numbers of sporocarps were recovered from the red topsoil that was purchased for use in greenhouse trials.

The trap pot method used to multiply the AMF for crude inoculum was effective, with a 30 times increase in the number of sporocarps in the topsoil that was used to produce the inoculum. The vigorous growth by the grass in the greenhouse led to higher numbers of AMF sporocarps being produced in the pots in the greenhouse when compared to the trap pits outside. However, the trap pits only produced 1.4 times less sporocarps than the pots in the greenhouse.

Crude inoculum produced on-farm therefore has much lower numbers of propagules than those of commercial formulations, but are produced at a fraction of the cost of commercial inoculum.

Sporocarps observed from the crude inoculum (Fig. 2) could be broadly classified into 13 morpho-groups based on colour and size. Based on morphological identification, most of these isolates belonged to the Glomeraceae family, but some Gigasporaceae were also present.

The highest diversity of AMF was found in the veld soil that was used to inoculate the trap pots and trap pits, with 14 different morpho-groups present. All of these groups were present in comparable ratios in the inoculum produced. The lowest diversity was found in the topsoil, with only five morpho groups, followed by that of the rain shelter, with seven morpho groups. The open field was slightly more diverse, with nine different morpho-groups.

The trap pot system used to multiply the AMF for inoculum successfully trapped the diversity of AMF present in the natural veld soil. Either system will provide adequate amounts and diversity of AMF for small-scale farmers. Species level identification of AMF is difficult based on morphology alone, however, most morphological groups could be classified in the Glomeraceae family, with some Gigasporaceae also present.

Table 1. The number of sporocarps recovered from 100g of soil.

Source	Number of sporocarps
Veld soil	4 644
Topsoil	78
Rain shelter	558
Open field	1 635
Trap pit	2 133
Trap pot	3 054
Final inoculum	2 623

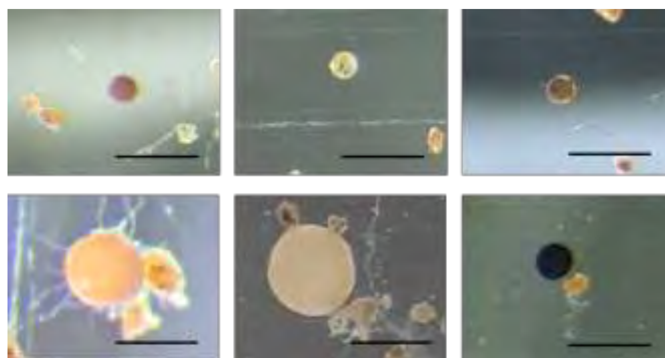

Figure 2. Diversity of AMF sporocarps observed, ranging from white to yellow, brown and black. Sizes vary from 50 µm to more than 200 µm. Scale bars are 200 µm.

The Glomeraceae family includes abundant genera such as *Glomus*, *Rhizophagus*, *Funneliformis* and *Septoglomus*, which have been reported on all continents and are the genera found most often in commercial inoculants. Members of the Glomeraceae have shown different levels of effectiveness in colonizing host roots and performing under various field conditions. For instance, under different management strategies, species of *Glomus* and *Rhizophagus* have reportedly outperformed other genera, such as *Gigaspora* and *Scutellospora*. (Veresoglou et al. 2011). The diversity of AMF present in the produced inoculum should therefore enhance the chances of successful colonisation of crop plants under different conditions.

Significant quantities of a taxonomically diverse inoculum can be produced using materials readily available to farmers. This technique saves the associated costs of processing and shipping, which are included in the price of commercially available inoculum. These factors, along with proven crop production improvements, point to the possibility of higher financial returns for farmers using AMF and the accompanying environmental benefits derived from reduced use of pesticides and fertilizers.

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Unlocking the benefits of good parental seed germination, flowering, and cross-pollination rate in potato breeding

Compiled by H.M Maluleke, S.M Laurie and N.W Mbuma

ARC-VIMP potato crossing programme

The choice of parents used in hybridization determines the efficiency of combining genes in plant breeding. Parent selection is based on the genotype's potential to produce high proportions of progenies with high trait values. The genetic combination of selected parents determines the genetic variability among progeny populations, which eventually is exploited during selection. Therefore, parent evaluation is critical in any potato (*Solanum tuberosum* L.) breeding program.

In the potato breeding program, local and foreign potato varieties, advanced breeding lines, and tuber-bearing *Solanum* species with specific desirable characteristics are used as parents. Imported varieties are also used as parents and wild germplasm as sources of resistance and tolerance to biotic and abiotic stresses as well as other important traits.

At the ARC-VIMP, the planting of parental genotypes for crossing takes place in a greenhouse in mid-August every year. Before planting, the soil is prepared and 21 bricks are placed per row on top of the soil, followed by the application of the fertilizers (N: P: K = 2:3:2 and LAN). After fertilization, six seed tubers per parent are planted on top of the bricks in two parallel rows and then covered with sawdust. The space between the parental rows and seed tubers is 50 cm and 15 cm, respectively. The seed tubers are planted on top of bricks to discourage tuberization and tubers are removed to promote flowering. The parental mini-tubers are fertilized once before planting and watered frequently until they germinate and produce flowers. The conditions in the greenhouses are regulated using wet walls, fans, and shade nets. The flowering initiation starts in the mid October and lasts until early December, depending on the parental germination and flowering rates in the greenhouse. If necessary, flowers are collected from lines evaluated in the field trials.

When the first florets open, anthers with pollen are collected and stained with an iodine solution as an indicator of pollen fertility. Flowers with no pollen grains, unstained or less than 50% stained pollen grains, are designated as females. Flowers with a pollen stain of greater than 50% are designated as males. Once the fertility levels of the flowers are known, the breeders decide which crosses to make. This process is a critical stage in the breeding programme, and utmost care is taken in deciding which combinations to make. The crossing is based on the random mating of parents, mainly depending on flowering. When making crosses, male flowers (anthers with pollen) are rubbed onto the female flowers (stigma) of the other plant with a soft hand brush. Five to ten days after pollination, berries will form indicating a successful cross. The cross that formed berries is then covered with a veggie net to prevent berries from falling off. Matured berries (Fig. 1A) are harvested and stored at room temperature to enable ripening. Once the berries have ripened, the true potato seeds (Fig. 1B) are extracted from the berries and dried to be ready for planting. True potato seeds are germinated in December every year in seedling trays (Fig. 1C) and when plantlets are 3 to 5 cm tall, are transplanted in pots (Fig. 1D) in February to develop mini tubers. The mini tubers are harvested in May and stored in the cold room. In September the mini tubers are planted in the field as the first generation of potato breeding lines (A-Group). Thus, the success of parental seed germination, flowering, and cross combination is of utmost importance in potato breeding programme to determine the populations to be evaluated in the field.

Forty-eight (48) parents were planted in the greenhouse in August 2022. These parents were obtained from the ARC *in vivo* gene bank (1) and *in vitro* ARC potato cultivar collection (47). The procedures for planting, trial management, and crossing are as described above. The data was recorded for germination, flowering and crosses from each parent planted. The parents were grouped as germinated, not germinated, flowered, and not flowered. The total number of crosses were recorded and grouped as successful (produced berries) and unsuccessful (no berries produced) crosses.

Figure 1. Showing A) Berries from crosses, B) true potato seed (TPS) from berries, C) seedlings planted from TPS, D) transplanting of seedlings into pots. Photo (A, C and D) by Dr Ntombokulunga Mbuma @ARC and B by https://www.cultivariable.com/instructions/potatoes/how-to-grow-true-potato-seeds-tps.

There were statistically significant differences within the parental groups in the number of parents that germinated/ not germinated and flowered/not flowered, as well as in the number of crosses that were successful and not successful. On average, the number of parental plants that germinated/ not germinated and flowered/not flowered were 6.67/6.61 and 3.31/3.4 out of the 48 parents planted, respectively (Fig. 3). The low germination and flowering rate could be due to the differences in genotypes since the greenhouse conditions are uniform. Different varieties can have different germination and flowering rates. This could be due to the different genetic make-up of the seeds, which affects their ability to germinate and flower. In addition, the optimum environmental conditions such as temperature, moisture, photoperiod, and soil type could vary among the parental genotypes resulting in different germination and flowering rates.

For example, high temperatures are known to reinstate the dormancy or even induce it in seeds (Geshnizjani, *et al.,* 2018). Other factors could include the physiological state of potato tubers and the dormant apical bud. Perhaps, the required period for breaking the dormancy in potato tubers varies with genotypes and the conditions of the pre- and post-harvest, thus, dormancy in the existing germplasm collection needs to be investigated. Previous studies have shown that the germination rate of potato tubers is critical for flowering and seed production (Kouakou *et al.,* 2018). The tuber's ability to germinate and produce shoots is essential for the development of flowers, which are necessary for the production of true seeds. Studies have also suggested that the number of flowers produced is in a negative proportion to the number of germinated tubers (Santos *et al.,* 2018).

The recorded higher number of parental plants that did not flower compared to number of flowering plants could be due to environmental factors such as the photoperiod and temperatures that occurs during the crossing seasons. Escuredo *et al*. (2020) also reported that high temperatures reduce flowering and can have a negative influence on plant growth and development.

The number of successful crosses was higher when compared to unsuccessful crosses, however, it has not reached the target of the potato breeding programme. Thus, a study focusing on the combining ability of parents in potato breeding is required. The unsuccessful crosses could be due to flower abortion attributed to high temperatures during the crossing season.

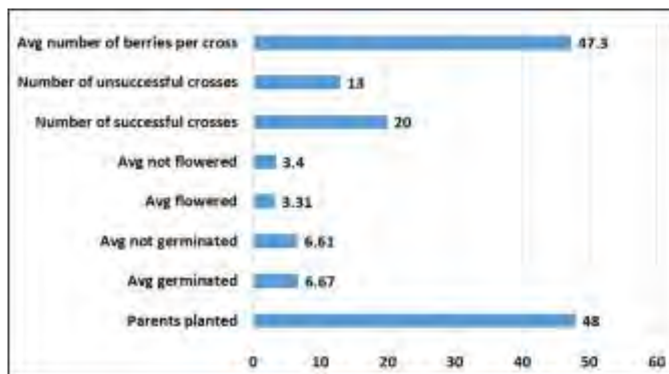

Figure 3. Number of parents planted in the greenhouse, the number of parents germinated, flowered and crosses that made berries in the ARC-VIMP potato breeding program.

Knowledge of the rate of potato germination and flowering is important in the potato-breeding programme in maximizing the number of successful crosses. The results achieved will guide future selection of parents and crossing strategies. A high number of successful crosses ensures that there is an adequate breeding population for evaluation and selection in the field. Thus, a successful breeding programme depends on the rate of germination, flowering and crosses, as well as the true seed production.

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Figure 2. Showing A) good germination, B) poor germination, C) flowering, and D) potato fruit berries.
Photos by Dr Ntombokulunga Mbuma @ARC

Can a subsurface irrigation system save water and improve crop production in water-scarce conditions?

Compiled by Hunadi A. Chaba, Hintsa T. Araya, Mariette Truter and Christian P. Du Plooy

Water scarcity in arid and semi-arid regions is a primary constraint to sustainable cultivation. However, ongoing population growth requires sustained growth of food production in the future, which in turn requires irrigation inputs that can support irrigated cultivation. In South Africa, water resources are considered a limiting factor in the development of the agricultural sector, given that the country is one of the driest countries in Africa and globally [1]. Agricultural production uses more than 60% of South Africa's freshwater, and irrigation accounts for 50% of the total water utilisation in South Africa [2]. Therefore, irrigation, such as subsurface drip irrigation, has been identified as a way of alleviating water scarcity in South Africa [1]. Subsurface drip irrigation (SDI) is the application of water below the soil surface via emitters with discharge rates like that of surface drip irrigation. It is essentially a drip irrigation network buried at a certain depth and is used to irrigate a wide range of plants around the world, including herbaceous crops (lettuce, celery, asparagus, and garlic), woody crops (citrus, apple trees, and olive trees), and others such as alfalfa, corn, cotton, grass, pepper, broccoli, melon, onion, potato, tomato, and so on [3]. The use of water surface drip irrigation systems has been increasing and yields various benefits, including the ability to apply water close to the plant root system without wetting the soil surface, which results in small losses by evaporation and, thus, high application efficiency [4]. Because the drip lines are not exposed on the surface, mechanical damage and solar radiation are minimized, facilitating crop management practices and increasing the system's longevity [5]. In South Africa, irrigation helps stabilize yields and provides farmers insurance when there is variability or insufficient rainfall to meet crop requirements. Irrigation also allows for all-year-round production, especially in the tropics and subtropics, where temperatures are favourable for some crop's growth [4]. Climate change may harm the growth and yield of some crops, without the introduction of appropriate irrigation infrastructure. Therefore, new irrigation methods, such as subsurface drip systems, should be developed to maximize water use efficiency and reduce operational costs (Fig 1). The ARC-VIMP team is investigating the potential of different subsurface irrigation systems (Figs 2 & 3) in conjunction with ethnobotanical intercropping systems. The study identified that the efficiency of subsurface drip irrigation could be similar to that of normal drip irrigation, but subsurface irrigation uses less water. It could save up to 25% - 50% of water compared to surface irrigation.

Figure 1. Subsurface drip irrigation system installation underway at the ARC.

Figure 2. Cowpea under the surface (top) and subsurface (bottom) drip irrigation systems.

Figure 3. Moringa intercropped with cowpea using a subsurface drip irrigation system.

Investment/cost of subsurface drip irrigation

The technology is widely variable, however, the cost of an SDI system ranges from R13 345,04 to R42 449,17 per hectare, depending on the specific type of technology, automatic devices, materials used, and the amount of labour required. Financing for equipment may be available from financial institutions via leasing operations or direct credit. Farmers usually cover installation, design and training costs that represent about 30 to 40 per cent of the final costs, depending on the size, characteristics and shape of the land, crops used, and the particular technology being applied. Generally, highly regulated black drip irrigation pipe (15 mm) is usually buried 20-30 cm below the surface, and the drip irrigation is normally connected to the main pipe with manual valves to control the opening and closing of the subsurface irrigation system. The dripper normally emits 2.0 *L* per hour, and the spacing between the two drippers is 30 cm. To install a dripline in a subsurface application, you will need a dripline with emitters, and the emitters must be designed in a way to permanently keep the roots from freely invading the emitter outlets. The dripline must also have an anti-siphon feature to keep soil from being sucked back into the emitter when the system turns off and a vacuum is created. Therefore, the subsurface drip irrigation system used depends on the crops being cultivated because the roots can reach deep into the soil.

Recommendations

Farmers are encouraged to use a subsurface drip irrigation system because it reduces water use and increases yield, allowing water to be available to other economic sectors. Thus, subsurface drip irrigation is promising. It shows a higher capability for minimizing water loss by evaporation, runoff, and deep percolation than other methods. Thus, the irrigation water saved may become available for other uses. It may also increase crop yields since it reduces fluctuations in soil water content and results in a well-aerated plant root zone.

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