

# Great minds gather to examine the future of groundnuts

By Adri Botha, chairperson, South African Groundnut Forum

**R**ole-players in the local groundnut industry gathered for the Groundnut Symposium 2022 hosted by the Oilseeds Advisory Committee (OAC) on 25 August at the South African Groundnut Forum (SAGF) in Potchefstroom. A wide spectrum of topics was discussed by esteemed experts in the field.

This article highlights the critical subject of local cultivar maintenance and future cultivar development outlooks.

## Cultivar DNA fingerprinting

During 2021, the groundnut industry, through the SAGF, the OAC and the Agricultural Research Council-Grain Crops Institute (ARC-GCI), collaborated to ensure the responsible maintenance of our most prominent groundnuts. One outcome of many discussions was the OAC funding a project that had originally been initiated by Dr Wikus Snijman of the ARC-GCI and Dr Renée Prins of CenGen.

With the ARC's groundnut team and individual members from the industry, specifically Lourens de Kock (SAGF vice-chairperson), the project aimed to generate DNA fingerprinting from different seed sources of the five major ARC-registered groundnut cultivars (*Table 1*): Akwa, Sellie Plus, Anel, Kwarts and Akwa Plus.

The objective was to identify a suitable set of microsatellite simple-sequence repeat (SSR) markers available in the public domain. The next step would be to optimise these markers using a single representative of each cultivar and to assess the ability of the markers to discriminate between the different cultivars. Finally would be the evaluation of the purity of individually germinated seed from three different sources: the gene bank, mother seed and breeder seed.

According to an earlier report by CenGen: "Leaf samples of four individual gene banks of each of the five cultivars

were sent to CenGen by the ARC-GCI, alongside seed of each of the three stated seed sources. The seed sources were germinated at CenGen, and emerging shoots were used as templates for DNA extractions. These individuals were thus destroyed in the process. The gene bank samples submitted as leaves will be harvested by the ARC-GCI team with their respective fingerprints recorded for future use.

"A set of 18 SSR markers were identified and ordered. Standard molecular genetics methodologies were used to extract the DNA. The data was generated on an ABI 3730XL instrument using a LIZ 500TM Size Standard and a 50cm capillary at the DNA Sequencing Unit of the Central Analytical Facilities of Stellenbosch University. The data was scored using GeneMapper v5 and exported to Excel."

Dr Prins shared her findings with the larger industry during the SAGF, further laying the foundation for discussion by industry and the ARC. Her findings can be summarised as follows:

- A total of 120 individuals across the targeted cultivars and sources were screened with the markers found to be informative during the first two stages of the project.

- A final fingerprint was generated with 12 SSRs, of which ten ultimately provided marker scores that could be used reliably.
- Nine heterozygous individuals were identified and removed, with three individuals that did not work well for any markers. Heterozygotes are an important source of variation within cultivars when seed is multiplied from these sources.
- The trimmed dataset was then used based on the specific profile obtained, with ten SSRs to assign a unique profile identification number to each individual.
- From a profiling basis, a major concern is that 22 profiles were identified instead of the expected five profiles for five cultivars, pointing towards impurity.
- Akwa and Akwa Plus are of serious concern due to the large variations recorded. Akwa Plus seed sources containing two specific profiles should be assessed phenotypically since one of them may be representative of the original cultivar released. In the meantime, the ARC, in agreement with the SAGF, will not release Akwa seed to breeders until this matter has been fully addressed.



According to Dr Prins, the way forward must take into consideration adding more individuals for profiling and expanding the marker set. Plans are already under way to immediately use this information in the seed multiplication process. The ARC has already advised that DNA fingerprint validation for seed multiplications of new cultivars will in future be conducted before release. This will ensure that true fingerprint profiles are available for future evaluation to protect the ARC against undue use of material and to indicate possible variations before release.

Dr Prins mentioned available technology and protocols which may be of future use in deciding the predominant genetic SSR, as well as the benefits of marker-assisted selection which may fast-track efficient breeding. Dr Solomon Ntladi presented on behalf of the ARC-GCI, accompanied by other key members of the team and management, including Dr Kingstone Mashingaidze.

Dr Ntladi highlighted seed and cultivar quality control and assurance, including factors such as the protentional sources of genetic impurity, which include mutations, residual heterozygosity and physical mixing. These risks are mitigated through field inspections and 'rogueing' at breeder seed production level and increased, future application of molecular markers in collaboration with the SAGF and CenGen to evaluate the purity of cultivars.

Referring to the findings of the DNA fingerprinting project as discussed by Dr Prins, the following plan has been put in place by the ARC for the maintenance of germplasm, nucleus and breeder seed.

#### **Germplasm**

In the short term, seed that was derived from the fingerprinted plants which displayed consistent patterns will be planted for Akwa, Anel, Kwarts and Sellie Plus and harvested in bulk. Due to the inconsistent pattern reported for Akwa Plus, more seeds will be planted. The ARC will also embark on a project to fingerprint the rest of the ARC-registered cultivars not included in the initial project.

#### **Nucleus**

The immediate action plan entails planting all existing germplasm and nucleus seed, as well as seeds of fingerprinted plants as reference checks for Akwa, Anel, Kwarts,

**Table 1: The following ARC-registered cultivars are of importance to the local industry.**

| Cultivar           | Date of registration | Current PBR* status | Current production |
|--------------------|----------------------|---------------------|--------------------|
| <b>Kwarts</b>      | 1991                 | Expired             | Commercialised     |
| <b>Anel</b>        | 1993                 | Expired             | Commercialised     |
| <b>Akwa</b>        | 1994                 | Expired             | Commercialised     |
| <b>Tufa</b>        | 2008                 | Active              | Commercialised     |
| <b>ARC-Opal 1</b>  | 2013                 | Active              | Not commercialised |
| <b>ARC-Oleic-2</b> | 2013                 | Active              | Not commercialised |
| <b>Akwa Plus</b>   | 2015                 | Active              | Not commercialised |
| <b>Sellie Plus</b> | 2015                 | Active              | Commercialised     |

\*PBR: Plant breeders' rights.

Tufa and Sellie Plus. The South African National Seed Organisation (Sansor) will be involved with field inspections.

#### **Breeder**

The long-term plan is to plant 250 germplasm kernels under irrigation, which is estimated to finally result in 750kg breeder seed per cultivar.

Dr Ntladi concluded by stating that their objective was to sustainably deliver pure breeder seeds to the industry to optimise the seed multiplication programme by collaborating with the groundnut industry.

#### **National breeding project**

Of equal importance to maintaining existing cultivars is the introduction of new varieties to stimulate an ever-shrinking local groundnut industry. New technology has not advanced to the same extent as is seen in competing summer crops. There are various reasons for this but considering the unique production environment in South Africa's main groundnut areas, not many international cultivars have shown sufficient adaptability to unseat our most planted cultivars just yet.

Dr Ntladi discussed the various factors considered in breeding a new South African cultivar. Firstly, a distinction must be made between early and medium maturing. Most current groundnut cultivars fall in the medium- to longer-maturing group, which limits producers in planting choices, keeping in mind changing weather and rainfall patterns. Shorter growers are therefore high on the breeding priority list.

It was presented to the symposium that breeding is done for genetic improvement and that in searching for continuous

genetic gains, breeding remains a continuous process. This will be achieved by planting at least two generations per year using both the glasshouse (winter) and fields (summer), single-seed descent (two generations in a glasshouse) and the use of molecular markers to increase breeding efficiency.

As far as the national cultivar trials are concerned, it was reported that the 2021 trials were planted in seven localities with 19 accessions (cultivars and breeding lines). Future plans include the evaluation of cultivar performance at different plant populations, the inclusion of ARC and other varieties, plant spacing of 90 x 10, 75 x 10, and 45 x 10, three irrigated and three dryland plantings, the use of confirmed pure seeds, and the planting of trials by the ARC agronomy team.

Dr Ntladi indicated that the national breeding project will be supported by increased collaboration with universities. Through CenGen, it will seek phenotyping of germplasm for various traits and apply results from molecular markers for diseases and align ARC grading protocols with that of the industry, finally strengthening partnerships throughout the local groundnut and agricultural industry.

The three presentations covered in this article address one, if not the most critical, aspect faced by the groundnut industry in South Africa – finding and caring for cultivars that put the industry in a position to sustainably compete with alternative summer crops and reduce the reliance of the local market on imports. 🌱

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