

REVIEW

Alternate wetting and drying irrigation for rice in Bangladesh: Is it sustainable and has plant breeding something to offer?

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Abstract

The crop management practice of alternate wetting and drying (AWD) is being promoted by IRRI and the national research and extension program in Bangladesh and other parts of the world as a water-saving irrigation practice that reduces the environmental impact of dry season rice production through decreased water usage, and potentially increases yield. Evidence is growing that AWD will dramatically reduce the concentration of arsenic in harvested rice grains conferring a third major advantage over permanently flooded dry season rice production. AWD may also increase the concentration of essential dietary micronutrients in the grain. However, three crucial aspects of AWD irrigation require further investigation. First, why is yield generally altered in AWD? Second, is AWD sustainable economically (viability of farmers' livelihoods) and environmentally (aquifer water table heights) over long-term use? Third, are current cultivars optimized for this irrigation system? This paper describes a multidisciplinary research project that could be conceived which would answer these questions by combining advanced soil biogeochemistry with crop physiology, genomics, and systems biology. The description attempts to show how the breakthroughs in next generation sequencing could be exploited to better utilize local collections of germplasm and identify the molecular mechanisms underlying biological adaptation to the environment within the context of soil chemistry and plant physiology.

Water Use and Rice

Irrigated rice production requires large amounts of water, with 1 kg of rice grain requiring 2500 L of water (Bouman 2009). One third of the World's developed freshwater is used to irrigate rice (Bouman 2009) with

this figure being half of all freshwater supplies for Asia (Kukul 2004). While transpiration of rice on a global scale equates to that of wheat or barley (500–1000 L of water to produce 1 kg of grain), rice loses a lot more water than aerobic crops through evapotranspiration and soil percolation due to paddy cultivation practices (Bouman

2009). Given that rice is a dietary staple for half the world with annual production of 463 mt in 2011 (IRRI 2012), then 1.2×10^{15} L of water is required for rice production globally. An increasing proportion of the water used in rice cultivation comes from unsustainable groundwater sources as the practice of irrigated dry season paddy rice cultivation is spreading in response to the demands of growing human populations. This type of cultivation is popular due to the increased crop yields that result from better regulation of water application and more favorable climatic conditions (e.g., less typhoon damage, better water management, warmer temperatures, and higher light intensities) (Faisal and Parveen 2004).

Current water intensive rice cultivation practices may decrease grain concentrations of essential micronutrients (e.g., zinc, copper, selenium, iron, and manganese), and elevate levels of the potentially toxic trace elements such as arsenic (a class one, nonthreshold human carcinogen) in rice. Rice is a major source of inorganic arsenic into the human diet (Meharg *et al.* 2009) as rice's anaerobic cultivation conditions facilitate the mobilization of inorganic arsenic into soil solution (Xu *et al.* 2008). Reducing water usage during rice cultivation can decrease arsenic accumulation 10-fold in grain (Xu *et al.* 2008; Norton *et al.* 2012). Some micronutrients such as zinc become less available in flooded soils, but its availability and uptake into grains increased substantially in aerobic soils, as in uplands (Wissuwa *et al.* 2008). Other elements such as iron become more available in flooded soils leading to toxicity as experienced in some coastal areas in Asia and in over 50% of paddy soils in Africa (Becker and Asch 2005; Cherif *et al.* 2009). The global challenge of sustainable rice cultivation, therefore, requires reducing the amount of water used for rice irrigation, while at the same time maintaining (or improving) grain yields and nutritional quality.

Alternate Wetting and Drying

To help reduce water consumption during rice cultivation there has been considerable interest in expanding the aerobic cultivation practices employed in upland rice to lowland environments where anaerobic, paddy cultivation is traditional (e.g., Bouman *et al.* 2005). However, the reduced yields and pest control problems (primarily nematodes and weeds) associated with aerobic cultivation need to be addressed (Kreye *et al.* 2009). One major recent advance in rice water management is termed Alternate Wetting and Drying (AWD). AWD combines the beneficial aspects of both aerobic and anaerobic cultivation. In Bangladesh it is being promoted by the Bangladesh Rice Research Institute (BRRI), the Rural Development Academy (RDA), the Department of Agriculture Extension (DAE) and Syngenta. In Bangladesh,

the method is based on inserting perforated tubes into the soil to measure the height of the water table in the field (Fig. 1). The first alternating wetting/drying cycle is deployed 10–15 days after transplanting and cycles are continued until the commencement of flowering. The wetting/drying cycle consists of flooding the field then allowing it to dry out 15 cm below the soil surface (as observed in the tubes); the field is then reflooded to 2 cm above the soil surface and then the next drying cycle begins. The length of each cycle will be dependent on a number of factors including the rate of water percolation through the soil, the weather, and size of the plants.

AWD can lower water use for irrigated rice by ~35% (Zhang *et al.* 2009), increase rice yield by ~10% relative to permanent flooding (Yang *et al.* 2009; Zhang *et al.* 2009), increase the nutritional status, and decrease toxic elements such as cadmium (by ~20%) that can be problematic in rice (Yang *et al.* 2009). However, in some studies, AWD either does not alter (e.g., Yao *et al.* 2012) or slightly lowers yield (e.g., Sudhir-Yadav *et al.* 2012). A review of reports on AWD yields shows a mixed picture depending partly on the severity of soil moisture deficit (Davies *et al.* 2011) (Fig. 2). AWD improves water use efficiency and can improve yield by increasing the proportion of tillers that are productive, reducing the angle of the topmost leaves, (thus allowing more light to penetrate the canopy) and modifying shoot and root activity, implying altered root-to-shoot signaling of phytohormones such as abscisic acid (ABA) and cytokinins (Yang and Zhang 2010).

Rice grown with AWD techniques can show higher yield than continuously flooded rice even though both treatments may have similar aboveground biomass (Yang and Zhang 2010; Zhang *et al.* 2010). This implies an increased harvest index, with increased grain yield due to



Figure 1. Perforated tube inserted into the soil to measure the height of the water table in the rice field for AWD in Bangladesh.

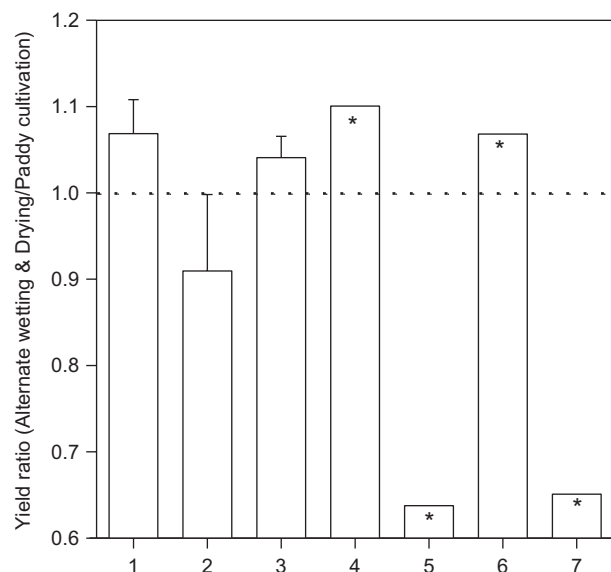


Figure 2. Crop yield ratio of AWD to paddy cultivation in two studies conducted with different nitrogen fertilizers, rice cultivars, and soil drying duration. Data replotted from Belder et al. 2004 (1–3) are means \pm SE of three different nitrogen fertilizer regimes conducted at (1) Muñoz, The Phillipines in 2001 (2) Tuanlin, China in 1999, and (3) Tuanlin, China in 2000. Data replotted from Yang and Zhang 2010 (4–7) are means from three cropping seasons (2006–2008) with the *japonica* cultivar Zhendao 88 (4, 5) and the *indica* cultivar Liangyoupeijiu (6, 7) at Yangzhou (China) when the field was rewatered when the soil at 15–20 cm depth reached a matric potential of -25 kPa (4, 6) and -50 kPa (5, 7), respectively. Significant treatment differences (from conventional paddy cultivation) are asterisked. In both studies, AWD was applied throughout crop development.

a reduction in redundant vegetative growth (nonproductive tillers by encouraging early tillering) (Yang and Zhang 2010), an increased percentage of filled grains (Zhang et al. 2010) and increased individual grain weight (Matsuo and Mochizuki 2009; Zhang et al. 2010). Remobilization of carbohydrates from stems to the grain (Yang and Zhang 2010) could represent another important mechanism of improving grain filling under AWD treatments.

Carbohydrate mobilization is likely to be under the control of plant hormone status, with ABA and cytokinins having positive impacts and 1-aminocyclopropane-1-carboxylic acid (ACC, the ethylene precursor) having negative impacts (Yang and Zhang 2010). Although root signals (such as plant hormones) can influence grain yield independently of leaf water relations (Westgate et al. 1996; Zhang et al. 2010), relatively little is known about the mechanistic basis of this response. The highly dynamic soil environment during AWD (decreased soil oxygen concentrations during flooding and decreased matric potential during drying) will produce dramatic

fluctuations in the root synthesis of chemical signals and their transport to the shoot. Flooding seems to increase shoot ACC status, and decrease shoot ABA and cytokinin status (Else et al. 2009) while soil drying increases shoot ABA (and possibly ACC) status and decreases shoot cytokinin status (Kudoyarova et al. 2007; Belimov et al. 2009). Consequently, each hormone has a unique relationship with soil water (or oxygen) status. Understanding the effects of AWD on the relationships between plant hormone status and instantaneous soil conditions is needed. Another issue is whether the soil conditions imposed during one cycle influence the subsequent response to the next cycle.

AWD is also expected to alter macro and micro nutrient availability and uptake. Aerobic growth has been shown to favor enhanced selenium accumulation in rice (Li et al. 2010), while decreasing arsenic uptake (Xu et al. 2008; Norton et al. 2012). Arsenic accumulation is increased in anaerobic soils as the inorganic arsenic is present as arsenite (as opposed to arsenate in aerobic soils), which is more readily taken up by plant roots (Brammer and Ravenscroft 2009).

While water savings are achieved through AWD, it is possible that these could be further improved by modifying the rooting behavior of the rice cultivars. Within a single cultivar (of barley), increasing the root mass exposed to a partial soil drying treatment exacerbated the physiological effects of the treatment on leaf growth (Martin-Vertedor and Dodd 2011). In comparing different rice cultivars, those with relatively shallow roots are likely to have a greater proportion of their root system in the aerobic and relatively droughted surface soils compared to a deep rooting cultivar. The placement of the roots relative to the timing and intensity of fluctuations in soil matric potential and redox potential will affect the impact of the AWD treatment on nutrient access, whole root system water potential and the signaling between root and shoot. A crucial issue for the optimization of AWD technique, therefore, is the extent to which the root systems of current cultivars bred for irrigated systems are suited to temporally and spatially heterogeneous soil moisture and oxygen.

Irrigated Rice Production in Bangladesh

In Bangladesh 145 million people are engaged in agricultural activities with rice representing 71% of crop production (Shahid 2010). Approximately 60% of the country's 28 m tons rice production is grown during the dry (*boro*) season (Shahid 2010) and more than 70% of that is irrigated using groundwater resources (Faisal and Parveen 2004). The environmental downside of *boro* season

cultivation is that agricultural pumping lowers the water table year on year as monsoonal recharge is insufficient to replenish the aquifers (Faisal and Parveen 2004; Ali *et al.* 2012). In zones near the coast, this is also leading to salinization of the aquifers. The situation is unsustainable and is predicted to worsen due to climate change even without consideration of population growth. A study by Shahid (2010) predicted that during this century the rate of aquifer depletion will increase because the daily extraction rate will increase even though total *boro* season irrigation will not change and the growth period will decline. Currently AWD is being promoted in Bangladesh and it is hoped that it will address some of the problems caused by the use of ground water for dry season irrigation. Also there is a potential decrease in the cost to the farmers as less water will need to be pumped onto the fields although crucially this depends on how farmers are charged for irrigation (e.g., by the area watered or by the amount of water used).

Potential Problems with AWD or its Introduction

It has been proposed that AWD could require additional labor for weeding; however, recent work demonstrated that AWD did not increase total labor use (Rejesus *et al.* 2011). For AWD, it is more important (compared to flooded or aerobic cultivation) that the field is level as differences in water depth will be experienced during every cycle. Elevated grain cadmium is problematic for rice grown under more aerobic conditions (Arao *et al.* 2009) yet mild and severe soil drying can reduce grain cadmium (Yang *et al.* 2009). Reducing cadmium accumulation in grain must be a priority for any AWD breeding program (Meharg *et al.* 2013). Moreover, soil drying might also affect the availability and uptake of certain nutrients, such as phosphorus, which is more available in flooded and anoxic soils (Dobermann and Fairhurst 2000; Kirk 2004). The long-term sustainability of AWD should be investigated to ensure that if the increased rice yields observed are due to more efficient nutrient mining from soils, this will not lead ultimately to soil nutrient depletion.

One major limitation to widespread AWD adoption is related to who are the main financial beneficiaries. Many farmers in Bangladesh pay a flat rate to pump owners to irrigate their land, based on area and not on quantity of water used or even number of times the fields are flooded. Thus, reducing about $\frac{1}{4}$ of the irrigation events will not save the farmer money. Unless the pump owners can be persuaded to charge less to a farmer implementing AWD this might represent a substantial socioeconomic hurdle to the widespread adoption of the method.

Supporting policies will be helpful to facilitate further adoption.

Soil Biogeochemistry

The AWD regime can be expected to affect the redox chemistry of soils, with metals in pore waters and the readily exchangeable solid phase pool varying dramatically, both temporally and spatially. This phenomenon has not been effectively studied. The techniques of diffusive equilibration/gradients in thin films (DET/DGT) can be readily deployed *in situ* to give precise information on metal concentrations and dynamics in pore waters (Tankere-Muller *et al.* 2007). As DET allows solutes in pore waters to equilibrate with the 95% water of a hydrogel, it is similar to dialysis. DGT employs a binding layer to the rear of the diffusive hydrogel layer. It is a dynamic technique, which continually removes solute, thereby introducing a controlled chemical perturbation, which allows the acquisition of flux and rate information relevant to the metal that can be readily released from the solid phase (Ernstberger *et al.* 2005). DGT can be deployed with either a Chelex-binding layer to measure trace metal cations (Garmo *et al.* 2003) or with a ferrihydrite-binding layer to measure oxyanions (Stockdale *et al.* 2009).

Genome-Wide Association Studies in Rice

The suitability of rice cultivars bred for irrigated rice production for AWD has not been studied. Given the description of AWD and its effect on soil chemistry and root–shoot signaling above, it is clear that traits that maximize yield under AWD may differ from those in conventional paddy irrigation. These traits might include nutrient uptake, root distribution in the soil profile or the dynamics of tillering and grain filling in response to soil drying. It is important that genetic studies are initiated to facilitate appropriate breeding for AWD. Genome-Wide Association Studies (GWAS) offer a rapid approach to identifying genes associated with phenotypic traits (Atwell *et al.* 2010; Baxter *et al.* 2010), or at least providing markers for these genes for assisted breeding. GWAS for rice populations is a relatively new approach. A 44,000 single-nucleotide polymorphism (SNP) chip for rice has been developed which has been used to conduct GWAS on a set of 413 diverse Asian cultivars of *Oryza sativa* named the “Rice Diversity Panel” for association analysis (Zhao *et al.* 2011). Huang *et al.* (2010) have advanced GWAS in rice by exploiting next-generation sequencing. They sequenced 520 Chinese rice land races, generating ~3.6 million SNPs to construct a high-density haplotype map which provides unparalleled marker

coverage. Both approaches could determine trait associations with SNPs at known candidate genes within regions of linkage disequilibrium of 72–200 kb covering between four and 14 genes demonstrating the accuracy of genetic mapping by GWAS. Despite this, there are two major problems that can be encountered with GWAS studies using wide collections of crop plants. First, population structure and the distribution of allelic diversity greatly limit the power to detect a number of important gene effects which can be overcome by concentrating the study on a set of material which is divergent for the trait of interest yet is not distributed into widely divergent sub-populations (Zhao *et al.* 2011). Second, variation in local adaptation mean that cultivars of diverse geographic origin differ widely in their growth rates and flowering time in individual locations and thus present considerable interpretational problems when such divergent populations are grown at one site.

Metabolic Networks

GWAS are an excellent approach to obtain a phenomenological description of how genomic variations correlate with important phenotypic traits, such as growth rate, grain yield, and nutritional value. To move toward a mechanistic understanding how differences in genotype translate into different phenotypes, the intermediate processes have to be described and understood. It is apparent that metabolism is central in translating genotype to phenotype, because metabolic activity is largely determined by the enzyme and transport properties which are encoded in the genome, but metabolism also determines how fast an organism grows and which biochemical compounds are accumulated in its biomass.

To study the metabolism of organisms as diverse as microbes, fungi, plants, and animals, genome-scale metabolic network reconstructions are now widely used (Baart and Martens 2012). These networks are typically constructed based on the fully sequenced genome (Fell *et al.* 2010) and transcript and proteome data can be exploited to define context- or tissue-specific subnetworks (Jerby *et al.* 2010). The analysis of these networks with constraint-based methods such as flux balance analysis (FBA; Orth *et al.* 2010) helps understand the structure and regulation of metabolic networks (Poolman *et al.* 2009; Nikerel *et al.* 2012), identification of essential genes (Joyce and Palsson 2008), predicting putative drug targets (Perumal *et al.* 2011), or supporting engineering of novel pathways (Basler *et al.* 2012) producing desired compounds of technological or economic interest. Despite the fact that no systematic approaches exist to date which aim at integrating GWAS into metabolic modeling, the combination of these datasets with genome-scale metabolic models

provide a promising opportunity to improve our understanding of the mechanisms which govern the phenotypic differences caused by genetic variations.

Integration of Biogeochemistry, Physiology, Genetics, and Modeling

There seems to be an opportunity to combine advances in soil biogeochemical analysis with next-generation sequencing and systems biology to gain a better understanding of the impact and sustainability of AWD in Bangladesh (and elsewhere). A multidisciplinary study could address the hypothesis that genetic variation exists that can be exploited in crop improvement by identifying cultivars, quantitative trait loci (QTLs) and candidate genes for adaptation to AWD, high grain nutrient content and low grain arsenic and cadmium under Bangladeshi agronomic (*i.e.*, soils, climate, and cultivars) conditions. It should test the hypothesis that AWD is sustainable by understanding the physiological and biochemical basis for improved yield and assess the impact of AWD on soil properties. Such an approach could be structured as follows (i) Generate an association mapping population of local cultivars specifically adapted for the *boro* season cultivation practices, using next-generation sequencing to produce markers; (ii) Conduct field experiments on these cultivars in Bangladesh comparing AWD to conventional flooding and measure adaptation to AWD (especially grain and biomass yield) and element concentrations in the shoots and grain; (iii) Characterize the impact of AWD on soil physics and chemistry, plant hormone balance, and gene expression; (iv) Conduct a thorough GWAS analysis of the data obtained to identify regions of the genome and candidate genes associated with adaptation to AWD cultivation and element distribution in rice; (v) Integrate the results into a genome-scale metabolic pathway model to establish links between genotypic variations and phenotypic differences. If successful, this would lead to breeding lines, candidate genes and pathways of uptake and metabolism linked to advanced genomics for future crop improvement in rice and other cereal crops, while field management practices to maintain yield in the long term will be advanced.

Generation of a suitable rice panel

It seems sensible for the objective above if the genotypes used were confined to the diversity of *boro* season cultivars and their geographically and genetically related *aus* cultivars. *Boro* cultivars have been identified as having high diversity, higher than other season-specific cultivars (Parsons *et al.* 1999) and are adapted to the main season for unsustainable irrigation in the target geographic

region. It has been demonstrated that *boro* season cultivars grown in flooded conditions display significant variation in yield, shoot, and grain element concentrations (Norton *et al.* 2009a,b, 2010), indicating their suitability for genetic dissection of these traits within this group of cultivars. In addition, *aus* cultivars have been recognized as the donors of many abiotic stress tolerance traits in IRRI's breeding program including flooding tolerance from FR 13A (Xu *et al.* 2006), tolerance to low phosphorus from Kasalath (Gamuyao *et al.* 2012), and drought resistance from N22 and Dular (e.g., Gowda *et al.* 2011). It would be important that the chosen accessions have diversity without duplication so some initial screening, using, for example, simple sequence repeat (SSR) markers or a small scale SNP array (see Zhao *et al.* 2010), could be used to screen a large set of material before selecting a core set of 300. It would also be important to ensure that the accessions have a narrow window of flowering time to avoid confounding interactions between drying-related soil processes and grain filling processes.

Field-based phenotyping

Ideally, the field evaluation of the rice panel should be conducted across years and locations, comparing conventional flooded irrigation with AWD cultivation. Consideration for sites should be the degree to which they represent large areas of the Bangladeshi soil types, the extent to which they might differ from each other as well as practical considerations of field size and ease of access. A field layout successfully employed previously (Norton *et al.* 2009a,b) where each test genotype is grown in a single 2 m long row with 10 single plant hills and is sown between alternating rows of a relatively short check cultivar that is used in the *boro* season (like BRRI Dhan 28) should be used. With two treatments and four replicates, this would require 80 plants. If more seed and space is available, bigger plots could be considered. Care must be taken to prevent water entering the AWD fields from adjacent areas during the drying cycle. Field sites should be assessed for soil hardness using a penetrometer (Cairns *et al.* 2004) to establish if conditions are representative of local farmers' fields and to know the way the AWD treatment affects soil hardness. The AWD treatment should be applied 14 days after transplanting and continue until half the plants show signs of flowering. Tillering should be measured fortnightly and flowering time recorded. At harvest, plant biomass and yield (and yield components) should be assessed. Macro and micro nutrient elemental composition of both the grains and straw should be determined including: boron, carbon, nitrogen, sodium, magnesium, phosphorus, sulfur, potassium, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc,

arsenic, selenium, rubidium, strontium, molybdenum, and cadmium. As the plants are growing in the field, in situ monitoring of soil and soil water chemistry should be conducted, ideally by using DET and DGT probes and rhizon samplers. Concentrations and the fluxes of iron, manganese, aluminum, cadmium, cobalt, chromium, copper, nickel, lead, zinc, arsenic, selenium, antimony, molybdenum, tungsten, and phosphorus should then be available through the DET and DGT, respectively, while the rhizon samples will allow sulfur and nitrogen concentrations to be assessed.

The physiology, transcriptomics, and soil chemistry of adaptation to AWD

From the field experiments, opportunities will arise to assess cultivars which display very different adaptabilities to AWD yet have rather similar genetic structure. Field and pot experiments could be conducted to determine the reason for these differences, principally if this is because of different abilities to take up soil nutrients or respond to plant growth hormones, and the degree to which these are related to root growth. For example, field-based analysis of hormone profiles could be conducted on a small subset of cultivars. The first year could be used to take frequent measurements of leaf ABA to determine critical time points within the AWD cycle while in the second year a wider suite of hormones ABA, ACC, cytokinins could be measured to determine the degree to which these hormones interact in regulating plant response to AWD. This hormone analysis could be combined with transcriptomics that would provide insights into genetic differences at the transcriptome level between adapted and nonadapted cultivars, to give a database for metabolic network analysis, and test expression levels of genes known to be responsive to plant growth regulators implicated in plant response and adaptation to AWD.

The root systems of the field-grown cultivars could be assessed using a high through-put methods while for a small subset of cultivars, detailed assessment of root system architecture should be evaluated using 1.2 m deep soil-filled rhizotron system previously described (Price *et al.* 2002) which allows root growth up to 7 weeks to be assessed.

Association mapping

The sequencing data of the 300 cultivars would be transformed into a SNP dataset using the approach employed by Huang *et al.* (2010); aligned to the Nipponbare reference or an *aus* subpopulation reference genome if available and verified using additional cultivars with high sequence quality and depth, SNPs filtered for singletons

and then missing data imputed using matching haplotypes. Association between phenotype and SNPs would be assessed using a mixed model approach which takes population structure into account (Hyun *et al.* 2008). The traits of greatest importance would be biomass, grain yield, productive tiller number, leaf angle, as well as shoot and grain elemental concentrations which will include element concentrations and total element uptake by the plant.

Metabolic network analysis

To explore possible mechanisms leading to increased yield in AWD cultivation, a genome-scale metabolic network model for rice could be developed. The model could be based on the annotated contents of the RiceCyc (Jaiswal *et al.* 2006) database which was established for *O. sativa japonica*, cultivar Nipponbare. Model development involves a detailed curation of the database and various cycles of consistency checks, until the mathematical description is suitable for constraint-based modeling approaches and is able to predict flux distributions correctly (Fell *et al.* 2010). The model would first be validated against data obtained from pot and hydroponic experiments under controlled growth conditions where nutrient (especially nitrogen, phosphorus, and sulfur) input and uptake are monitored in six contrasting cultivars and the biomass composition (amino acids, nucleotides, lipids) of shoots and roots determined at intervals with standard biochemical techniques. Transcriptome data obtained from the field experiments would be used to identify the active metabolic pathways by a computational method (Jerby *et al.* 2010) developed for tissue-specific networks. Data on the biomass composition and soil nutrient content will be used to calculate metabolic flux distributions for different cultivars, different soils, and different cultivation techniques. Comparing the flux distributions among cultivars together with mapping the SNPs onto specific enzymes in the metabolic network, will link genotypic variations to the resulting phenotypic differences. It would be computationally investigated which metabolic genes have the strongest influence on overall biomass production. These gene predictions would be tested by growing selected cultivars with SNPs in the relevant genomic region under the different irrigation systems in field experiments.

Outcomes of the Project

In the short term (2 years) a project like that described above would establish if (i) AWD is sustainable or if it potentially depletes limited nutrient resources in the soil; (ii) AWD reduces the problem of arsenic accumulation in soils and rice grain; and (iii) there is genetic variation for

adaptation to AWD. This information could guide agricultural policy in Bangladesh and probably in the bordering parts of India and other countries in south east Asia with similar climate, geochemistry, and rice cultivars.

In the medium term (3–5 years), the project should identify the best cultivars, QTLs and candidate genes for adaptation to AWD which can be used throughout the Bengal region in breeding better cultivars. The results on soil chemistry and plant nutrient uptake would provide strategies to explore maximizing the sustainability of AWD (i.e., identify a difference in farm inputs) which could be tested by agronomists. Confirming that water-saving strategies also reduce grain arsenic would enable rice producers worldwide to reduce the grain arsenic in local and exported rice and rice products. The results would also provide strategies to ensure cadmium in rice is minimized.

In the longer term (5+ years) the effect of individual candidate genes could be fully explored and strategies to utilize them in wider plant breeding (including orthologs in other cereals) could be evaluated. Phytohormonal studies would identify the role of root-to-shoot chemical signaling in adaptation to varying soil chemistry and matric potential thereby providing hypotheses for wider agronomic practice (e.g., design of root systems to match predicted soil water content). The panel of 300 sequenced *aus* and *boro* cultivars would provide a valuable resource for both researchers interested in identifying candidate genes related to climate change (drought, salinity, heat, and cold tolerance) and researchers interested in the diversity of cultivars from the Bengal area.

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Conflict of Interest

None declared.

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