# SRDC Capacity Building Project
# Final Report

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| **Project title:** | Enhancing the plant breeding outcomes of sugarcane cytogenetic techniques (SD11001) |
| **SRDC project number:** | BSS339 |
| **Participating people and/or organisation(s):** | Dr Nathalie Piperidis, BSES Limited |
| **Project contact(s):** | Dr Peter Allsopp |
| **Acknowledgement of SRDC's funding:** | The project participant/s acknowledge receipt of project funding from the Australian Government and the Australian Sugarcane Industry as provided by the Sugar Research and Development Corporation. |


**Summary**

The first part of this project was to attend the annual Plant and Animal Genome XIX (PAG) conference held in San Diego, USA, from January 14-19, 2011, and to present a paper at the associated International Consortium of Sugarcane Biotechnology (ICSB) workshop. The ICSB workshop was co-chaired by Dr Paul Moore from the Hawaiian Sugar Planters' Association and Dr Erik Mirkov from Texas A&M University. My presentation "Cytogenetic and Molecular Marker Characterization of *Erianthus* Backcross Hybrids" was the first of the workshop and attracted considerable interest, especially from the Brazilian participants. Since the intergeneric clones proved to be genuine, the sugarcane community is quite interested in these Australian clones, especially because our results revealed recombination between the two genomes in some clones. With the constantly growing interest in biofuels and the future requirement of increasing biomass production, it is likely that the *Erianthus* intergeneric clones will become of greater interest for plant breeders. Following discussions with Dr Jorge Da Silva (Texas A&M), Dr Derek Watt (SASRI), Dr Bernard Poitier (SASRI), Dr Neil Glynn (USDA Canal Point), Dr Chifumi Nagai (Hawaii ARC) and Dr Paul Moore, it was clearly evident that a number of groups across the world are also trying to generate similar types of intergeneric hybrids and/or would like to gain access to the Australian material. I recommended that further discussions should be held with Dr Phillip Jackson (CSIRO) at the ISSCT Breeding and Germplasm workshop to be held in Brazil in May 2011 regarding access to this material. More questions were also asked about field data related to BC3 hybrids, but these data are still confidential and could not be disclosed. In conclusion, these discussions reinforced that the Australian sugar industry holds a unique position with the availability of well-characterised generations of *Erianthus* hybrid material.

Further discussions during the conference also made it clear that other sugarcane research and development organisations are establishing new cytogenetic studies and/or new laboratories, particularly in Brazil. Sugarcane researchers are now realising the enormous potential benefits of cytogenetic studies. For the last 5 years, the BSES Mackay cytogenetic laboratory was the only one in the world dedicated solely to sugarcane studies. BSES maintains a strong and ongoing collaboration with Dr Angelique D'Hont (CIRAD) that is still producing significant and constructive findings within the sugarcane genome. Some specific

discussions were held with Dr Watt regarding the development of potentially collaborative cytogenetic projects in the future, as well as with Dr Nagai.

The second part of this project was a study visit to the laboratory of Dr Bernd Friebe at the Wheat Genetic and Genomic Resources Centre (WGGRC) in Kansas. At the WGGRC there are approximately 50 highly qualified researchers currently working on the wheat genome. Wheat, an essential worldwide crop, is a very good candidate for cytogenetics research techniques and it is widely use to characterise:

- deletion/addition lines for disease resistance
- interspecific hybrids between wheat and rye, for example

Wheat is an excellent model species to conduct C-banding studies as the wheat chromosomes are very large (compared to sugarcane!). Such research would not be successful in sugarcane because of the high number and the small, similar size of the individual sugarcane chromosomes. Wheat is also a species of choice to perform bio-fortification, for example. One of their research areas is to use *Aegilops* species to concentrate genes of nutritional importance in a particular clone of wheat. In wheat, crosses between closely related species or other genera can be controlled with the Ph1 gene, which could inhibits intra/inter recombination. Similar genes have not been described in any other species.

I was introduced to the technique of fluorescent in-situ hybridisation (FISH) karyotyping of maize chromosomes by Dr Tatiana Danilova, who has just been recruited to perform the same type of work on wheat. These discussions led me to believe that this type of work should be attempted on sugarcane. Similar work is being reported on diverse species such as barley, and I will enquire further in order to develop a research project to examine the feasibility of such work for sugarcane.

My visit to the centre was too brief because there is so much to learn from Dr Friebe and his team. He has been working at the centre since 1979 and has achieved much in the area of cytogenetics. The scientists there impressed me with their generosity and honesty regarding their work and discussions regarding laboratory methods and techniques. Dr Friebe has since sent me an email after my return to Australia saying that “it was great having you here and we also enjoyed your visit a lot. Your material is much more challenging and I was really impressed about the great work you are doing with these dots.” (where the “dots” refer to the sugarcane chromosomes).

**Itinerary**

- 14/01/2011-21/01/2011: Mackay – BNE – LA – San Diego
  Attend PAG Conference, January 15-19
  ICSB workshop: 10-12am, January 16

- 26/01/2011-28/01/11: Los Angeles – Manhattan-Kansas
  Visit WGGRC Laboratories – January 27-28

- 29/01/2011-31/01/2011: Los Angeles- BNE- Mackay

**Summary of conferences attended or meetings held, including persons met and summary of discussions and outcomes:**

**Plant and Animal Genome Conference**
The five-day conference consists of many workshops, plenary lectures and poster exhibitions. Below is a brief description of the important presentations and discussions held at the sessions that I attended.

**Genomics of Genebanks workshop:** One the most interesting presentations was "DNA information facilitates utilization of tree fruit genebanks" by Dr Cameron Peace. His main message was that allelic diversity is not useful if not used and the primary users are obviously the plant breeders. All of his data were compared to rocks with hidden gems and the message was to find the sparkle in our rocks with a number of guiding principles such as: providing performance/ predictive DNA information, the need for efficient DNA technologies, the need for appropriate germplasm, etc. The "take home" message was that, at the end of the day, high quality methods and phenotypic information is critical to reduce unpredictable results and allow accurate conclusions to be drawn. I also attended the presentation of Dr Suzanne McCouch: "Capturing positive transgressive variation from wild and exotic germplasm resources" which consists of enhancing the exploration and utilisation of the natural variation in rice.

**Bioenergy Genomics workshop:** All the talks in this workshop focused mainly on species that are being bred now for their efficiency to become bioenergy crops. The main species represented were *Sorghum*, switchgrass, *Miscanthus* and maize. Dr Andrew Paterson mentioned during his presentation that the attractiveness of *Miscanthus* has to do with the small number of chromosomes of this species compared to sugarcane. The domestication of *Miscanthus* for biofuel production has to be accelerated and will be done by improving the knowledge of its genome.

**ICSB workshop**: This was the main focus of my attendance and was held on the Sunday morning. Apart from my presentation there were four others, with one presenter unable to attend. The abstract of my presentation is located at the end of this report in Appendix A as well as the PowerPoint presentation in Appendix B. The standout presentation was that of Dr Angelique D'Hont entitled, 'High homologous gene conservation despite extreme autopolyploid redundancy in sugarcane". This presentation revealed that in sugarcane there is a high retention of redundant functional genes in contrast to a general absence of transposable element (TE) colinearity; meaning that there is multiple copies of functional gene across haplotypes while TE are not so aligned together side by side in the haplotypes. Their work also confirmed the high gene micro-colinearity between sugarcane and sorghum, making the *Sorghum* sequences a good template for the assembly of the gene rich part of the sugarcane genome. They also stated that one sugarcane haplotype can serve as reference for the other hom(oe)ologous haplotypes regarding gene content. These results are certainly good news for the current project of sugarcane genome sequencing and were emphasized by the following presentation from Yogesh Parmessur (Mauritius) titled "Exploiting sorghum resources in the molecular mapping of sugarcane". Another genetic map involving the French cultivar R570 is under construction and they have already observed evidence of gene and chromosome duplication events leading to the expansion of the *Saccharum* genome. Another haplotype study was presented by Jisen Zhang concerning the sucrose synthase gene (SuSy). Five haplotypes annotated in sugarcane were studied on three *Saccharum* species (*S. officinarum*, *S. spontaneum* and *S. robustum*). The results showed a reduced genetic diversity in *S. officinarum* and confirmed that *S. officinarum* is more closely related to *S. robustum*, thus confirming previous studies and hypotheses that *S. officinarum* originated from *S. robustum*. The last talk was presented by Jorge da Silva on the "Development of new intergeneric cane hybrids, Miscanes, as a source for biofuel production". Miscanes is a new term and indicates material derived from a

cross between sugarcane and *Miscanthus*. Data on F1 generations were presented and the progeny have been assessed with AFLP and TRAP markers. It was not clear to me if they actually proved that these F1 were genuine hybrids. Two years ago, the da Silva team thought they had some true hybrids but GISH analysis revealed that this was not the case. No GISH work has yet been attempted on these latest releases.

**Sugarcane Genome sequencing initiative:** This workshop is a new addition for sugarcane researchers as the sequencing of the sugarcane genome is a relatively new research topic. I was disappointed by the lack of uniformity of the presentations and was hoping that we would see an overview of the progress to date on the collaborative project of the R570 BAC sequencing, but this was not the case. The presentations were of a high standard, but were more focused on presenting material and methods rather than results. At this stage, it is still unclear how the different organisations involved in the sequencing are selecting the BACs to be sequenced. In their defence, it is an early stage of this project and we must remember not to compare sugarcane with other species that have already seen their genome sequenced several times.

**Comparative genomics workshop:** The first presentation was by Jianming Yu entitled "Comparative analysis of genome and chromosomes evolution across 128 species with sequenced genomes". This paper was very informative and develops the subject of upper/under limit size variation for chromosomes in diploid eukaryotes. Mini-chromosomes for example are not really stable in any genome as their inheritance is random with a low rate of transmission. The second presentation was entitled "Extensive intra-specific structural variation among maize genes", and was an introduction to array-base comparative genome hybridisation, where an entire genome is fluorescently-labelled and then hybridized on genomics arrays. This method can detect transgressive segregation and reveal/or not epistasis phenomenon and eQTL (expression of gene as a trait) for example. The third presentation "Radiation hybrid mapping in wheat and its utilisation in detailed comparative analysis of grass genome" emphasised how the wheat genome is malleable and in this case gamma rays were utilized to create small chromosomal breakages as opposed to what has been performed previously such as large chromosomal deletions. The last talk attended in this workshop was "CoGe: **Co**mparative **Ge**nomics". The presentation focused on results from the CoGe's website for generating tools to reveal patterns of genome evolution using examples from several domains of life (plants, plasmodia, animals, bacteria, and viruses). For example the whole genome syntenic dotplots takes two genomes from different species and lays them out end-to-end along different axis and if two genomic regions are related to one another through common descent from the same ancestral genomic region, then they will maintain a collinear arrangement of genes from that ancestor. While genomes can change, genes can move to new genomic positions, and duplicate genes lost, this pattern of collinear gene arrangement will be discernible for long evolutionary time periods and can be used to infer that two genomic regions are related through common ancestry (synteny). CoGe is publicly available at http://genomeevolution.org and tutorials are also available.

The Monday session started with plenary sessions. The first presentation was by Robert Beachy, entitled "Opportunities and challenges in food agriculture: who will set priorities for research and translation?" This is a very important topic and served to highlight many concerns of agricultural researchers. He emphasised the fact that plant breeders are in high demand and will remain so for a long time. Further plenary lectures were attended on Tuesday on the "Global transcription analysis of symbiotic bacteria" and "DNA methylation dynamics in human epigenomes" and Wednesday on "Mutation and evolution" by Dr Michael Lynch.

The poster sessions were of a very high standard, but very intense due to the large number of posters of interest and having not enough time to fully read and try to comprehend the intent and importance of the work. Because I could not attend the cytogenetics workshop

(as it was on at the same time as the ICSB workshop), the poster session was a good opportunity to catch up on some of the cytogenetic posters such as “Determining the genomic constitution in *Festulolium* cultivars” which is a very similar study to my work. *Festulolium* is an intergeneric hybrid between *Lolium* and *Festuca* and they used GISH techniques to characterize these intergeneric clones. The poster “Molecular cytogenetics of 25S and 5S ribosomal RNA genes and dual-color GISH in Strawberry (*Fragaria* spp.)” was also very informative as again, some of the research methods involved were similar to the work that I perform in Mackay.

The industry exposition or trade display was very good because it allowed delegates to visit the displays anytime, and it was a good opportunity to catch up with newly released technologies, equipment and products. Many of the new technologies are concentrating on genome sequencing. The scale of the sequencing effort of the whole genome research community is very impressive. In virtually every workshop that I attended, sequencing is what has emerged the most and the displays reflected the worldwide effort to improve the speed and accuracy of genome sequencing, while also trying to reduce the overall costs.

As I have in previous PAG conferences, I attended the **Challenge program workshop.** This workshop is driven by the need to help and educate people living in the poorest countries to cultivate land by themselves, but also to collaborate on developing adaptive crops for the drastic climatic conditions they are facing. Dr Andrew Borrell from UQ presented a very interesting paper on “Developing drought-adapted *Sorghum* germplasm for Africa and Australia: stay-green trait beneficial in tall and short backgrounds”. He anticipated very well the questions that were raised about breeding for drought tolerant sorghum varieties through flooded Queensland!

This year, the workshop on **Genomics assisted breeding** was very intense and one of the most interesting presentations was made by Dr Bicheng Yang from the Beijing Genomics Institute. His talk was entitled “Genome-wide patterns of genetic variation among elite maize inbreeds”. He broadened his presentation further by and changed his title from maize to crops and this was really justified. With his last few slides he revealed the Chinese institute’s capacity which includes up to 4000 individual staff members of whom 1000 are bio-informaticians, and revealed that the institute runs 150 Illumina sequencing units! Thus he concluded that molecular breeding is “the faster breeding” than traditional breeding, but nevertheless conceded that traditional breeding is always the starting point for these kinds of investigations.

As usual, the conference was very well attended with nearly 2500 scientists registered. Based upon the excellent feedback I received after my presentation, it seemed that Australian sugarcane research is highly regarded. My main regret about the PAG conference is that many workshops of interest are held concurrently and because of this, it is not possible to attend all sessions or workshops of interest. Some of the workshops finish very late, some at 8pm and most days, delegates have presentations to attend beginning at 8 am. Overall, the conference is very intensive, but provides a tremendous opportunity to learn about the latest developments in a wide range of subjects held over the 5 days.

**Economic, Environmental and/or Social benefits to the Australian sugar industry, the community and/or the participants that will accrue from the project:**

It has been almost 6 years now since BSES Limited invested to develop a cytogenetic research laboratory in Mackay focused on sugarcane studies, which was co-funded by the CRC-SIIB. Collaborations have been developed and undertaken with researchers within Australia (CSIRO), as well as France (CIRAD) and China (GSIRI). Further working relationships are being developed with other Australian cytogenetic researchers. In

summary, important outcomes have been delivered during the last 5 years and have been published in two separate international journals. One of most important outcomes produced was the characterisation of three generations of *Saccharum/Erianthus* intergeneric hybrids by GISH techniques. The first part of this research was presented at the ICSB workshop during the PAG meeting in 2008, with the second part presented at this year's meeting as part of this funded project.

This project has provided a travel opportunity to allow me to immerse myself in a very large research community, and has provided a renewed enthusiasm and energy to work on my current projects and to design new projects based on our research findings and existing/new collaborations. Unfortunately, Mackay is a very isolated place scientifically, and does not provide me with ready opportunities to interact with other scientists involved in molecular genetics research. The PAG conference was the perfect place for me to talk about my work and try to get a feeling for what is important at the moment in the world of genome research. Another important benefit was to be able to present a paper outlining our recent research findings at the ICSB workshop which is held within the PAG conference every year. Because we work in a closed environment it is difficult to have a true understanding of our own research position within a worldwide context. Nevertheless, our research was acclaimed by the sugarcane community as important on a global scale and has provided valuable exposure to the work performed at BSES. It has also confirmed that the work we are conducting is well regarded, and has reinforced that Australian sugarcane research (and BSES in particular) is at the leading edge of technology in this area.

During my time at the WGGRC, I had a number of discussions with many scientists who are currently involved in similar research, and we spent much time discussing technical problems that are encountered and their solutions. I was also able to share my views with other scientists and provide my own advice or opinions on their research. New leads for our current and future research directions were developed from the important positive feedback obtained.

**Means of communicating the findings of the project to relevant stakeholders**

Discussions with staff and researchers from BSES and CSIRO have been held to share important findings from this conference. The presentation that was given at the ICSB workshop will also be presented at BSES Brisbane and to some CSIRO researchers.

A poster will be presented at the ASSCT meeting to be held in Mackay, 4-6 May 2011. A companion paper on the BC3 hybrid results is also being prepared and should be published before the end of 2011 in the highly regarded journal "Genome".

The PAG Final Abstracts guide is available on request and a copy will soon be forwarded to the BSES library to be available as a research resource. The abstracts also are available online from the following link: http://www.intl-pag.org/19/abstracts/index.html.

**Recommendations on how knowledge or information gained can be used or transferred to projects or the industry (at a local and/or industry-wide level):**

I believe that my trip to the PAG conference followed by a visit to the WGGRC has been very worthwhile and successful. First of all, I now realise more fully the impact of the work we have performed on the generation of *Erianthus* hybrids across the global sugarcane community. Further, this has also put BSES and the Australian sugarcane industry in a good position at the international level. Clearly, we are now working with the most advanced *Erianthus* hybrids in the world, and we are also in a good position to "trade" them with other

“wanted” clones such as the Miscanes for example that were presented by Jorge Da Silva. I believe that further collaboration on an international level is very important to ensure that the Australian sugarcane industry will be at the forefront of biofuel and biomass production areas of research.

The visit to the WGRRC in Kansas has reinforced that sugarcane is a particularly challenging genome to work with having seen leading edge results on wheat and held discussions using the wheat genome as a comparison. Despite the challenges of small and unknown numbers of chromosomes in most sugarcane clones, we have published very good research results which generated some very interesting discussions with people who were impressed by the work achieved so far.

The trip has exposed me to some very interesting and important research on maize, called genomic karyotyping, presented to me by Dr Tatiana Danilova. Dr Danilova is now starting to apply the same techniques to wheat. I have since read a paper on the applications of similar research in barley and melon and I am currently considering the options and opportunities for this type of work in sugarcane. The research can be described simplistically as trying to find a limited number of probes that will be sufficient to differentiate every chromosome in a clone. In sugarcane, it will actually differentiate homology groups and all the different homo(eo)logues as well. We already have two of the eight to ten probes that I believe we need to perform this project. Further discussions and collaboration will be necessary on the subject with Dr Angelique D’Hont to better establish a working research plan. We are already involved in a collaborative project with Dr D’Hont and aim to co-publish a paper on the BAC FISH results that have been obtained over the last two years.

## Appendix A

### Cytogenetic and molecular marker characterization of *Erianthus* backcross hybrids

Nathalie Piperidis[1], Karen Aitken [2] and George Piperidis[1]

[1] BSES Limited, PMB 57, Mackay Mail Centre, Mackay, Queensland, 4741, Australia.
[2] CISRO

For more than a decade now, sugarcane breeders have considered *Erianthus* species with high interest as a new source of germplasm. Indeed *Erianthus* is a relatively close genus of *Saccharum* that shows good ratoonability, vigour, tolerance to environmental stresses, and disease resistance. Fertile hybrids from intergeneric crosses between *Saccharum officinarum* and *E. arundinaceus* have been generated and so far three backcross generations have been studied. We used molecular markers and cytogenetics techniques to characterize the chromosome composition and the mode of chromosome transmission through four generations (F1, BC1, BC2, and BC3) of interspecific hybrids between sugarcane and *E. arundinaceus*. In the F1 generation n+n transmission was observed. Unexpectedly, a 2n+n transmission was revealed in the BC1, while n+n was restored in the BC2 and BC3 generations. No recombination events were observed between *Saccharum* and *E. arundinaceus* chromosomes in BC1 and BC2 clones; however, translocation events were observed in two of the 14 BC3 clones studied. As only three to six *Erianthus* chromosomes were inherited in the BC3 clones, we also used SSR markers to assign them to sugarcane homology groups.

**Appendix B**

# Cytogenetic and molecular marker characterization of *Erianthus* backcross hybrids

16 January 2011

Dr Nathalie Piperidis
*BSES Limited*

BSES
Sugarcane for the future

BSES QCANES
Varieties for your future

BSES QCROPS
Growing your bottom line

sutech solutions

## Talk overview

- Previous results: F1, BC1, BC2
- Characterisation of the BC3
  - *Cytogenetic*
  - *Molecular marker*
- Perspectives

# Aim / Expected outcomes

## Characterisation of $F_1$, $BC_1$ and $BC_2$ intergeneric hybrids by GISH

- **TRANSMISSION** of chromosomes during crossing
- **EXTENT** of chromosome loss from *Erianthus arundinaceus*
- **RECOMBINATION ?**
  - Develop breeding and selection strategies for utilising *Erianthus arundinaceus*

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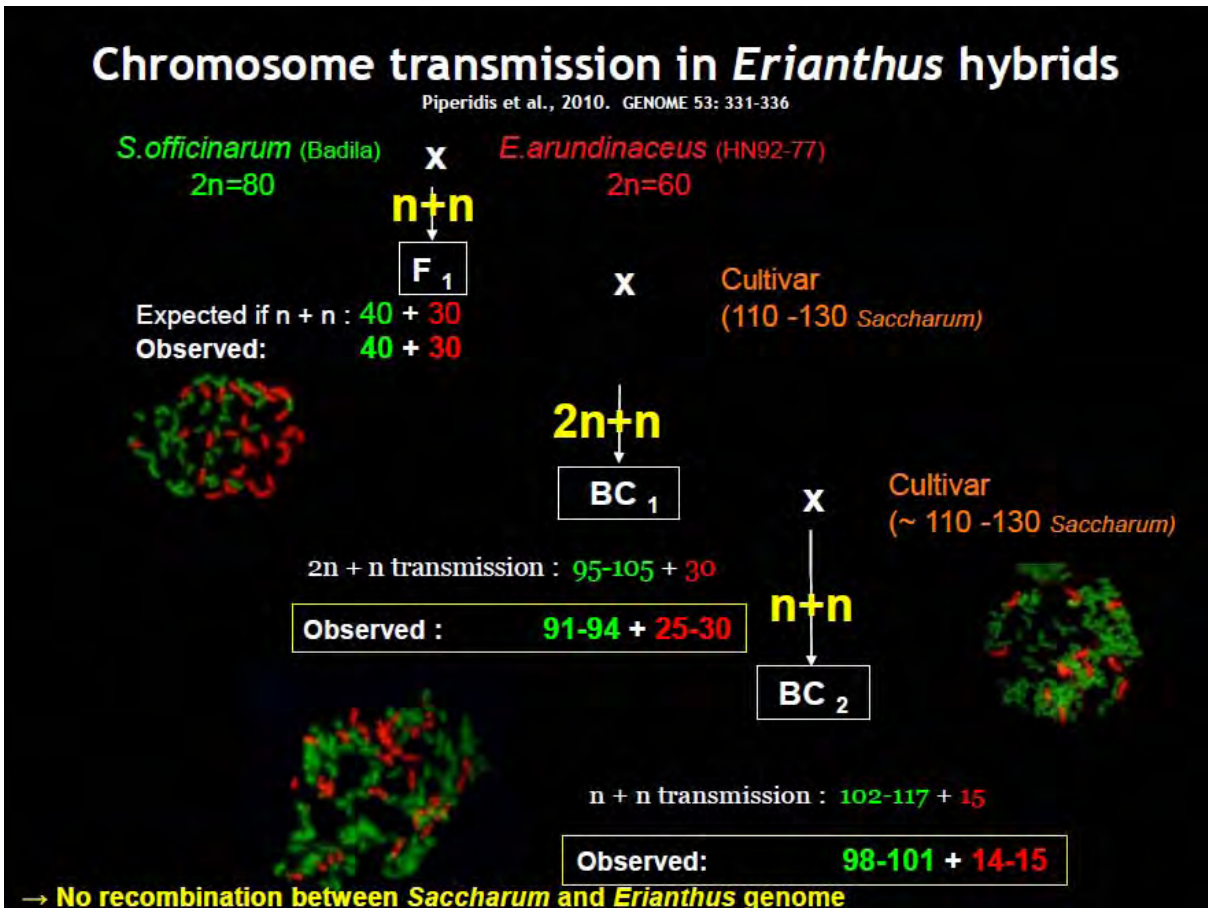

# Talk overview

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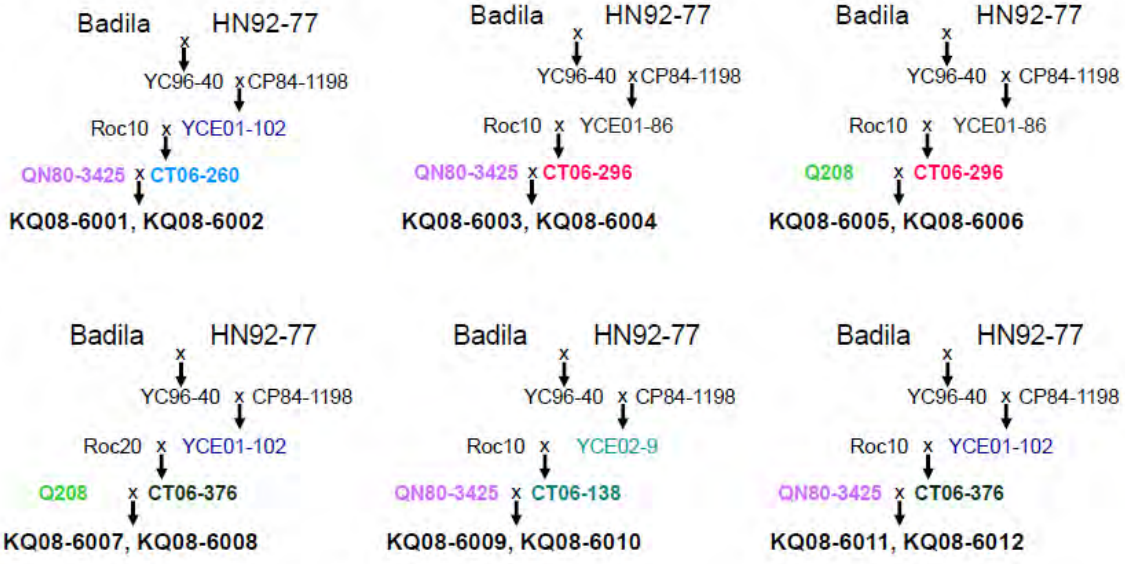
# $BC_3$ Pedigree

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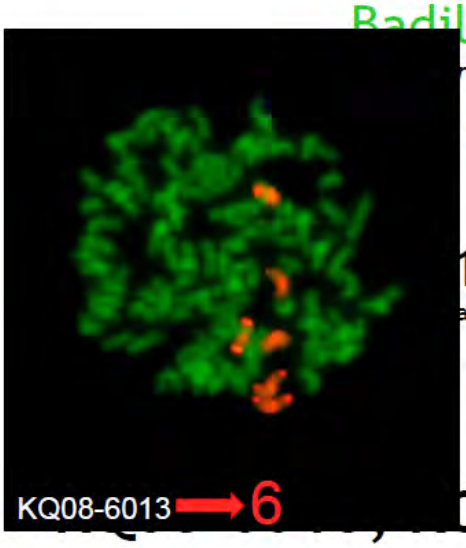
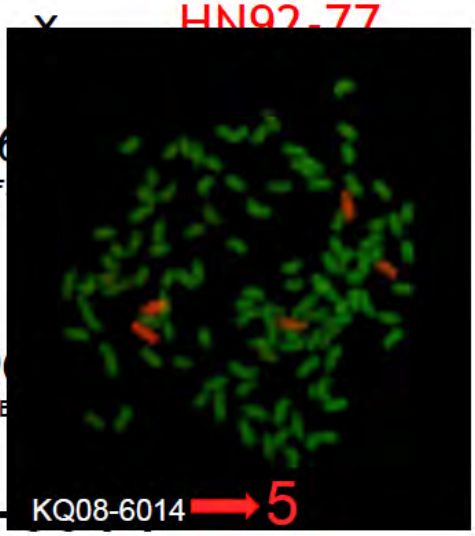
# *Erianthus* BC3

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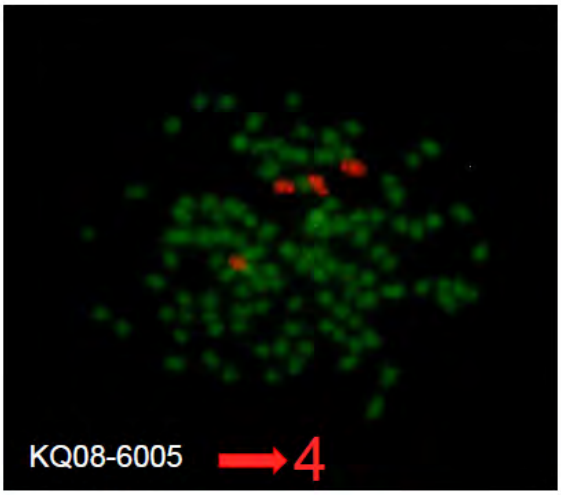
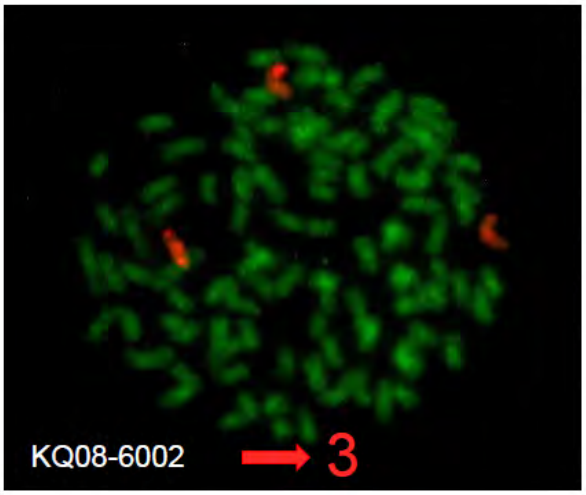
# *Erianthus* BC3

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# Chromosome counting in $BC_3$

| Clone names | *Saccharum* C. | *Erianthus* C. | Total C. |
|---|---|---|---|
| KQ08-6001 | 112 | 5 | 117 |
| KQ08-6002 | 105-106 | 3 | 108-110 |
| KQ08-6003 | 110-115 | 6 | 116-121 |
| KQ08-6004 | | | |
| KQ08-6005 | 102-107 | 4 | 106-111 |
| KQ08-6006 | | | |
| KQ08-6007 | 107-108 | 3 | 110-111 |
| KQ08-6008 | 104-107 | 3 | 107-110 |
| KQ08-6009 | 100 | 4 | 104 |
| KQ08-6010 | 109-110 | 4 | 113-114 |
| KQ08-6011 | 103 | 6 | 109 |
| KQ08-6012 | 107-108 | 6 | 113-114 |
| KQ08-6013 | 110-112 | 6 | 116-118 |
| KQ08-6014 | 112-113 | 5 | 117-118 |

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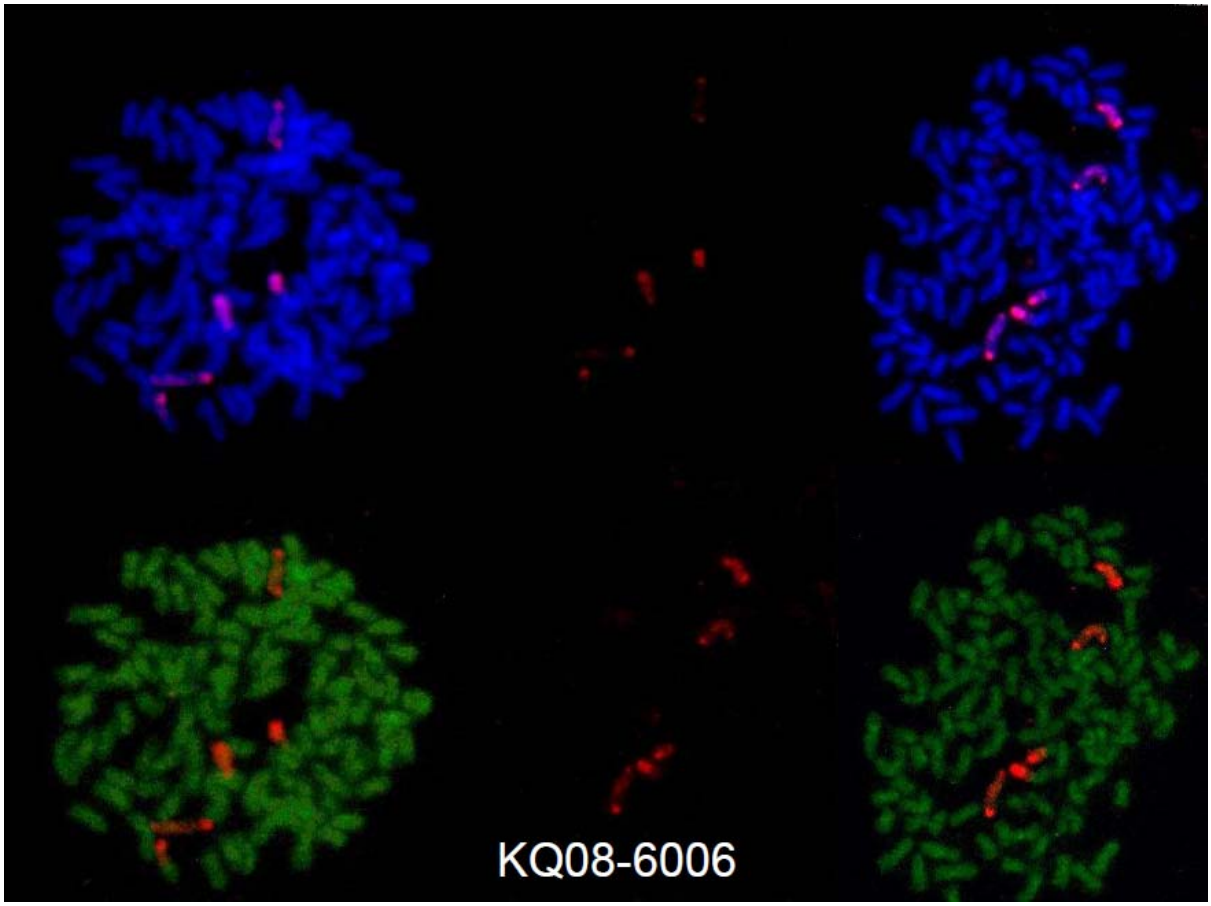
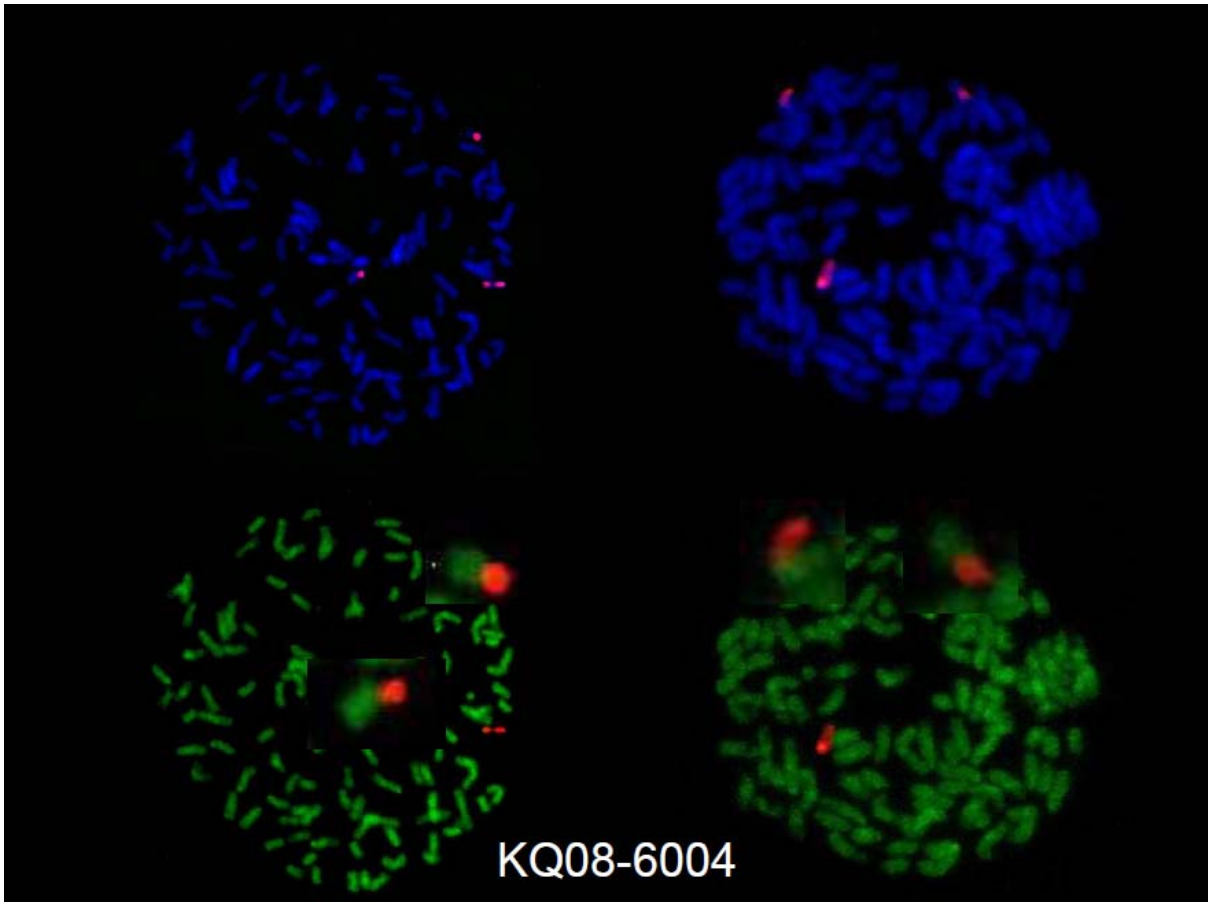
Sugarcane for the future



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| KQ08-6001 | 112 | 5 | 117 |
| KQ08-6002 | 105-106 | 3 | 108-110 |
| KQ08-6003 | 110-115 | 6 | 116-121 |
| KQ08-6004 | 112-113 | 3 (1 + 2* 1/2) | 115-116 |
| KQ08-6005 | 102-107 | 4 | 106-111 |
| KQ08-6006 | 107 | 5 (3+ 2* 1/2) | 112 |
| KQ08-6007 | 107-108 | 3 | 110-111 |
| KQ08-6008 | 104-107 | 3 | 107-110 |
| KQ08-6009 | 100 | 4 | 104 |
| KQ08-6010 | 109-110 | 4 | 113-114 |
| KQ08-6011 | 103 | 6 | 109 |
| KQ08-6012 | 107-108 | 6 | 113-114 |
| KQ08-6013 | 110-112 | 6 | 116-118 |
| KQ08-6014 | 112-113 | 5 | 117-118 |

# Summary and Questions

- Reciprocal translocation in 2/14 clones
  → Can we reveal a genome specificity for the translocation?

- Chromosome composition:
  - *Saccharum* content: 102-115
  - *Erianthus* content: 3 to 6, 1+ 2*half, 3 + 2* half
  - Total: 104 to 121

  → **Can we assign *Erianthus* chromosomes to a specific HG?**

- Implication for breeding strategies?

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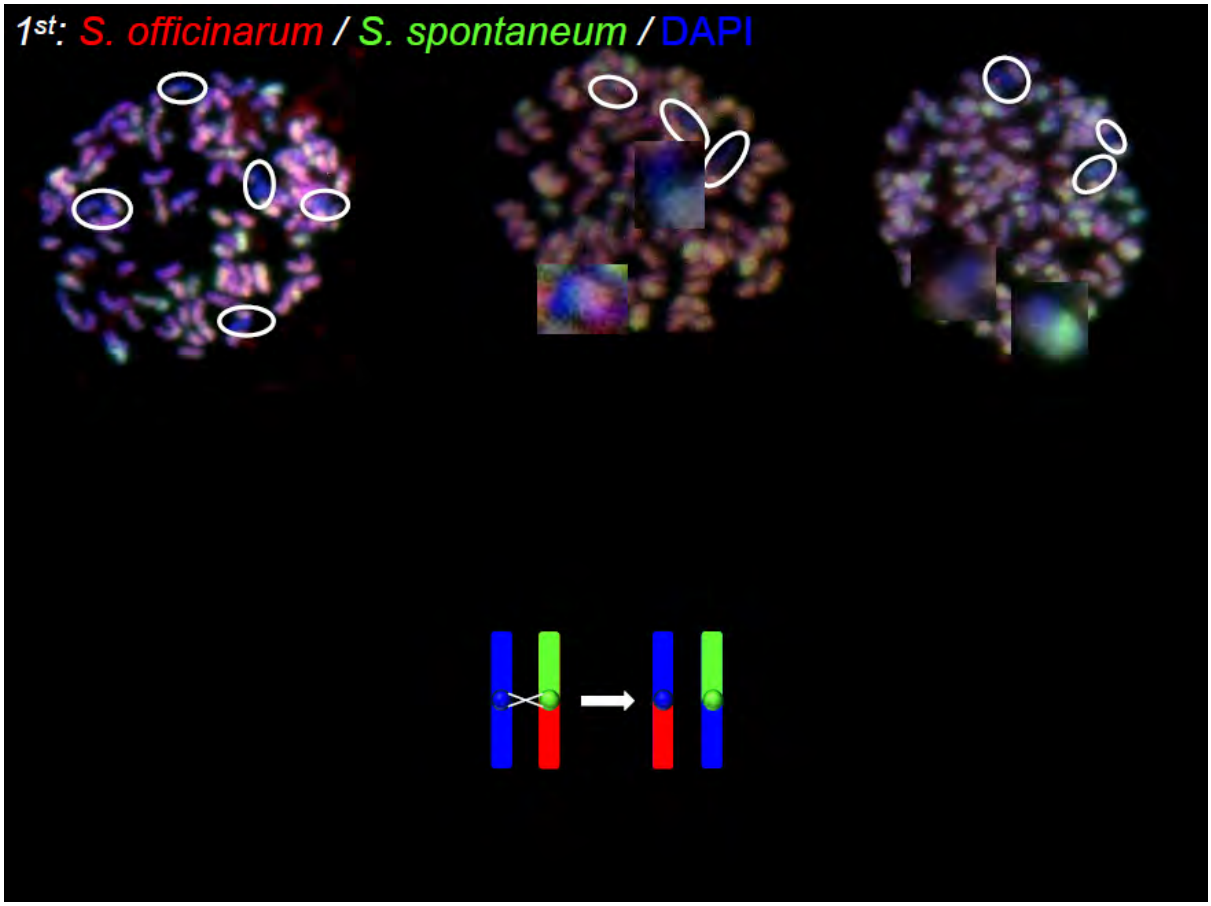

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# Talk overview

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- Characterisation of the BC3
  - *Cytogenetic*
  - *Molecular marker*
- Perspectives

# Molecular marker

| SSR | sorghum HG | Q165 HG | R 570 HG |
|---|---|---|---|
| | Number of *Erianthus* Chromosomes | | |
| SMC1120 | 1 | 4 | I |
| mSSCIR14 | 1 | 4 | I |
| mSSCIR47 | 2 | 3a | VI |
| mSSCIR60 | 2 | 3a | VI |
| mSSCIR 34 | 3 | 5 | II |
| mSSCIR 35 | 3 | 5 | II |
| mSSCIR41 | 3 | 5 | II |
| mSSCIR21 | 4 | 1 | VII |
| mSSCIR36 | 4 | 1 | VII |
| mSSCIR12 | 5 | 2 | VIII |
| mSSCIR28 | 5 | 2 | VIII |
| mSSCIR26 | 6 | 2 | VIII |
| m483 | 7 | 9 | ? |
| mSSCIR24 | 8 | 8 | ? |
| mSSCIR74 | 9 | 7 | ? |
| mSSCIR33 | 10 | 6 | III |

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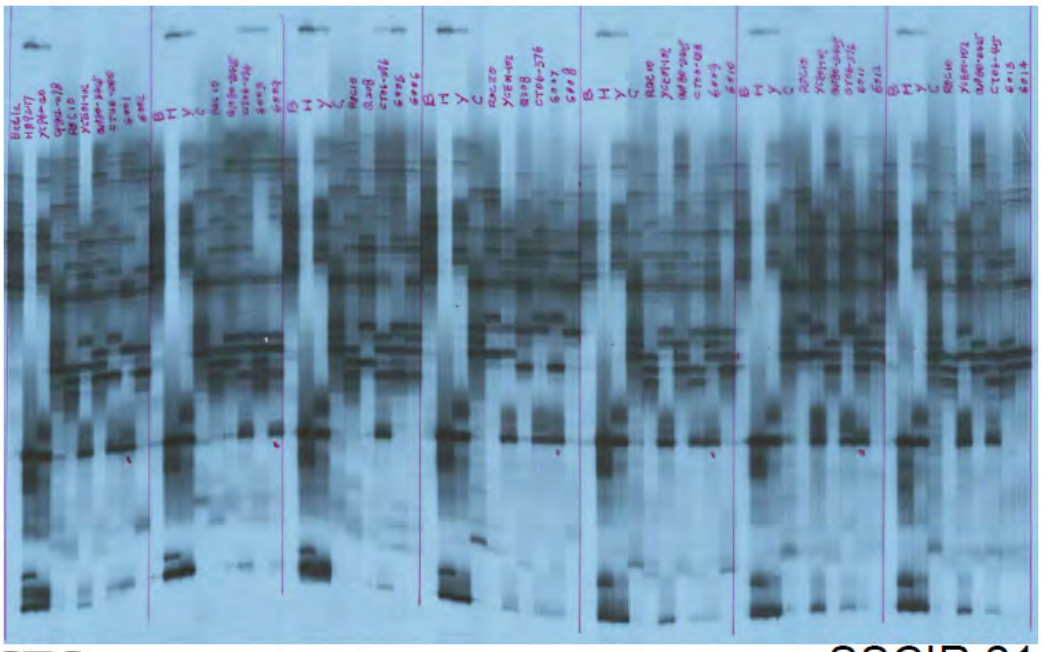
# Molecular marker results



mSSCIR 21

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# Molecular markers results

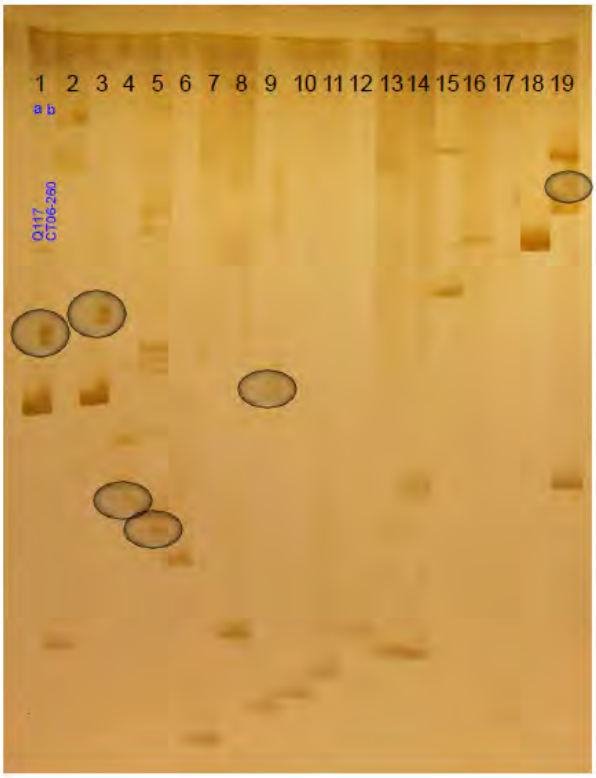
| SSR | sorghum HG | Q165 HG | R 570 HG | KQ08-6001 (5C.) | KQ08-6002 (4C.) | KQ08-6003 (6C.) | KQ08-6004 (3: 1+2*1/2) | KQ08-6005 (5C.) | KQ08-6006 (5: 3+2*1/2) |
|---|---|---|---|---|---|---|---|---|---|
| mSSCIR 34 | 3 | 5 | II | 0 | 0 | 1 | 0 | 0 | 1 |
| mSSCIR 35-1 | 3 | 5 | II | 0 | 0 | 1 | 0 | 1 | 1 |
| mSSCIR 35-2 | 3 | 5 | II | 1 | 0 | 0 | 1 | 0 | 0 |
| mSSCIR 35-3 | 3 | 5 | II | 0 | 0 | 1 | 0 | 0 | 1 |
| mSSCIR41-1 | 3 | 5 | II | 1 | 0 | 1 | 1 | 1 | 1 |
| mSSCIR41-2 | 3 | 5 | II | 1 | 1 | 1 | 1 | 0 | 0 |
| mSSCIR21 | 4 | 1 | VII | 1 | 0 | 0 | 1 | 0 | 0 |
| mSSCIR36 | 4 | 1 | VII | 0 | 0 | 0 | 1 | 0 | 0 |
| mSSCIR26 | 6 | 2 | VIII | 0 | 0 | 0 | 0 | 0 | 0 |
| mSSCIR74 | 9 | 7 | ? | 0 | 0 | 0 | 0 | 0 | 0 |
| mSSCIR33-1 | 10 | 6 | III | 0 | 0 | 0 | 0 | 0 | 0 |
| mSSCIR33-2 | 10 | 6 | III | 0 | 0 | 1 | 0 | 1 | 0 |
| mSSCIR33-3 | 10 | 6 | III | 1 | 1 | 1 | 0 | 1 | 1 |

# Molecular markers results

| SSR | sorghum HG | Q165 HG | R 570 HG | KQ08-6007 (3C.) | KQ08-6008 (3C.) | KQ08-6009 (4C.) | KQ08-6010 (4C.) | KQ08-6011 (5C.) | KQ08-6012 (5C.) | KQ08-6013 (6C.) | KQ08-6014 (5C.) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mSSCIR 34 | 3 | 5 | II | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| mSSCIR 35-1 | 3 | 5 | II | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| mSSCIR 35-2 | 3 | 5 | II | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| mSSCIR 35-3 | 3 | 5 | II | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| mSSCIR41-1 | 3 | 5 | II | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| mSSCIR41-2 | 3 | 5 | II | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| mSSCIR21 | 4 | 1 | VII | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| mSSCIR36 | 4 | 1 | VII | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| mSSCIR26 | 6 | 2 | VIII | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| mSSCIR74 | 9 | 7 | ? | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| mSSCIR33-1 | 10 | 6 | III | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| mSSCIR33-2 | 10 | 6 | III | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| mSSCIR33-3 | 10 | 6 | III | ? | ? | 1 | 1 | 1 | 1 | 0 | 1 |

# Silver stain

- 19 single copy genes in *Sorghum*
- PCR on Q117 and CT06-260
- Number of polymorphism in lane 1ab, 3ab, 4ab, 5ab, 9ab,19ab
- Primer pairs 1 and 3 → gene MAX3
- PCR on the 14 BC3 clones and their parents

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# Silver stain results

- Gene MAX3 is mapped on *Sorghum* HG 6
- Correspond to HG VIII in R570

- Results show an amplification of the gene in clone:
  - KQ08-6006
  - KQ08-6007
  - KQ08-60010
  - KQ08-60011

- mSSCIR 26 (R570 HG VIII) show an amplification of a marker:
  - KQ08-6008
  - KQ08-60010
  - KQ08-60011
  - KQ08-60013

| Clone | SoMAX3F3-SoMAXR3 | mSSCIR26 |
|---|---|---|
| HN92-77 | 0 | 1 |
| Badila | 0 | 0 |
| YC96-40 | 1 | 1 |
| ROC10 | 0 | 0 |
| YCE01-102 | 1 | 1 |
| QN80-3425 | 0 | 0 |
| CT06-260 | 1 | 1 |
| KQ08-6001 | 0 | 0 |
| KQ08-6002 | 0 | 0 |
| QN80-3425 | 0 | 0 |
| CT06-296 | 1 | 0 |
| KQ08-6003 | 0 | 0 |
| KQ08-6004 | 0 | 0 |
| Q208 | 0 | 0 |
| CT06-296 | 1 | 0 |
| CT06-376 | 1 | 1 |
| KQ08-6005 | 0 | 0 |
| KQ08-6006 | 1 | 0 |
| KQ08-6007 | 1 | 0 |
| KQ08-6008 | 0 | 1 |
| QN80-3425 | 0 | 0 |
| CT06-138 | 1 | 1 |
| KQ08-6009 | 0 | 0 |
| KQ08-6010 | 1 | 1 |
| QN80-3425 | 0 | 0 |
| CT06-376 | 1 | 1 |
| KQ08-6011 | 1 | 1 |
| KQ08-6012 | 0 | 0 |
| QN80-3425 | 0 | 0 |
| CT06-415 | 1 | 1 |
| KQ08-6013 | 0 | 1 |
| KQ08-6014 | 0 | 0 |

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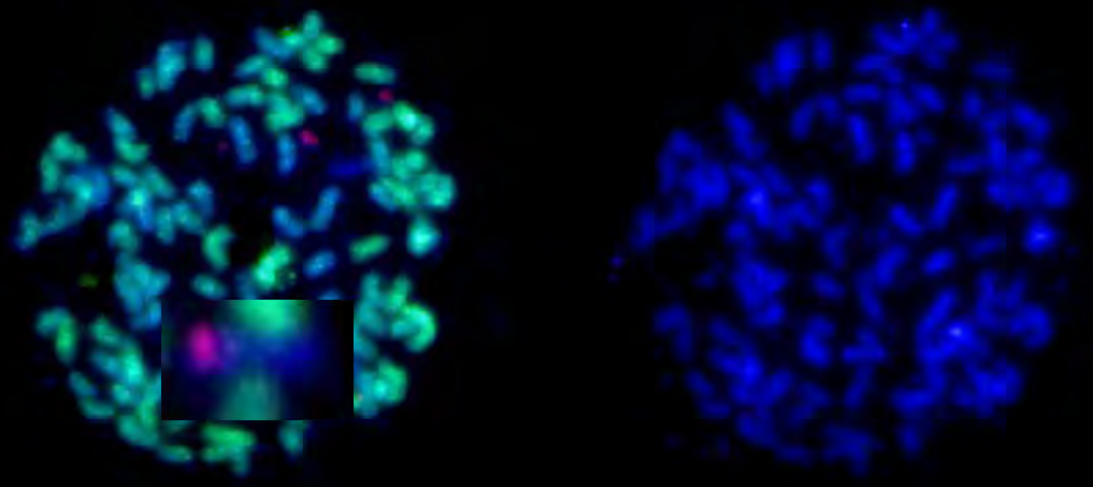
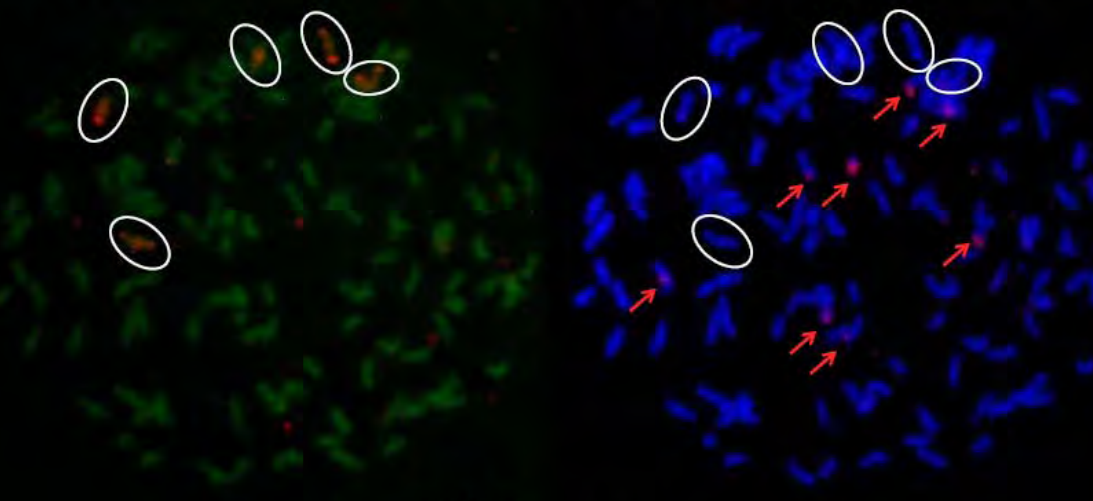
# Localisation of rDNA on KQ08-6007



# Localisation of rDNA on KQ08-6014

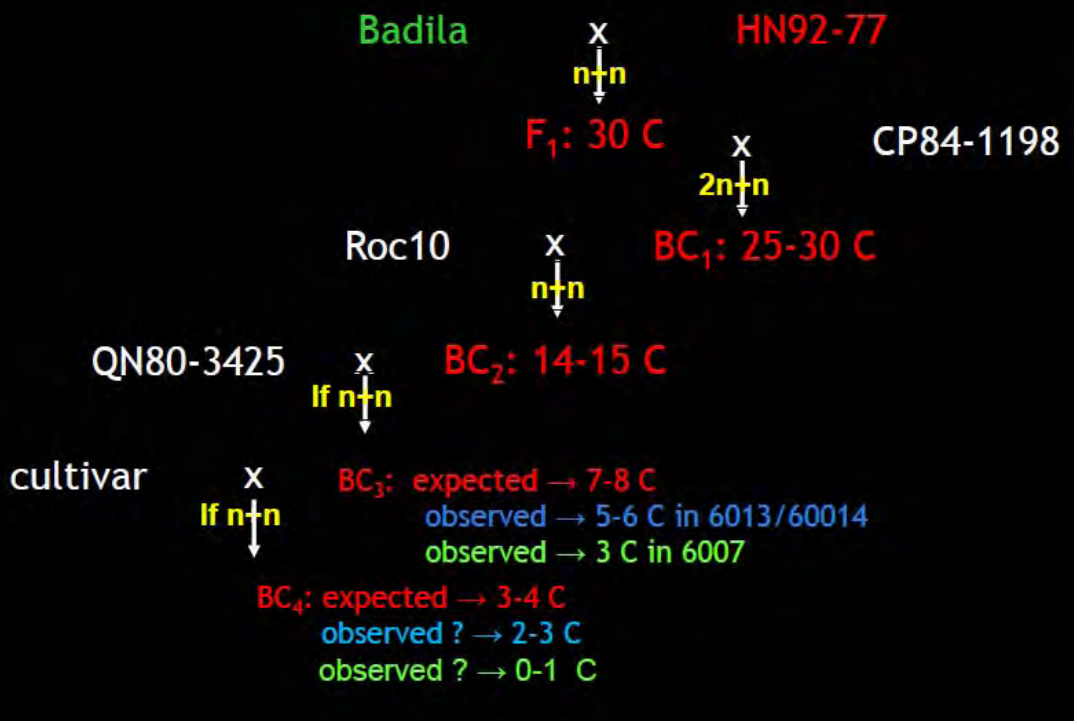


| Clone | SoMAX3F3-SoMAXR3 | mSSCIR26 | rDNA |
|---|---|---|---|
| KQ08-6006 | 1 | 0 | |
| KQ08-6007 | 1 | 0 | Yes |
| KQ08-6008 | 0 | 1 | |
| KQ08-6010 | 1 | 1 | |
| KQ08-6011 | 1 | 1 | |
| KQ08-6013 | 0 | 1 | |
| KQ08-6014 | 0 | 0 | No |

# Talk overview

- Previous results: F1, BC1, BC2
- Characterisation of the BC3
  - *Cytogenetic*
  - *Molecular marker*
- Perspectives

# Breeding perspective with the *Erianthus* hybrids



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