



Josef G. Knoll-Europäischer Wissenschaftspreisträger 2016

Josef G. Knoll-European-Science Award Winner 2016

Willmar Leiser “Sorghum Breeding Strategies for Phosphorus-Limited Environments in Western Africa: From Field to Genome Level”, University of Hohenheim, 2014

Summary

A growing world population juxtaposed with dwindling phosphorus resources present new challenges to current and future agricultural production. The burden of depleting phosphorus resources is particularly felt in sub-Saharan Africa (SSA). The expected doubling of the sub-Saharan African population by 2050 and the widespread poor soil fertility will pose an enormous task to future food security in SSA. Plant breeding can be considered as one major factor to improve agricultural production under these harsh low-input conditions. Nevertheless, until recently there have been no thorough breeding efforts to enhance crop production for low-phosphorus soil conditions in SSA.

Phosphorus (P) is a key component of DNA, cell membranes and cellular energy, hence vital to every form of life. There is no substitute for P in food production and it is considered as the possibly most limiting mineral nutrient for plants across all arable land. Currently, 90% of all mined rock phosphate is used in food production and its worldwide use is constantly increasing due to a higher demand in food, feed and fuel production. Worldwide P reserves will be exhausted in 40-400 years, depending on the source of information and the estimated worldwide demand for this scarce mineral. Already in 2008 P fertilizer prices were skyrocketing and are since then on a level at least 2.6 times higher than in 2006. While farmers worldwide are economically affected by increasing fertilizer prices, smallholder farmers in SSA are hardest struck by such strong increases. In SSA fertilizer prices are often relatively higher than in other developing countries, mostly due to lacking infrastructure and inefficient supply chains. Already nowadays, fertilizer application rates are very low in SSA with levels mostly below 5 kg P ha⁻¹, thus leading in some regions e.g. West Africa to P deficits of the agricultural production system. Furthermore, most of the SSA soils are highly weathered low pH soils with a high P retention level, thus fixing most (70-90%) of the applied P as plant unavailable phosphate. Therefore, there is a strong need for developing plant varieties that are more P efficient -that is, crops that produce more with less external input.

Sorghum (*Sorghum bicolor* L. Moench) is the world's fifth and Africa's second most grown cereal crop. Sorghum is a staple crop of SSA and is mostly grown in resource poor regions under low-input cropping conditions, with the largest share in West Africa (WA). Farmers in WA mostly cultivate sorghum in less fertile fields, knowing that sorghum can more dependably produce grain than can maize under such conditions. Nevertheless, limited soil P availability is a serious and frequent constraint to sorghum growth and productivity across the range of environments in WA. Although sorghum has a grain yield potential of several tons per hectare in WA, average grain yields are only about 1 t ha⁻¹ since 1960, due in part to low

soil fertility and low-input production systems. WA sorghum is known to experience P stress below a threshold of 7-10 ppm plant available soil P (Bray-1P) content. Most of smallholder farmers' fields and especially women fields show P levels below this threshold, therefore sorghum productivity is directly impeded by these low-P soil conditions. Increasing sorghum productivity by applying mineral P fertilizer is currently no viable option for most of the smallholder farmers in WA. The lack of financial resources, high prices, risk aversion and inadequate rural infrastructure hinder many WA farmers' use of fertilizers, resulting in average annual fertilizer application rate below 5 kg P ha⁻¹. Plant breeding is therefore a major tool to overcome this hurdle of sorghum production under smallholder farmers' conditions. Until recently there were no broader sorghum breeding efforts to directly address these low-input production conditions. Therefore, there is a strong need to develop sorghum varieties, which are better adapted to these predominant low-P cropping systems, hence serving millions of smallholder farmers and helping to assure food security in WA.

Although the issue of low-input cropping systems in WA has been known for decades, plant breeders in WA, but also in many other parts of the world, try to avoid genotype selection under low-input (e.g. low-P) conditions. This is mostly due to the fact, that response to selection under low-input conditions is often considered as less efficient based on an expected lower heritability, due to an expected higher experimental error and a lower genetic variation. The former is mostly due to higher spatial variation of environmental factors e.g. soil fertility, which cannot be compensated by external inputs in low-input systems, and small fertility differences can have very large effects on plant growth especially under low-input conditions thus genotypic variation and selection can be biased by environmental factors leading to a higher unexplained residual variance. Therefore, environmental effects need to be controlled by design and analysis for effective genotypic selection. Different field designs and corresponding analyses have been created in the last century, mostly controlling environmental heterogeneity by blocking structures and replications. These techniques have their limitations if spatial variation cannot be captured by the applied design. Various spatial adjustment techniques have been developed (e.g. autoregressive models) and have been shown to significantly reduce residual error, hence increase heritability and therefore make selection more efficient especially in abiotic stress environments. At the beginning of this study, there was no knowledge if these methods were advantageous specifically under low-input conditions, which impact they have on genotypic selection and how they can be best employed in a breeding program targeting low-input conditions.

Plants evolved two basic adaptation strategies for soils with low plant available P levels: higher P acquisition efficiency from soils and improved internal physiological P use efficiency. A higher P acquisition can be achieved by root exudates (e.g. organic anions, phosphatases), greater root biomass, changes in root architecture (e.g. root angle, root hair, aerenchyma, finer roots) and by symbioses with mycorrhiza. A higher internal P use efficiency (e.g. more plant biomass with less P uptake) is characterized by reduced growth rate, better internal translocation, alternative respiration pathways and modified carbon cycles. Both adaptation strategies show a large genotypic variation in several crops and particularly in rice several quantitative trait loci and genes have been identified to be involved in P-use efficiency. Up to this study, there was no knowledge on the genetic diversity of sorghum for P uptake and P use efficiency, and the underlying mechanisms and genomic regions which are responsible for overall improved P-use efficiency.

This study presents an unprecedented largescale multi-environment experiment, including 29 good-quality field trials, from 2006-2012 in three WA countries, namely Mali, Senegal and

Niger. In these field trials 187 WA sorghum genotypes were evaluated for their performance under P-sufficient and P-deficient conditions. Additionally, several pot and field trials under P-limited conditions were conducted using the entire set or a subset of the genotypes to investigate P-uptake and internal P-use efficiency mechanisms. The DNA of the 187 genotypes was genotyped using a genotyping-by-sequencing approach yielding 220 934 SNPs, which were evenly distributed across the entire genome. Furthermore, gene specific molecular markers were developed for the Aluminum-tolerance gene *SbMATE* and for homologs of the major rice P-efficiency gene *PSTOLI*. All 187 genotypes were evaluated for these *SbMATE* and *PSTOLI* specific markers. Both, the genome wide and the gene specific markers were subjected to a genetic association study, and the genome wide markers were also used to evaluate the potential of genomic selection for P-efficiency traits.

The overall main goal of this study was to establish a breeding strategy for sorghum targeting P-limited environments. In order to establish such a strategy, the following objectives were defined: (I) to evaluate the impact of statistical spatial models on genotypic selection in low-input field trials, (II) to develop a selection strategy for sorghum targeting P-limited environments, based on quantitative genetic parameters and (III) to identify genomic regions influencing sorghum performance in P-limited environments using modern genomic tools.

The major findings of this study can be summarized as following:

Spatial models can increase the precision and efficiency especially of low-input field trials and may lead to different genotype rankings. Hence spatial models and/or adequate field designs are necessary tools for efficient genotype selection under low-input conditions and must be considered in a breeding program targeting P-limited conditions.

Sorghum performance is severely impeded by low-P soil conditions and shows large grain yield and plant height reductions and delayed flowering. Nevertheless, WA sorghum is generally well adapted to low-P soil conditions and shows a large exploitable genetic variation for P efficiency. Direct selection under low-P conditions is feasible, necessary and more efficient than indirect selection under high-P conditions and should be pursued in a breeding program targeting P-limited environments. Landrace genotypes are more specifically adapted to low-P conditions and show a higher P acquisition capacity, Durra and Guinea race sorghums show a similar specific low-P adaptation, hence these genotype groups are very promising source germplasm for further breeding efforts. Photoperiod sensitive genotypes show less delay in heading, a higher P acquisition rate and a specific low-P adaptation, hence should be considered for climate and low-P resilience breeding. Selection for low P concentration of grain can be used to enhance internal P use efficiency, therefore decreasing further soil P mining. WA sorghum shows a large genetic diversity, hence providing a valuable source for genetic studies examining the underlying genetics of low-P adaptation.

There are many genomic regions involved in sorghum adaptation to low-P soil conditions. Nevertheless, some regions could be identified as major contributors, showing large effects on and strong associations to genotypic performance. Molecular markers in sorghum homologs of the major P efficiency gene *PSTOLI* from rice stably enhanced P-uptake and crop performance through an increased root growth of sorghum under low-P soil conditions and can be used in marker assisted selection for grain yield production under P-limited conditions. Furthermore, it was observed that grain yield production under P-limited conditions and Al-tolerance are pleiotropically regulated by the same genomic region and most probably the same gene *SbMATE*. Molecular markers of this region and within the gene *SbMATE* should

be used for marker assisted selection to simultaneously enhance the tolerance to two of the most serious abiotic stresses for sorghum in WA, Al toxicity and P deficiency. WA Guinea race sorghums are an excellent source not only for low-P specific alleles, but also for Al-tolerance and represent therefore an excellent source germplasm for allele mining and marker assisted selection. Genomic selection appears to be a very promising approach to further increase the response to selection. But methods giving more weight to single molecular markers should be considered.

The laid out results show that breeding sorghum specifically targeting P-limited conditions is necessary and feasible using advanced statistical models and modern genetic tools, and should be pursued as a major selection criteria in WA sorghum breeding programs. Nevertheless, only by combining agronomic and socio-economic measures with plant breeding efforts, millions of WA smallholder farmers can be reached and major yield increases can be expected in the near future.

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