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Final report

Project	**Improving post-rainy sorghum varieties to meet the growing grain and fodder demand in India – Phase 2**
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1 Acknowledgments

This final report put a close to two successive project phases funded by ACIAR and acknowledgement goes primarily to ACIAR's continued support to the objectives of the project. We are grateful to the confidence that was put in the project team, especially at the time of approving the second phase in a context where the target region of the project was no longer a priority for ACIAR investment in India.

In conjunction with this, our thanks go to the ACIAR officers who have successively monitored the projects' proposal submission and activities, with great involvement. These are chronologically Dr Christian Roth, who played an active role at the time of the first phase proposal development. This was followed by Dr Paul Fox who was a keen and engaged officer during much of the first phase. And then finally by Dr Eric Huttner who was also keen and engaged and who played an active role in the approval of the second phase.

Thanks in particular to two ICRISAT's colleagues, Dr Dave Hoisington who was keen to promote one of the first case studies for the use of DNA markers to target a complex constraint, and Tom Hash who had developed the initial sets of sorghum material that has served the basis of this project.

This project has been one of several targeting similar constraints and issues in sorghum and we are thankful to the close exchanges, interactions, and discussions that we have had in all those years with scientists of these other projects. The pinnacle of these exchanges came in the form of a special session at the 2018 Sorghum in the 21st Century Conference (9-12, Cape Town, South Africa).

And last, but not least, these 10 years or so since the beginning of the first phase have been a rich, enthused, and engaged forum for discussion with scientists from ICRISAT, ILRI, IIMR, UQ (and DAFF) and all should be thanked for their involvement. In a certain way, the project's constituents were those of a "breeding team", encompassing a diverse skill set interacting in a breeding crucible toward the development of a user-driven breeding end-product. This can be taken as a case study of what the Excellence in Breeding platform of the CGIAR is now starting to promote widely at the CG level. Project's constituents should all be thanked to have been part of this harbinger.

2 Executive summary

This project was an extension to a previous 4-year phase where the proof-of-concept was established on the value of introgressing genome segments conferring the capacity to maintain green leaf area under terminal water stress conditions (stay-green quantitative trait loci, QTL) for improving both the grain and the stover productivity of sorghum during the post-rainy season (Rabi) in India. Importantly, several QTLs improved multiple nutritional quality parameters of the crop residue, used as cattle feed, without having trade-offs with grain and stover productivity. Higher stover quality also conferred a price premium and led to higher milk productivity or weight gain. The first phase showed that improving different components of the sorghum value chain simultaneously was possible. Yet, stay-green QTL effects are genetic background dependent. A thorough characterization of the stress patterns in the target regions also showed highly variable patterns of stress, requiring specific breeding/agronomic solutions to specific stress scenarios.

The second phase capitalised on these findings, to introgress stay-green QTL segments into the background of six sorghum cultivars adapted to the target region in India, in a close partnership between ICRISAT and IIMR, and using crop simulation modelling to design optimal genetic and agronomic management packages for specific stress scenarios defined in Phase 1. It then expanded the modelling work to the West-Central Africa (WCA) region and initiated the development of genetic stocks needed to target traits underlying the stay-green phenotype and identified in Phase 1. The objectives of the second phase were:

1. Introgress two stay-green QTL (3A and 3B) into the six most popular cultivars currently used by farmers in the post-rainy sorghum track of India, and use crop modelling to design optimum genotype-by-environment-by-management options.
2. Expand the environmental characterization of stress scenarios to West Africa, and develop new breeding populations – BackCross Nested Association Mapping (BCNAM) with the most popular post-rainy sorghum cultivars of India as recipients and new donors for beneficial traits from phase I (funding from CRP DC).

In Objective 1, the QTL interval of two critical stay-green QTLs, *Stg3A* and *Stg3B*, was refined. From initially a 139 SNP assay from available genotyping-by-sequencing (GBS) data, 41 polymorphic SNPs for *Stg3A*, and 8 SNPs for *Stg3B*, were used to introgress these two QTLs in six Rabi-adapted cultivars. The most advanced introgressions have reached the BC3F4 or BC4F1 stage while some BC3F3 lines have started being evaluated. Field trials have also tested the effects of stay-green introgressions, nitrogen treatment, and plant density on grain/stover yield and quality, the purpose being to better understand the Gene x Environment x Management (GxExM) interactions, and to better target key nodes of the entire sorghum value chain. A revision of the ex-ante analysis done in Phase 1, using robust modelling outputs rather than subjective informant responses, showed a higher return to investment from developing two different ideotypes, either grain or stover, fitted to district-specific end-user demand, rather than a one-fits-all technology. Grain nutritional aspects have become an important aspect of our research, since a beneficial effect of *Stg* introgressions on grain quality was shown.

Under Objective 2, a version of the APSIM crop model that takes into account tillering effects has been developed and tested successfully. The APSIM algorithm has also been improved to model long duration cultivars of the West African region developing a large number of leaves. This has allowed us to expand the environmental characterization to the West-Africa (WCA) region. The development of BCNAM for West Africa is continuing actively, in parallel to the development of other BCNAM materials in Ethiopia using the same donors (as part of a USAID project). Until these are ready, phenotyping of existing BCNAM progenies from WCA in a high throughput phenotyping platform has started to map traits underlying the stay-green expression, and have shown co-mapping between *Stg* loci and aspects of the plant vigor and grain size.

3 Background

Post-rainy sorghum is important for about 5 million households in India. Being cultivated at the end of the rainy season on poor and shallow soils, where sorghum production is constrained by water limitation, sorghum has virtually no contender crops, and livelihood improvement needs to be focused on the crop value-chain itself. Both grain and stover residues indeed play an almost equally important role in the sorghum value chain, and the price of stover is linked to stover quality. Furthermore, the second phase of our project was set in the context of the beginning of the CGIAR Research Program (CRP) on Dryland Cereals, and in the context of a USAID initiative on sorghum targeted to sub-Saharan Africa, both targeting closely related issues in terms of constraints and of developing integrated solutions.
The purpose of this project was therefore two-fold: (i) generate cultivars with higher productivity and quality under such limitations; and (ii) generate knowledge to speed up the generation of improved cultivars for similar constraints across the world. This second phase proposal was then anchored on an ACIAR-funded Objective 1 focused on a targeted delivery of improved cultivars, and on a CRP-funded Objective 2 aiming at building on the knowledge acquired in Phase 1 to expand its use and approach globally.

During the project's first phase, the introgression of stay-green QTLs into senescent sorghum lines improved grain yield, stover yield, and stover quality traits, without trade-offs between these traits. There were also synergistic associations between stover productivity and stover quality. Introgression of these QTLs, initiated in the background of farmer-preferred lines (M 35-1, Parbhani Moti, Phule Vasudha and CRS1), was proposed to be extended to RSLG262 and CRS4 in the ACIAR-funded Objective 1. Re-sequencing of introgression lines developed during the first phase, plus fine-mapping information on different stay-green QTLs and re-sequencing data for 40 diverse sorghum lines was available to develop a set of closely linked markers for precise introgression of Stg3A and Stg3B segments. A very solid *ex-ante* assessment had shown highly beneficial impacts of the proposed improved varieties, even in the most conservative scenario, for both the producer and the consumer. Then the modelling work has identified a number of promising traits or agronomic management alterations, and interactions (GxExM) capable of improving the monetary return of the value chain.

From the first phase, the mechanisms underlying the expression of the stay-green phenotype have been identified: (i) improved water extraction; (ii) improved transpiration efficiency (TE); and (iii) reduced leaf area and large genetic variation for these traits has been identified. Weather patterns in the sorghum production area had been analysed and showed the existence of sub-zones with different stress severity patterns. Simulation of the effects of some of the underlying traits predicted an increase in yield and yield resilience (less frequency of crop failure), with the highest effects being specific to certain stress patterns. Then the CRP-funded Objective 2 aimed at (i) developing and using backcross nested association mapping (BCNAM) populations in the background of a few post-rainy season varieties with germplasm variants donors for these traits to harness their genetics and develop promising breeding materials. This anticipated a likely shift from breeding "stay-green QTL" to breeding QTLs involved in the key underlying mechanisms, and/or combinations of these to optimize production; and (ii) Using crop simulation modelling in the West Africa region to characterize stress patterns and prevalence and to guide the choice of key traits and/or management options for these different scenarios.

The work was aligned with the CGIAR Research Program (CRP) on Dryland Cereals, and aimed at a global integration of sorghum breeding efforts for water-limited environments, with short to mid-term support from ACIAR and long-term with support from the CRP.

4 Objectives

This project had both a mid-term goal (Objective 1) supported by ACIAR, and a long-term goal (Objective 2) supported by the CRP on Dryland Cereals. The mid-term goal was to capitalize on the achievements of the first phase of the project and breed improved cultivars by continuing the development of introgression of stay-green QTL in the backgrounds of post-rainy season landraces and varieties, testing these for the suite of targeted traits, and progressing towards the most promising ones and to the testing stage via on-station trials. Front Line Demonstrations (FLD) on farmer's fields, to be organized by DSR (now IIMR), were initially planned but could not fit into the time frame. The long-term goal was then to take stock of the main findings from the first phase to set a framework for an efficient delivery of improved cultivars for regions facing water limitation. This was proposed to be based on the development of BCNAM populations in WCA and Asia using some of the germplasm donors of key traits identified in Phase 1, and on the use of crop simulation modelling to expand on the learnings from the first phase to the West Africa region. The BCNAM development was proposed to be the common denominator for several integrated disciplinary efforts, i.e. the modelling to characterize the regional drought scenario and guide the choice of key traits, the physiology to assess recurrent parents and locally adapted materials for key traits, and the genetics to identify QTL for key traits from the BCNAM populations. These two mid- and long-term objectives are described as follows:

Objective 1. Defining the best package to develop improved cultivars. Refine post-rainy season backgrounds for stay-green expression, agronomic/stover quality traits, and underlying traits using suitable control conditions, and initiate a "seed delivery" mechanism in the scope of multi-location testing, for which DSR has a well-established system in place for mass seed multiplication and delivery. Specifically: (i) select best markers for each of the key QTLs to breed improved cultivars, (ii) further advance and select the best backcross lines in post-rainy season backgrounds for stay-green expression, agronomic/stover quality traits, and underlying traits, (iii) initiate a "seed delivery" mechanism in the scope of multi-location testing and on-station demonstrations; (iv) test the most promising GxExM packages for crop productivity and crop/residue nutritional value using crop simulation modelling; (v) refine the ex-ante analysis of the first phase with robust modelling output to design district-based ideotypes.

Objective 2. Improve breeding efficiency by better targeting key traits and trait donors. Develop BCNAM populations using trait variants (for TE, water extraction capacity, altered canopy development) from the first phase in genetic backgrounds from India and WCA, and expand the modelling work from the first phase to the WCA region. The objective was to develop the material needed to quickly identify promising mapping regions responsible for high TE (VPD responsive) trait, and link up/coordinate similar types of activities for West Africa with partners of the CRP, and with the East African region through a UQ-led project funded by the BMGF and ACIAR (BCNAM development, parameterization of key cultivars, weather scenario analysis, cross linkage on trait analysis, etc.).

Planned outputs (as per the proposal): **(i)** Pre-release post-rainy season lines with improved grain and stover yield, high stover quality; **(ii)** Seed delivery pipeline framework put in place (seed production and distribution system of IIMR under ICAR project); **(iii)** Additional pre-breeding lines coming from the BCNAM efforts; **(iv)** Effective marker system/assays for efficient introgression of key regions, better knowledge of optimal QTL combinations, and genomic regions involved in key underlying mechanisms; **(v)** Sorghum breeding efforts integrated across regions and multi-institution partners.

5 Methodology

Objective 1. Defining the best package to develop improved cultivars.

1. Identify better/perfect SNP markers for the *Stg3A* and *Stg3B* QTLs – this used GbS and fine-mapping information for each targeted stay-green QTL, cross checking information with the UQ group who targets the same QTLs, and developed a SNP assay to test for polymorphism in the parental materials. The available GBS data of field validated best performing Introgression Lines (ILs), *viz*., K260 and K359, along with parental genotypes was used for calling SNPs. Only functional SNPs (by utilizing annotations) were identified from target region of 56 Mbp to 72 Mbp. A set of 139 SNPs were developed using LGC KASPar® assay. Further evaluation of these SNPs resulted in identification of 69 polymorphic SNPs, out of which we have only selected 30 SNPs spanning the target region which were consistently polymorphic across several MABC.
2. Development of introgression lines and assess intermediate BC products – The introgression work targeted six post-rainy season landraces/OPVs, using an R16 derivative containing *Stg3A* and *Stg3B* loci. A routine MABC workflow was followed for developing BC_2- and BC_3- based segregating material. The foreground selection (for confirmation of target QTL introgression) and limited background selection (to evaluate background-recurrent parent recovery) was carried out at each of BC_n-generation. The selected $BC_{2/3}F_{3:4}$-progenies will be evaluated during postrainy 2018-19.
3. Conduct multi-location trials– This used the IIMR network of testing locations in the target region and the main objective was to identify the most promising products. The choice of testing location also took into account the different stress scenarios identified in Phase 1, ensuring that each of these was represented in the testing sites.
4. Identify critical trait-by-management packages – The crop model APSIM was used to test the value of traits or trait combinations on the grain and stover productivity, looking at trade-offs between grain and stover productivity. This was also backed up by field experimentations testing different density, nitrogen, and water stress factorial combinations to test the robustness of the model.
5. Undertake feed trials – This was initially planned to be done in the most promising "proof-of-concept" entries of the first phase. It was then decided to wait for the final product development. Replacement activities then established a linkage between grain quality attributes and stay-green QTLs, using NIRS (near-infrared spectroscopy) assessments to measure quality indicators of the stover residue (e.g. metabolizable energy, cellulose, lignin, and N content) and of the grain (e.g. amino acids, protein, and starch). Samples for NIRS came from factorial yield trials using introgression lines from the first phase and combinations of density, N fertilization and water stress treatments.
6. Refine socio-economic coefficient of the ex-ante analysis – While the ex-ante analysis of the first phase was based on informants' opinion of the likely benefit from the stay-green technology on different aspects of the sorghum value chain, the analysis was re-done using the output of simulations as an estimator of these benefits. Since different stress scenarios were identified in Phase 1, then different ideotypes were tested representing the range of end-user demand (between stover type and grain types).

Objective 2. Improve breeding efficiency by better targeting key traits and trait donors.

1. Develop BCNAM populations – The principle was to undertake crosses between key trait donors identified from Phase 1 project (high transpiration efficiency, high water extraction capacity) and post-rainy season landraces/OPV, followed by a backcross

with the latter to restore as much as possible the agronomic characteristic of the farmer-preferred background. Similar work was undertaken in the WCA region, using locally preferred cultivars and the same key trait donors, in the scope of a USAID-supported project.

2. Expand the use of crop simulation to the WCA region – This consisted in assembling weather databases from target regions in WCA, parameterize representative adapted mega-cultivars using existing reports from field trials carried out in the region, and characterize stress patterns and prevalence using the crop simulation model APSIM and following similar work undertaken in the Phase 1 of the project.
3. Map QTLs for key traits underlying stay-green – The trait target was the capacity to restrict water losses under high VPD conditions and this was phenotyped using a high throughput phenotyping platform developed at ICRISAT and consisting of weighing trays containing plants to measure plant transpiration under different VPD regimes, and leaf area from 3D laser scanning technique. Genetic material used was a set of existing 957 BCNAM progenies in which parental contrast for the trait was previously established.

6 Achievements against activities and outputs/milestones

***Objective 1:** Defining the best package to develop improved cultivars. Refine post-rainy season backgrounds for stay-green expression, agronomic/stover quality traits, and underlying traits using suitable control conditions, and initiate a "seed delivery" mechanism in the scope of multi-location testing and on-station demonstrations, for which DSR (now IIMR) has a well-established system in place for mass seed multiplication and delivery.*

no.	activity	outputs/ milestones	completion date	comments
1.1	Identify better/perfect SNP from the GbS data for each stay-green QTL	A 96-SNP assay on a KASPar or Illumina Bead-Xpress platform	Mid Year 1 (month 6)	A set of 26 SNPs (designed on KASPar platform) for *Stg3A* (covering genomic region from 56112177bp to 61953206bp) and four SNPs for *Stg3B* (covering genomic region from 69739036bp to 71360153bp) on SBI-02 are polymorphic between the recurrent parents and donors. Markers are used in a routine basis for Stay-green QTL introgression in other projects such as Govt. of Karnataka funded project on post-rainy season sorghum improvement. In addition to SNP platform, 37 SSRs targeting Stg3a and 11 SSRs targeting Stg3b were designed. Using publicly available and new SSRs, 14 SSRs for Stg3a and 6 SSRs for Stg3b were shortlisted as polymorphic markers for foreground selection scheme.
1.1	Continue the stay-green QTL introgression into six post-rainy season landraces	A set of individual stay-green QTL into 4 backgrounds initiated in Phase 1	Mid Year 2 (month 18)	Different genetic stocks at different stages of backcrossing are available, depending on recurrent background, up to BC3F4 in the 4 genetic backgrounds (CRS1, M35-1, Parbhani Moti, Phule Vasudha) targeted in the introgression work at ICRISAT.
1.1		A set of individual stay-green QTL into 2 additional backgrounds initiated in Phase 2	Mid Year 3 (month 30)	Different genetic stocks at different stages of backcrossing are available, depending on recurrent background, up to BC4F1 in the 2 genetic backgrounds (CRS4 and RSLG262) targeted in the introgression work at IIMR.

no.	activity	outputs/ milestones	completion date	comments
1.2	Assess intermediate BC products for stay-green and traits expression	Knowledge of trait expression (both drought-related and stover quality) of most promising intermediate products of the 4 recurrent background initiated in Phase 1	End Year 2 (month 24)	Seeds being produced in BC3F2 progenies for testing during post-rainy season 2017-18.
1.2		Knowledge of trait expression (both drought-related and stover quality) of most promising intermediate products of the 2 recurrent background initiated in Phase 2	End Year 3 (month 36)	Stay-green expression confirmed in BC3F3 progenies. Seeds produced in BC3F2 progenies and tested during the post-rainy season 2016-17.
1.3	Conduct multi-location trials of most promising products	Promising stay-green QTL introgression lines identified of the 4 recurrent background initiated in Phase 1, based on field evaluation	End Year 3 (month 36)	With the one year delay in the generation of introgression lines, only a single site evaluation (ICRISAT Patancheru) has been done.

no.	activity	outputs/ milestones	completion date	comments
1.3		Promising stay-green QTL introgression lines identified of the 2 recurrent background initiated in Phase 2, based on field evaluation	End Year 4 (month 48)	Done during the 2017-18 season, being the sole purpose of the no-cost extension year. Field evaluations of 13 BC3F4 in two genetic backgrounds (8 in CRS 4 and 5 in RSLG 262) were conducted at three locations (Bijapur, Solapur and Hyderabad). Preliminary analysis of data from Solapur and Hyderabad centres indicate some promising lines over the recipient parents, but final results would be available after the pooled data analysis of three locations. The data indicated that staygreen QTLs (Stg 3A and Stg 3B) introgression is leading to a significant improvement in green leaf area retention (GLAR) at physiological maturity, gain yield (GY), stover yield (STY), water extraction/ water use (WE) and transpiration efficiency (TE) under postflowering drought stress (WS) in both the genetic backgrounds (RSLG 262 and CRS 4). Yield components improved more in CRS 4 (Gy-54%, STY-63%) than RSLG 262 genetic backgrounds (Gy-35%, STY-47) under WS. Enhancement in GLAR and yield components seems to be due to improvement in WE and TE as measured in lysimeters. Improvement in WE was more in RSLG 262 than CRS 4 and it was the opposite for TE. Lines like C3 & C7 in CRS 4 genetic background & R3 in RSLG 262 genetic background were promising and need to be evaluated on more locations for another two seasons to confirm these results.

no.	activity	outputs/ milestones	completion date	comments
1.4	Use APSIM to identify critical trait-by-management packages needed for increase yield in each sub-zone of the targeted regions	Optimum packages identified for each of the drought scenarios of the post-rainy season sorghum area	End Year 2 (month 24)	Replicated field trials with a combination of genetic and agronomic management criteria. Density × N treatment packages investigated taking into consideration productivity output together with product quality aspects. Economically significant variation in quantitative/qualitative traits as well as their interaction with stay-green x E x M interactions has been identified. These have been connected to socio-economic analysis. In addition, not initially planned, relationship have been established between grain nutritional content and stay-green QTLs.
1.4		Optimum packages identified for each of the drought scenarios of the WCA region	End Year 4 (month 48)	Parameterization is now complete for 2 adapted cultivars in WCA covering the north-south gradient of adaptation. Final parametrization will be completed once APSIM-sorghum is upgraded to the most recent and working version. Environment characterization is also almost completed and a manuscript from this work being developed.
1.5	Undertake feed trials with best entries from first phase and most-promising entries from second phase	Confirmation of the stover quality effect of certain stay-green QTL on the weight gain of sheep	End Year 2 (month 24)	These activities were initially put in the proposal but it was later on considered that the stover quality analysis should be done only on the final product of the second phase, rather than on the "proof-of-concept" products from the first one. In replacement, much work has been done on assessing the effect of GxExM combinations on stover quality but also on grain quality.
1.5		Information on milk productivity of 1-2 new promising introgression line	End Year 4 (month 48)	These activities were initially put in the proposal but it was later on considered that the stover quality analysis should be done only on the final product of the second phase, rather than on the "proof-of-concept" products from the first one. In replacement, much work has been done on assessing the effect of GxExM combinations on stover quality but also on grain quality.

no.	activity	outputs/ milestones	completion date	comments
1.6	Tracking sorghum grain and fodder value chains	Potential beneficiaries along the value chains due to new improved technology	End of year 2	Completed at the end of Year 3 (Districts in the Rabi sorghum area have been segregated into those dedicated predominantly to grain or stover production. This information has also been used to further refine the ex-ante analysis)
1.6	Revisit *ex-ante* assessment of potential benefits based on refined parameters	Refined ex-ante assessment of the technology, having scenario-based analysis of potential	End of year 3	Completed at the end of Year 3 (Ex-ante activity has been refined using APSIM modelling outputs and then shows an increase in the benefit from the stay-green technology, compared to when informant input was used). In addition, it was demonstrated that more benefit could be accrued from the new technology by developing two types of products (grain or stover type) and targeting district with likely preferred product, rather than by developing a sole dual purpose type for all districts.

PC = partner country, A = Australia

Objective 2: To Improve breeding efficiency by better targeting key traits and trait donors. Develop BCNAM populations using trait variants (for TE, water extraction capacity, altered canopy development) from the first phase in post-rainy season backgrounds with the objective of quick identification of promising entries and of mapping regions responsible for high TE (VPD responsive) trait, and link up/coordinate similar types of activities for West Africa with partners of the CRP, and with East African region through a UQ-led project funded by the BMGF and ACIAR (parameterization of key cultivars, weather scenario analysis, cross linkage on trait analysis, etc…). The modelling and environmental characterization activities in WCA will be an expansion of the BMGF-ACIAR-funded activities in ESA.

no.	activity	outputs/ milestones	completion date	comments
2.1	Develop BCNAM populations in post-rainy season lines using key trait donors from Phase 1	Ms3 versions of most popular sorghum cultivars (M35-1 and Parbhani Moti in India, CSM63-E and others for WCA and ESA) across regions are available for developing large BC1F2 populations	End Year 2 (month 24)	F1s developed by Plant x Plant crosses for Parbhani Moti were made with 20 donor Parents after confirming the true hybridity 17 crosses for Parbhani Moti were advanced during Rabi 2016-2017.
				The BC1F1-progenies were advanced by selfing during postrainy season 2017-18.

no.	activity	outputs/ milestones	completion date	comments
		BC1F3 populations available for phenotyping	End Year 3 (month 36)	Several BCNAM populations have been imported to India from Mali (in Lata3 and in CSM63E background) for phenotyping.
2.2	Assemble weather data from WCA and ESA and parameterize representative mega-cultivars of WCA region	Assemble weather datasets from WCA region	Mid Year 2 (month 18)	APSIM module capable of handling genotypes with large number of leaves developed. A number of studies focused on the WCA region have been undertaken to assist breeding program, including an environmental characterization of the stress patterns, and an analysis of most promising agronomic management alterations to improve crop productivity.
2.2		Parameterization input for several cultivars available for APSIM Drought stress scenario characterized in target region	End Year 2 (month 24)	Effect of sowing windows tested and paper being developed to report these outputs.
2.3	Mapping QTLs for key traits underlying stay-green	Phenotyping data on 1-2 BCNAM population for the transpiration response to high VPD	End Year 4 (month 48)	957 progenies of BCNAM populations in 13 populations in Lata3 background tested for the canopy development dynamics and transpiration response to increasing VPD.

PC = partner country, A = Australia

7 Key results and discussion

A breeding team

This was not an intended output of the project but this turns out to be an important output, if not the most important output. In that project a team of scientists with a varied basket of skills worked together toward the development and the economic analysis of a product that they jointly and initially designed, i.e. a sorghum crop package fitted to the post-rainy sorghum crop production environment, with grain and stover quality and quantity being the main traits needed depending on specific districts. This is, in essence, what the Excellence in Breeding (EiB) platform of the CGIAR intends to promote since 2017, with enthusiastic support from the donor community.

A SNP assay for rapid introgression of Stg3A and Stg3B

SNPs were extracted from available GBS data of lines K260 and K359W separately to cover the region 56Mbp to 72Mbp of chromosome 2 for *Stg3A* and *Stg3B* QTLs. Filtering was done depending on the annotation for not amplified, downstream, upstream, intron, intergenic region and low coverage synonymous SNPs to obtain potential SNPs. A set of 139 SNPs were designed from available GBS data of K260 and K359W separately to cover the region 56Mbp to 72Mbp of sorghum chromosome 2 (SBI-02) for *Stg3A* and *Stg3B* QTLs. A set of 133 SNPs were passed in preliminary *in-silico* evaluation and developed using the KOD (KASPar on Demand) facility from company LGC, UK. Parental polymorphisms were undertaken at ICRISAT-HQ to find polymorphic SNPs between combinations of donor and recurrent parents. A set of 69 polymorphic SNPs were short-listed. Eventually, a set of 26 SNPs (designed on KASPar platform) for *Stg3A* (covering genomic region from 56112177bp to 61953206bp) and four SNPs for *Stg3B* (covering genomic region from 69739036bp to 71360153bp) on SBI-02 are polymorphic between the recurrent parents and donors. In total, 17 populations of BC3F1:2s; 10 populations of BC1F2:3 for all 4 recurrent combinations; 3 populations of BC2F1:BC3F1 families of Parbhani Moti, and respective F1s, BC1F1s, BC2F1s and BC3F1s families of all combinations were evaluated to track QTL segregation pattern across generations. **In summary, a set of 30 SNP markers for *Stg3A* and *Stg3B* are now available and being used on a routine basis for introgressing these two critical stay-green segments into the background of post-rainy sorghum cultivars.**

Marker-assisted backcross introgression advancement of *Stg* QTLs and initial field evaluation

This has been done in a close collaboration between ICRISAT and IIMR, targeting 6 cultivated backgrounds.

At IIMR, the varietal choice for post-rainy sorghum depends on the soil depth and many farmers grow CRS4, a variety suited for medium to deep black soils with a grain yield of 2.5-3.0 t/ha and fodder yield of 5 t/ha or RSLG262, a variety suited for shallow soils with a grain yield of 1.5-1.8 t/ha and fodder yield of 4.5-5 t/ha. The stay-green trait has been introduced as 2 QTLs for *Stg3a* and *Stg3b* from the donor line B35. During the 2016 rainy season, 100 BC3F2 progenies of CRS4 and 165 BC3F2 progenies of RSLG262, generated earlier, were advanced and genotyped with 15 *Stg3a* and *Stg3b* QTL linked markers. Plants with QTL introgressions at both QTL positions (2-QTL lines) or with only 1 QTL were identified and advanced to BC3F3s. During the post-rainy season 2016-17, 79 BC3F3s of CRS4 and 46 BC3F3s of RSLG262 were planted and were advanced to BC3F4s. In a parallel genetic material advancement, 89 CRS4 and 72 RSLG262 BC2F3s were advanced to BC2F4s. Around 117 progenies (42 of CRS4 and 53 of RSLG262) of BC1F4 were grown and advanced to BC1F5s. A total of 24 BC4F2s of CRS4 and 48 of RSLG262 were advanced to BC4F3s. Selected progenies of BC3F3s (30 in CRS4 background and 20 in RSLG262 background) were evaluated during post-rainy season for various stay-green expression traits under physiology trials (see below).

At ICRISAT, the introgression in four different genetic backgrounds at ICRISAT is one backcross generation behind the introgression work at IIMR. Introgression of *Stg3A* and *Stg3B* QTL targeted popular cultivars M35-1, Parbhani Moti (= SPV1411), Phule Vasudha and CRS1. These have been advanced to BC3F1:2 (for SPV1411) and BC3F2:3 (for reminder of 3 genetic backgrounds). Plant × Plant crosses for 12 plants were made among 3 populations of Parbhani Moti to advance from BC2F1 to BC3F1. Simultaneously BC2F1 plants not used in crossing were advanced to BC2F2 by selfing.

Testing of BC3F3 progenies for stay-green - Progenies of CRS4 and RSLG262 were evaluated for stay-green expression in the IIMR fields during post-rainy season of 2016-17 under well-watered and water stress conditions. Most of the derivatives of CRS4 were found to be improved for their green leaf area retention at maturity and for the drought susceptibility index. C44, C61, C21, C58 and C13 were identified with improved green leaf area and lower drought susceptibility index (DSI) under WS. Progenies C27, C22-2 for grain yield and C58, C7, C42 and C22-2 for stover yield were identified. Similarly, backcross progenies of RSLG262 were found to exhibit improved drought tolerance as measured in terms of green leaf area, grain yield, and stover yield.

During the 2017-18 season field evaluations of 13 BC3F4 in two genetic backgrounds (8 in CRS 4 and 5 in RSLG 262) were conducted at three locations (Bijapur, Solapur and Hyderabad). The data indicated that staygreen QTLs (Stg 3A and Stg 3B) introgression led to a significant improvement in green leaf area retention (GLAR) at physiological maturity, gain yield (GY), stover yield (STY), water extraction/ water use (WE) and transpiration efficiency (TE) under postflowering drought stress (WS) in both the genetic backgrounds (RSLG 262 and CRS 4). Yield components improved more in CRS 4 (Gy-54%, STY-63%) than in RSLG 262 genetic backgrounds (Gy-35%, STY-47) under WS. Enhancement in GLAR and yield components seems to be due to improvement in WE and TE as measured in lysimeters. Improvement in WE was more in RSLG 262 than in CRS 4 and it was the opposite for TE. Lines C3 & C7 in CRS 4 genetic background & R3 in RSLG 262 genetic background were promising and will need to be evaluated on more locations for another two seasons to confirm the results.

In summary, a large number of pre-breeding materials in the backgrounds of the six most popular post-rainy sorghum cultivars have reached the stage of yield assessment in the field. This material will be the basis for the new improved cultivars containing end-user demand traits for the target regions. These initial evaluations have been very promising, in particular confirming the phenotypic expression of the stay-green trait and some productivity advantage over recurrent background cultivars. Efforts will be needed in the years to come to ensure these lines are further advanced and tested in multi-location trials.

<u>Physiological understanding of stay-green adaptation - Effect of *Stg3B* confirmed in target region</u>

Modelling stay-green and validation against series of field trials - For the purpose of modelling the “stay-green” related traits, APSIM canopy functions have been reworked to mechanically capture the canopy growth dynamics (transition from “total plant leaf area” function to “individual leaf growth” function) and “maximum transpiration rate” function has been better incorporated into the model to realistically capture the trade-off on biomass accumulation linked with this trait. The concept functions and coefficients capturing the dynamics of tillering (another important mechanism underlying stay-green expression) have been tested against observed data and are ready to be coded into the APSIM sorghum (and millet) framework by our Australian partners. The model with the till-date most advanced functions has been run against 3 years of field trials to test the effect of stay-green mechanisms (canopy growth, transpiration rate) identified in yield-superior stay-green isogenic lines (senescent S35 against stay-green IL 7001, and senescent R16 against stay-green IL K359w). The yield advantage/trade-offs of stay-green traits under WS/WW conditions were reasonably well captured by the model and helped further understanding of stay-green-×G×E interactions. These results are being prepared for publication (Fig. 3). The same functions were further run in the simulation grid and used as input for activity 1.6. – "economic modelling". **In summary, the crop simulation model APSIM has been improved to cater for three of the main features controlling plant water use, i.e. the canopy development, the capacity to restrict transpiration under high VPD, and tillering. This now allows to simulate and test fairly complex staygreen-×G×E interactions, which are critical to determine the optimum fitness of staygreen introgressions.**

Exploring linkages between stay-green and grain quality attributes

To further explore the interactions among different traits, stay-green introgression lines and mapping population parents were grown in a Lysimeter Facility (details at http://gems.icrisat.org/lysimetric-facility/) to study the agronomic, nutritional and physiological traits in details. Indeed, we found that some of the stay-green ILs performed better compared to senescent parents in terms of drought adaptive traits like transpiration efficiency but also grain quality, especially the ILs from S35 genetic background. In this study we also realized that NILs having higher rates of canopy expansion (which was studied previously) produced larger grains. Since it is known the grain quality profile could be influenced by the differences in grain size we decided to test the association between grain size and plant vigor in a suitable Recombinant Inbred Line (RIL) population. For this study we selected a RIL population developed earlier from a cross between N13 and E36-1, where the parents differed in grain size (E36-1 is another stay-green donor). This N13 x E36-1 based RIL population was also initially developed because of its segregation for stay-green and striga resistance. Furthermore, we screened these RILs for early vigor traits (at LeasyScan facility; http://gems.icrisat.org/leasyscan/) and evaluated their grain size from an independent trial. We used the QTL co-localization approach to detect any functional associations between measured traits. We found one particular position on LG4 which harboured early vigor and grain size QTLs and a similar position was reported before as stay-green 4 locus (Fig 1.5.2.1). Furthermore, another "late vigor QTL" co-localized with striga resistance scores and phenology-related traits on LG5 (fig 1.5.2.1). These results suggest that different components of plant vigor are functionally linked to i) stay-green expression, ii) grain size and iii) striga resistance. An abstract on these results was presented at the Inter-drought V conference, then rewarded as the best poster within the corresponding session, and is currently being written for publication. **In summary, a grain size (which is linked to quality) locus co-located with a vigor and *Stg4* locus, and a vigor locus co-located with a striga resistance locus.**

Refinement of the environmental characterization

Improvement of the Rabi sorghum cultivation area coverage with weather & soil data information – The environmental characterization of the first phase was done with weather information from 14 weather stations spread out across the target post-rainy sorghum region, and used generic soil information in terms of texture. Here we have developed and validated a grid of weather information. For the purpose of Rabi sorghum modelling with APSIM, the weather data generator (MARKSIM) was used to generate weather data (NASA weather information could be used as well). The simulation grid 100x100km of weather-soil-plant information has been successfully set, run and evaluated against observed sorghum production and presented at the conference (NASA-2014 "New Dimensions in Agro meteorology for Sustainable Agriculture" at G.B. Pant Agricultural University, Pant Nagar on October 16 - 18, 2014). Such a modelling grid is now being used for sensible evaluation of sorghum crop production and is used as input for socio-economic model to assess the value of stay-green technology (rather than subjective informant opinions). The data is available at www.gems.icrisat.org . **In summary, a 1º-by-1º grid (about 100x100km) of weather data has been generated for a large part of agricultural India and used to refine environmental characterization of Phase 1, providing a lot more details in patterns than were initially found in their contours. This grid allowed us to include detailed soil information and is a resource that is usable for all crops. This grid now allows us to map potential model outputs with district-level socio-economic information, enabling socio-economic analysis of the impact of the stay-green technology and to better target breeding interventions.**

Testing of GxExM packages for productivity, nutrition, return

Field studies of selected stay-green material, senescent lines, elite cultivars (10) and elite breeding material (25+25 B-&R-lines, hybrids) were conducted over several years during the post-rainy season. Trials included factorial treatments of water stress, N-application and plant population. This time-series data is being prepared for publication, along with GxExM trials from prior years. In short, there was a significant influence of GxE interactions on agronomic as well as grain quality traits. These studies also showed that stay-green Introgression Lines (ILs) had consistently enhanced grain nutrient densities compared to senescent parents along with improved agronomic traits especially under water stress environments. There was also an influence of the N treatment and of the N-by-density interaction on a number of quality attributes, allowing us to conclude that finding the best crop package for specific stress scenarios will require a careful combination of genetic and agronomic factors, using crop simulation modelling as a guide in this choice. **In summary, crop simulation modelling, validated by field observations, allowed us to test the effect of complex genetic and management combinations on the productivity and quality of both grain and stover yield. This allowed us to recommend the most promising crop/management packages, suited for each of the stress scenarios identified, and targeted for the most likely end-user demand, based on the socio-economic information.**

Development of tillering algorithm within APSIM sorghum

This year we progressed the development of the tillering algorithm which is now ready to use in further studies. One of the long pending tasks was indeed the development of a "dynamic tillering algorithm" and its incorporation into sorghum-APSIM in order to capture this essential stay-green mechanism. After a lot of efforts, the dynamic tillering algorithm has been refined, coded, tested against the data on Australian sorghum cultivar (Buster) and Indian cultivar (Maldandi; M35-1; example Fig. 1.4.1.1) and the prototype of APSIM is now available. Here it is important to understand that this algorithm is very unique and based on the continuous data and knowledge accumulation since the 1980s (from our Australian collaborators). This dynamic tillering algorithm is the first of its kind ever developed and its use may be a break-through in the mechanistic modelling of all tillering crops. To inform the broader community, the article describing the same is being prepared by Prof. GL. Hammer. **In summary, a dynamic tillering module has been developed for the crop simulation model APSIM, allowing the simulation of sorghum genotypes that have a) different propensities to tiller, and b) an accurate simulation of the leaf canopy development, a critical factor conditioning plant water use and eventually water availability during grain filling.**

Modelling the effect of N-fertilization on Rabi sorghum production in India and use of modelling platform to support local breeding program.

We used the modelling platform to evaluate the effect of particular crop management practices across the sorghum Rabi tract and disseminated the generated resources to assure their further use in sorghum breeding programs. We decided to test the resources developed within this activity in collaboration with IIMR which is also involved in farmer's advisory services. It appeared that the common advice to Rabi sorghum farmers frequently includes the improvement of N fertilization to the crop, hence we decided to simulate the effect of an unlimited dose of N application on stover and grain production across the Rabi sorghum belt in India using the existing modelling framework. Unlimited N-fertilization practice is also frequently used in breeding on-station trials. In our simulation exercise we compared the recommended N practice (20+20 kg/ha N) with on-station common practices (50+100 kg/ha DAP+N). Our simulations predicted that high N fertilization would result in higher frequencies of grain yield loss while marginally improving the stover production across the majority of the Rabi sorghum belt. Indeed, well-fertilized crops led to a larger canopy establishment earlier in the season, resulting in a higher transpiration demand by the crop, leading to earlier water depletion from the soil. Therefore, on-station crops faced water stress earlier in the season with less moisture available to facilitate grain-filling processes. This modelling exercise demonstrated that the high N dosages would likely result in yield penalties across the majority of post-rainy sorghum production areas. This also means that on-station experimental set-ups, including high N fertilization, have little relevance to developing elite post-rainy material for India [detailed report at http://www.icrisat.org/simulating-postrainy-sorghum-yield-response-to-on-station-n-management-in-india/].

In summary, the results of the activity above justified the necessity to integrate crop modelling into breeding programs as a decision-making support to design relevant crop improvement strategies for complex cropping systems. These results included the training of a young scientist from IIMR, who works also in collaboration with the breeders at her institute and this training was quite successful in terms of output and interest. Therefore, our further plans include refining the existing modelling platform and its dissemination to national breeding programs. We intend to continue supporting the capacity building of national programs beyond the duration of this project and apply for additional support to enable broader and practical use of project outcomes.

Ex-ante assessment and refinement

In the Phase-1 of the project, an ex-ante analysis was carried out to determine the potential benefits of the stay-green technology and to quantify the welfare benefits and returns to investment for the technology. This analysis showed a very high return on investment of the Staygreen technology. The analysis was carried out in a broad manner without taking in account the specificities and heterogeneity of the nature and characteristics of the population who is adopting the technology, and the diversity of agricultural production conditions in the potential areas of technology adoption. In this analysis we made two major assumptions: 1) **homogeneity of the livelihood of population and production conditions** adopting the stay-green technology, on which the technology will have an impact, and 2) **same technology is transferred to all the population**, irrespective of their heterogeneity in livelihoods and production conditions on which the technology will have an impact. However, in reality a) the livelihood of the population and the production conditions on which the stay-green technology will have an impact are heterogeneous, and b) there can be more than one type of stay-green (SG) technology to suit the above-mentioned heterogeneities.

In the Phase-2 of the project, we tried to address these issues in the ex-ante analysis by incorporating two elements of specificity at a further disaggregated level than in the earlier Phase-1 analysis. The first specificity was with respect to the composition of the source of livelihood of the population who adopts this technology. In the Rabi-sorghum production region, where the stay-green technology can potentially make an impact on the livelihoods of the population, livestock is an important source of livelihood and is critical for managing risks and uncertainties in agricultural production. The income from livestock forms a major share of the household income and acts as a cushion absorbing risks and uncertainties from volatility of agricultural incomes. Hence, based on the importance of livestock, we have used the per capita livestock population to disaggregate the region into two: a) those having predominantly livestock-based production systems for their livelihood (they will prefer a technology providing more stover than grain) and, b) those being less reliant on livestock-based production systems for their livelihood (they will prefer a technology providing more grain than stover). Households in each region can adopt possible stay-green technologies (varieties) options, depending on their priority, and essentially varying for three factors: dependency on livestock, the ratio grain / stover of the proposed varieties, and the severity of the stress that they encounter.

The second specificity was with respect to the nature of the technology. In the region where there is predominance of livestock (measured by livestock population per capita) there will be a preference towards a technology (SG1) having higher stover yields than a technology with higher grain yield. In other regions, the population will have a preference for a technology that has higher grain yield (SG2). Hence it made sense to have two different technologies, one being a “stover type” with high stover yield and another one being a “grain type” with high grain yield. Our hypothesis was that generation and adoption of specific technologies (SG1 and SG2) by target population disaggregated by their preferred livelihood option will vary, and the aggregated returns and welfare benefits will give higher returns to the technology and create higher welfare benefits in comparison with adoption of a single technology (SG) for the entire the population. In the ex-ante analysis for Phase-2, we have incorporated these two specificities in the analysis and has analysed the potential and net benefits from SG technology in three scenarios. They are the following:

Scenario 1: Ex-ante analysis with a single SG technology (with a Grain: Stover ratio of 1:2) across all regions and population using actual data on area and production.

Scenario 2: Ex-ante analysis with multiple SG technologies (SG1 with a Grain: Stover ratio of 1:2.5 for region with high livestock intensity and SG2 with a Grain: Stover ratio of 1.5:2 for region with low livestock intensity) across two different regions (based on intensity of livestock population) and using actual data on area and production.

Scenario 3: Ex-ante analysis using APSIM simulation outputs on grain and stover productivity increases from stay-green technology (a result of Activity 1.4). Based on our knowledge of stay-green technology, we designed five virtual stay-green genotypes using APSIM: Base (maldandi);

Bad1 (grain crop type for severe water stress)

Bad2 (stover crop type for severe water stress)

Good1(grain crop type for mild water stress)

Good2 (stover crop type for mild water stress).

Cash flows under each scenario have been generated and can be consulted in the yearly reports. In the first two scenarios we have carried out the ex-ante evaluation of the impact of research investment in stay-green post-rainy season sorghum. A summary table presenting the major results from ex-ante analysis of different scenarios of SG technology is given in Table 1. The results below confirm the hypothesis that when targeting specific segments of the sorghum value chain (grain or stover), the net production value of a single technology was less than the sum of the cumulated values of specific technologies (Table 1).

Table 1. Summary Of Results From Ex-ante analysis of Returns to Investment for Stay-green Sorghum Technology, *by case, by scenario*					
Scenario	**Case**	**Description of scenario**	**IRR (%)**	**NPV (Million Rs)**	**BC Ratio**
Scenario 1	One case only	Single SG technology (with a Grain: Stover ratio of 1:2) across all regions and population using actual data on area and production	46	1766	31.74
Scenario 2	Case 1	SG1 with a Grain: Stover ratio of 1:2.5 for region with high livestock intensity using actual data on area and production.	34	588	11.24
	Case 2	SG2 with a Grain: Stover ratio of 1.5:2 for region with low livestock intensity using actual data on area and production	43	1397	25.32

In summary, the ex-ante analysis from the first phase, where input data on productivity improvement by the stay-green technology was sole informant opinion, was refined by using crop simulation outputs as input to the ex-ante analysis. In addition, and thanks to the weather data grid that was developed, this analysis took into account the livestock population in the different districts (as a proxy for preference toward a "grain" or a "stover" plant type). Results confirmed the positive return on investment of the stay-green technology, and showed that developing two stay-green types, based on end-user demand, would increase the return on investment compared to developing only one technology (sum of ROI for Case 1 and Case 2 for Scenario 2 being superior to ROI of Scenario 1).

Effect of stay-green QTLs on stover quality and grain nutritional quality

The choice has been made not to invest in the feeding trials that were initially slated in the proposal, since there is already well-established evidence that the enhanced stover in-vitro digestibility, as found in some stay-green ILs (Blummel et al 2015), correlates well with either animal weight gain or milk production (Blummel et al 2006). Instead, the efforts have been re-directed to understand whether the grain nutritional quality is also affected by stay-green traits (water-use related mechanisms), what are the exact mechanisms influencing grain quality, and how the altered grain quality affects the final sorghum products acceptability.

Sorghum stay-green mechanisms haven't been investigated in relation to the grain nutritional value before. Our initial results indicated the grain nutrition profile (e.g. protein, lipids and starch) was affected by the stay-green introgression in several of the stay-green ILs. We progressed with development of necessary techniques and understanding of how some water-saving processes affect nutritional characters (e.g. link between tryptophan content in grain and tillering propensity; relation of protein content in grains to photosynthetic capacity (abstract submitted for International Agrobiodiversity Congress, India). A field trial has been dedicated to test new protocols to better capture dynamics of these relations. Within this activity, we also initiated protocol development for evaluation of stay-green sorghum grain products acceptability by consumers (http://www.icrisat.org/sinking-your-teeth-into-sorghum-rotis/). This activity on nutrition was not planned in the original project proposal and provides useful results to increase the potential impact of the project's main output: the improved sorghum varieties. Improved sorghum production (both, quantity in conjunction with quality) may further increase the profitability of sorghum cropping systems in India. Progress has also been made toward the development of Near Infra-Red spectroscopy calibrations to enable rapid analysis of the main grain macronutrients, to analyse GxExM effects on macronutrients and continue the exploration of the physiological basis for enhanced nutritional density of stay-green material. Reliable calibrations for sorghum grain protein and fat contents were developed and validated.

In summary, the analysis of the nutritional profile of the stay-green product showed that, beside the effect of stay-green introgressions on the stover quality found in Phase 1, there was a clear effect of these introgressions on the grain nutritional status. In addition, it was also very clear that these effects depended on complex interactions between genetics, management, and water stress aspects. Being a critical criteria for end-users, these results clearly indicate that early testing of grain quality aspects will be needed during the breeding of the stay-green technology.

Development of BCNAM populations

This second objective of the project was initially supported by the CRP-Dryland Cereals and because of severe budget cuts in the CRP-Dryland Cereals, advancement of crosses performed has been very limited. Yet, out of 20 crosses involving drought tolerance donors, screened in Phase 1 for their high transpiration efficiency (TE) and high capacity for water extraction, and crossed with Parbhani Moti, a total of 17 crosses were advanced to produce BC1F1s during Kharif 2016-2017 by confirming the true hybridity. The donor accessions involved IS3971, IS929, IS1127, IS2367, IS5720, IS8348, IS10876, IS10978, IS14556, IS16044, IS16173, IS20709, IS23988, IS31693, IS3147, IS15428, and IS20387.

The BCNAM development efforts using these donors paralleled similar efforts in the West-Central Africa region. In that scope, the assessment of the transpiration response to increasing VPD conditions in a small set of BCNAM population parents from WCA revealed a promising contrast between some of them. For instance Grinkan was insensitive to increasing VPD, whereas B35 and Lata3 were sensitive giving at least 2 BCNAM populations in which parental lines do contrast for that response (Grinkan x Lata3 and Grinkan x B35). As a consequence, a larger set of BCNAM population parents was tested for the transpiration response to increasing VPD to try to broaden the range of variation between parental lines. From this work, a number of existing BCNAM populations were identified with parental contrast for the transpiration response to increase in VPD (a proxy for a higher TE) and these have started to be used to map genomic regions underlying a better TE.

In summary, the development of BCNAM has been recognized as an efficient way to introgress important trait characteristics in popular cultivated background, therefore giving the dual advantage of generating genetic stock for a precise and efficient mapping of genomic regions involved in traits of interest, while being also a source of pre-breeding material (the crossing with trait donors being in the background of farmer-preferred materials). BCNAM using post-rainy sorghum cultivars has started and similar work is in a more advanced state (in WCA) in the scope of other projects.

Development of specific algorithms for the APSIM crop simulation model to capture the long-cycle genotypes

These activities were part of Objective 2, dealing with an expansion of the modelling work to the WCA region, using the approach and method used in Phase 1.

CSM63E and CSM335 are popular cultivars in two agro-ecological regions of WCA. These have been successfully parameterized and reported. The long life cycle crop type (represented by IS15401) has not been completed yet because the APSIM-sorghum algorithms couldn't reflect the crops with more than 26 leaves (Leaf No > 26). Therefore, new algorithms were developed and coded into APSIM v.7.7. These codes were tested using other long-cycle genotypes (Souroukoucou and CSM388; with the detailed data provided by Dr. M Vaksmann, our colleague from Institut d'Economie Rurale, Mali). CSM388 (120-140 days) and Souroukoucou (150-210 days) are both from Mali, and are Guinea-gambicum and Caudatum respectively. The observed leaf numbers (green point) were quite close to the predicted leaf numbers. The observed actual green-leaf area index derived from the leaf numbers could not be represented properly as data on senesced leaves were not available. Overall, we have a new basic algorithm to capture the long-cycle plant type, although a rigorous fine-tuning and validation are still required, for which more detailed data needs to be collected.

Simulations have been used to evaluate the drought stress trajectories occurring at specific locations across Mali for particular plant types (represented by CSM335 and CSM65E). This was done using daily APSIM outputs of water supply and demand ratio (drought index) ± 400°C.day around flowering and averaged every 100°C.day. These seasonal trajectories were analysed using PCA followed by cluster analysis. Four water-limited environment types were identified by cluster analysis of water stress index trajectories for both plant types across the sorghum production belt of Mali (Figure below; http://www.icrisat.org/identifying-climate-smart-sorghum-lines-for-mali/).

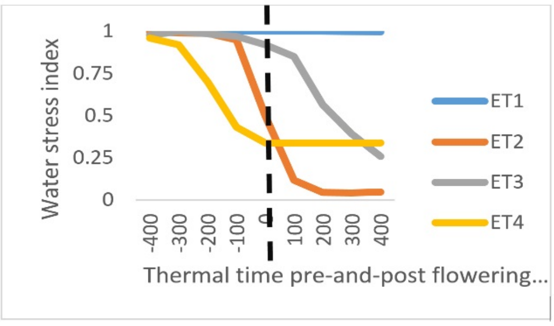

Figure. Four environment types were identified by cluster analysis of simulated water stress index across the sorghum production belt of Mali for the genotype CSM335. This daily index corresponding to the ratio between water supply and demand, was centred around flowering (represented by the vertical line) and averaged every 100°Cd from emergence to 400°Cd after flowering. A ratio of 1 indicates no water stress, while a ratio of 0 corresponds to a full stress.

To express the probability of drought stress on sorghum across Mali, the APSIM outputs had to be further weighted by the data availability within particular isohyates. This work is in progress to analyse the occurrence of particular drought stress types typical for each of the genotypes within the isohyate and its effect on plant production. The outputs are being worked into the form of publication. Simultaneously, MARKSIM (weather generator) grid covering Sahelian region (Senegal, Mali, Burkina Faso) is being evaluated against the wider set of observed weather information. The main purpose of this exercise is to saturate the Sahelian region with high density or reliable weather information and consequently also saturate the modelling grid. The simulations across 22 sites covering Mali sorghum production belt have been set (using the database resources developed in previous years) and evaluated against available sorghum production records.

In summary, the parameterization of popular cultivars for the different agro-ecological zones of Mali has been undertaken. This has allowed the characterization of the stress pattern in this region, highlighting four types of stress. These simulations offer a benchmark that will be used to better target the cultivars and the trait and management characteristics that are needed in each of these scenarios to optimize economic returns and reduce risks. Assessment of the effect of management options (sowing date, N fertilization, density) have been tested across the range of sites.

8 Impacts

8.1 Scientific impacts – now and in 5 years

The development of stay-green introgression lines in different backgrounds is on-going and will allow a thorough assessment of the background effects that have been reported earlier (Vadez et al., 2011 – Funct. Plant Bio.). This will also allow us to proof-test the stay-green QTL in a real-case situation, with improved lines in the background of Rabi-adapted and farmer-preferred cultivars. Thinking retrospectively, this project has applied some of the principles of the Excellence in Breeding platform (years before that platform was initiated) by having a proper definition of the breeding product profile (especially combining traits demanded by end-users such as the stover and grain quality), the suite of molecular tools for rapid introgression, trait-specific phenotyping for key traits (stay-green and underlying mechanisms), and more than anything by having assembled a breeding team encompassing a wide set of skill toward a commonly-designed and user-drive product.

The development of the BCNAM population is a longer term effort with potentially additional benefits from the donor parents because, while we expect these donors to confer some of the traits explaining the expression of a stay-green phenotype, there are other unknown traits that may also come up from these populations. In addition, we have here in the making a large set of breeding stocks, being developed in the scope of several projects and in the background of cultivars adapted to different regions (India, West Africa, East Africa) with a set of common donors, creating the basis for large scale genetic analysis.

The use and combination of a modelling approach into the breeding and agronomic management effort is also forward-looking. Here, the dogma of breeding for broad adaptation across large regions is getting dented by the demonstration from the modelling output that specific stress scenarios within a target region requires specific and custom-designed solutions to optimize return and reduce risk. We are currently testing the combination of genetics and agronomic management (plant density, N fertilization) on productivity and residue quality, where we also found effects on the grain nutritional quality. This opens a new avenue that shows a close connection between these domains.

In addition, we are expanding this work to the West Africa region, following up on the case developed for the Rabi region in India. Here we made progress in the prediction of phenological stages of photoperiod sensitive cultivars. We have made progress in the characterization of the stress scenarios in Mali and, in conjunction with another project (USAID Innovation Lab on Climate Resilient Sorghum), we are moving towards a regional characterization of stress patterns.

We have also made amazing progress in the understanding of traits underlying the expression of the stay-green phenotype (essentially revolving around the control of transpiration under high vapor pressure deficit (VPD) and around the dynamics of the canopy development). We have developed high throughput methods to measure these traits at a high rate and are now gearing up to use these methods (see on-going progress with the LeasyScan platform at www.gems.icrisat.org) to assess BCNAM populations segregating for these traits, toward the identification of genomic regions involved in these critical traits.

Last, but not least, this proposal is about a close integration of different disciplines in the biological sciences, which also extends to the socio-economic dimension of the benefit of technology (see the ex-ante analysis generated during Phase I). This is rather rare and a unique blend, which we think is in the spirit of the modernization of breeding programs currently taking place in the CG system, with breeding becoming more a transparent process with input from different disciplines at different stages of implementation. We believe that improving breeding efficiency is indeed about picking the best of different disciplines. Here it is essential to acknowledge that the stress scenarios differ across time and geographical scales and that adaptive traits can't be the same everywhere, as we see in our data. What is proposed here links up a) the need to understand the mechanisms of adaptation to the most relevant stress type, b) the use of modelling as a tool to better understand the complexity of biological systems, and c) the use of genetics to speed up the selection process once intelligent selection procedures have been designed. The output of that work consists in improved cultivars whose socio-economic benefit can be assessed.

8.2 Capacity impacts – now and in 5 years

Capacity enhancement is taking place currently in India where senior molecular breeder R Madhusudhana, working in close collaboration with S Deshpande, are training younger molecular breeders on the introgression work in relation to the first objective. Another young scientist from IIMR is also being trained in crop simulation modelling with Dr Jana Kholova. Capacity enhancement is also on-going in sub-Saharan Africa, currently with the training of PhD student M Diancoumba on modelling and on the physiology and genetics of stay-green. A number of short term students (MSc thesis) were also engaged in this project over time.

We think the capacity impacts will not be so much for the number of scientists, young or confirmed, having been trained through this project, but rather our own learning, as members of this project, of being part of a multi-disciplinary effort toward a commonly-designed product.

8.3 Community impacts – now and in 5 years

While the project is too upstream to have impact at the level of farming communities, it has had an impact on a community of scientists involved in research around the sorghum value chain. As described earlier, the impact has been in having different disciplines, from different institutions, coming together to define a strategy to improve segments of this value chain, and in so doing, developing a language to better understand each other. Living examples of this on-going process are the fact that the revised ex-ante analysis took into consideration the segregation of production into grain and stover, and that crop simulation took outputs as input to the economic analysis.

In time, perhaps a 5-20 year timescale, drought-adapted sorghum lines developed in this project should impact farming communities. In collaboration with IIMR and regional universities, a pipeline is being developed for this drought-adapted material to ultimately be available to smallholder sorghum farmers in at least four states of India (Andhra Pradesh, Telangana, Maharashtra and Karnataka). Potential beneficiaries of stay-green technologies for enhanced grain and stover products are smallholder farmers, grain and fodder traders, the food industry (bread & biscuit manufacturers) and the peri-urban dairy industry. Streamlining the efficiency and effectiveness of rolling out these stay-green technologies will be critical to the rate of adoption. Removing constraints to this roll-out should be a critical follow-up activity of this project.

8.3.1 Economic impacts

No economic impact yet but promising ex-ante analysis using refined input to the economic analysis.

Although the products from that project are not ready yet and would still need to be released, the proof-of-concept of the value of the technology is now well established. A refinement of the ex-ante assessment made in the Phase 1, using now APSIM modelling output, more precise than the informant's own perceptions, clearly shows that large potential economic benefit of the technology and a high return to investment of the technology, especially after segregating the benefits into either a benefit for grain or for stover productivity.

8.3.2 Social impacts

The eventual benefits would target the approximate 5 million households that depend on post-rainy sorghum production. While it is unlikely that these benefits will flow-through to smallholder farmers in the next 5 years, it is likely that specific products (grain and/or stover) will have benefits in specific target environments in the medium to long term (10-30 years).

8.3.3 Environmental impacts

No detectable environmental impact although an increase in the agricultural productivity of cattle feed would normally reduce the pressure on common grazing grounds and as such be beneficial for the natural environment. Stay-green technologies should enable scarce water resources to be used more efficiently by improving the balance between water supply and demand, resulting in increased water availability during grain filling.

8.4 Communication and dissemination activities

A number of publications have come out of this work (see the list below). Several workshops and meetings have taken stock of the activities, enabling exchange between partners for possible re-orientation of the activities. These were usually annual meetings, held during the post-rainy sorghum season. Some of these meetings have also been enlarged to include participants of other sorghum project as a means to provide feedback and cross-pollinate activities from these other projects. A special session was held at the 2018 Sorghum in the 21st Century Conference in Cape Town, South Africa, to feedback the project's outputs to the wider sorghum community and have exchange with other projects of the same nature.

9 Conclusions and recommendations

9.1 Conclusions

This project has achieved a large number of outputs at different levels in the research-development continuum. This is, in a large part, the fruit of multi-disciplinary and interconnected efforts, focused on a product that was commonly designed. There are now SNP markers that can be routinely used for the introgression of key stay-green QTL, i.e. for *Stg3A* and *Stg3B*. A large number of pre-breeding lines generated through routine introgression have reached the yield evaluation stage and have shown great promise. An important insight gained in the first phase is that introgression of a QTL would not have the same effect in all genetic backgrounds, implying that intermediate phenotypic evaluation remains very important. A key element of this project has been the tight involvement of crop simulation modelling to better target breeding and agronomic efforts. Several stress patterns have been identified and have shown that breeding targets would be different in each of these scenarios – this is a paradigm shift in how one conceives breeding. The traits needed in each of these scenarios to achieve productivity gains have been identified. For example, the overall importance of breeding for lines capable of limiting transpiration under the high evaporative demand conditions of the post-rainy season was demonstrated. Simulation has also shown the importance of trade-off effects between traits having antagonistic effects on different parts of the sorghum value chain. Another critical aspect of this project has been to embed, early on, a careful socio-economic ex-ante analysis of the stay-green technology. This analysis has shown the benefit of the technology but has pointed to the importance of designing final breeding products according to the end-user preferences (in our case being either a grain or a fodder ideotype). This phase of the project has also opened, together with crop simulation, the domain of grain quality. We have shown that certain grain quality indicators were strongly influenced by stay-green QTLs and that these QTLs interacted with either environmental (water regime) and/or management (density, N) factors. All in all, addressing the problem stated in the title of this proposal from a value chain angle was hugely rewarding in helping to define disaggregated targets.

9.2 Recommendations

The inner perception of the project's participants is that the project has been highly successful, both in terms of scientific production and of applicable output. This would not have been possible without the long-term support from ACIAR. Therefore, our first recommendation to the donor agency would be the importance of securing long-term funding as much as possible and against mid-term deliverables.

One key ingredient of the project has been the close interaction between disciplines and the fact that the final product toward which the efforts were aiming had been defined commonly. This helped keeping a common perspective to the final target while delving into disciplinary specific domains. We believe the collective outputs of the project, set in the scope of a multi-disciplinary breeding team, would be worth a peer-reviewed publication to document the process and output flows.

The ex-ante analysis has been a key element in the cross-disciplinary effort because it has forced each of the members to think outside his/her comfort zone, and to think of what impact the technology could have. This has made us realize that even small improvements would be worth the investment. This, in turn, has given us the necessary confidence to progress with these long-term efforts. Therefore, embedding an ex-ante analysis in otherwise highly-technically oriented project looks like a safe way to stay "grounded".

Finally, we believe a key follow-up to this project is to monitor the pipelines that will deliver grain and fodder products to smallholder sorghum farmers in India's Rabi environment. This could involve some strategic input by project staff (IIMR, ICRISAT & UQ), particularly over the next 5 years, to increase the likelihood of adoption of drought-adapted material. For example, Front Line Demonstrations (FLDs) on farmer's fields, to be organized by IIMR, will be an important step in making the new drought adapted lines available to farmers.

10 References

10.1 References cited in report

10.2 List of publications produced by project

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