**Breeding rice for a changing climate by improving adaptations to water saving technologies**

Maria Cristina Heredia1, Josefine Kant2, M. Asaduzzaman Prodhan3, Shalabh Dixit1, Matthias Wissuwa3\*

1International Rice Research Institute (IRRI), Los Baños, The Philippines

2Forschungszentrum Jülich (FZJ), Jülich, Germany

3Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

\* corresponding author

**All authors conceptualized, wrote and revised/edited the paper**

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**Abstract**

Climate change is expected to increasingly affect rice production through rising temperatures and decreasing water availability. Unlike other crops, rice is a main contributor to greenhouse gas emissions due to methane emissions from flooded paddy fields. Climate change can therefore be addressed in two ways in rice: through making the crop more climate resilient and through changes in management practices that reduce methane emissions and thereby slow global warming. In this review we focus on two water-saving technologies that reduce the periods lowland rice will be grown under fully flooded conditions, thereby improving water use efficiency and reducing methane emissions. Rice breeding over the past decades has mostly focused on developing high yielding varieties adapted to continuously flooded conditions where seedlings were raised in a nursery and transplanted into a puddled flooded soil. Shifting cultivation to direct seeded rice (DSR) or to introducing non-flooded periods as in alternate wetting and drying (AWD) gives rise to new challenges which need to be addressed in rice breeding. New adaptive traits such as rapid uniform germination even under anaerobic conditions, seedling vigor, weed competitiveness, root plasticity, and moderate drought tolerance need to be bred into the current elite germplasm and to what extent this is being addressed through trait discovery, marker assisted selection and population improvement is reviewed.

**List of Abbreviation:**

AWD- Alternate Wetting and Drying, DSR- Direct Seeded Rice, GHG- greenhouse gas, LR- Lateral Root, TPP- Traditional Transplanted Puddled Plantation

**1. Introduction**

Rice is the most important food crop being the primary food source to nearly half the world’s population (GRiSP, 2013). Rice is typically associated with Asia where 90% of the world’s rice is currently produced, but rice consumption has especially increased in Africa during the last decades, making rice is a truly global crop of tremendous importance for global food security.

Rice production systems have traditionally been distinguished into upland and lowland systems, the former being conducted under aerobic field conditions and relying typically on rainfall alone as a source of water (Bouman et al. 2007). In contrast, lowland systems traditionally employ the paddy system of rice cultivation which involves land leveling, puddling and bunding to maintain a uniform water level that would fully and uniformly submerge the soil and thereby create anaerobic soil conditions. A further distinction within lowland systems is between irrigated and rainfed rice. The traditional crop establishment in lowland rice is through transplanting of seedlings that had been raised in a seedling nursery, but upland rice is sown directly into the field. While these are the typical characteristics of the three main rice production systems, increasing pressure from socio-economic factors and climate change are driving factors for modifications of these systems (GRiSP, 2013) and these modifications will be discussed in detail in following sections.

**1.1 Rice and climate change. What is the impact of one on the other?**

Climate change is expected to mainly affect crop production because of increasingly irregular rainfall patterns and due to increased frequency and intensity of high temperature events (Porter et al. 2014). High temperature events with canopy temperatures above 35°C induce spikelet sterility and thereby reduce yields, especially in very humid climates where transpiration cooling is not effective (Hasegawa et al. 2001; Julia and Dingkuhn, 2013). Thus, air temperatures above 40°C at flowering can be tolerated in dry climates as transpiration cooling can reduce canopy temperatures by 9°C (Julia and Dingkuhn, 2013). The steady increase in temperatures does have additional negative effects and rising nighttime temperatures that diminish carbohydrate reserves through increased respiration are a main concern (Peng et al. 2004).

Tolerance of extreme temperatures during flowering has gained considerable interest in rice breeding. One strategy of mitigation has been to focus on early-morning flowering (EMF) to escape heat stress and donors have been identified among accessions of *Oryza officinalis*, a wild relative of rice (Ishimaru et al. 2010). Subsequently a major QTL (*qEMF3*) has been mapped and introgressed into major rice varieties, shifting flower opening by up to 2 hours towards the early morning (Hirabayashi et al. 2015). Likewise, donors and QTL for true heat tolerance at flowering have been identified (Ye et al. 2011 and 2015). For discussions on heat tolerance in rice the reader shall be referred to excellent reviews on this topic (Jagadish et al. 2015; Wassmann et al. 2009).

Rice grown under rainfed upland or lowland conditions has always been exposed to incidences of drought and drought tolerance and escape have therefore been objectives in rice breeding for decades. Climate change estimates suggest droughts will increase in frequency and intensity (Dai, 2013) and this will put rainfed rice production under increased strain while also reducing the water available for irrigation (Shaw, Nguyen, Habiba & Takeuchi, 2011). Breeding for tolerance to drought in lowland rice is facing some peculiar challenges not encountered in other crops. Heavy monsoon rains typically leave standing water, causing reduced soil conditions throughout most of the rice season, even in rainfed lowland fields. In an O2 deprived environment it can be advantageous to concentrate roots in shallow and therefore more oxidized soil layers (Colmer, Cox & Voesenek, 2006). This contrasts to drought conditions where deeper rooting is advantageous to maintain water uptake from lower soil layers (Uga, Sugimoto & Ogawa, 2013). For tropical rice it is very common that excess water causes prolonged flooding during part of the season, while a different part of the season is affected by drought (Shaw, Nguyen, Habiba & Takeuchi, 2011). Breeding a crop with a root system adapted to flooding and water shortages in relative short succession clearly is a challenge.

1.1.1 Current rice growing practices and their environmental footprint

Methane (CH4) is a potent greenhouse gas (GHG) and anaerobic soil conditions encountered in flooded paddy fields promote methanogenesis (Figure 1), which is the reason lowland rice production alone causes 11% of non‐carbon dioxide GHG emissions from agriculture (Smith et al., 2007). The global warming potential (GWP) of rice is 2.5 - 5.5‐fold higher compared with other cereal crops as a consequence of these CH4 emissions (Linquist et al., 2012). Reducing CH4 emissions from rice can be achieved by a shift to cultivation/irrigation practices that maintain paddy soils in a less reduced state. In a meta-analysis evaluating different options of managing irrigation and drainage, Hussain et al. (2015) concluded a mitigation potential of around 30-60% is feasible through mid-season drainage or shorter but repeated drainage cycles as in the alternate wetting and drying (AWD) method (Figure 1). As drainage shifts the soil to a more aerobic state, GHG emissions may shift from CH4 to N2O and the overall mitigation potential would dependent on the extent N2O emissions can be controlled. Two other factors with the potential to mitigate CH4 emissions are the management of crop residues and the choice of rice varieties as varietal differences in CH4 emissions appear to exist (Hussain et al. 2015).

The second environmental footprint of concern is the high water consumption of irrigated rice, estimated to account for 34–43% of the world’s irrigation water (GRiSP, 2013). A rice crop requires between 600 – 960 mm of water (from irrigation and precipitation) to produce between 4.4 – 9.2 t ha-1 rice, which translates to a water productivity of between 0.5 – 1.2 kg grain m−3 water input (Belder et al. 2004). Thus, up to 2000L water are required to produce one kg of rice. Such high rates of water consumption are not sustainable in many rice-growing regions where irrigation water is becoming increasingly scarce due to effects of climate change and competition from non-rice water uses (Bouman et al. 2007).

**1.2 Water saving technologies AWD and DSR**

Reducing the amount of water consumed in rice production and maintaining rice soils in a less reduced state to mitigate methane emissions has spurred research on water saving technologies over the past 3 decades. Variations of intermittent drainage have been tested and among these a procedure termed alternate wetting and drying (AWD) has been promoted widely (Bouman 2007; Carrijo, Lundy & Linguist, 2017). AWD is a rice cultivation method that follows a repetitive cycle of irrigating and drying the paddy field (Carrijo, Lundy & Linguist, 2017). The interrupted irrigation saves between 23 - 29% water compared to continuously flooded rice, increasing water productivity by 19 – 29% (Belder et al. 2004; Carrijo, Lundy & Linguist, 2017). AWD aims at improving water productivity at no loss in grain yield, however, yield penalties in the range of 6-23% are typical and varietal differences appear to exist (Carrijo, Lundy & Linquist, 2017).

Even more water saving potential can be realized in a shift away from the classical paddy system of transplanted seedlings towards direct seeded rice (DSR). DSR can be practiced in puddled or dry soil and on standing water in the form of water seeding (Figure 2). The highest water saving potential of up to 40-60% is realized in dry-DSR (Singh et al., 2017) where rice is essentially grown under aerobic conditions similar to other upland crops. It should be mentioned that the strongest drivers towards DSR are not environmental but socio-economic, because classical manual transplanting is labor intensive and will be abandoned where labor is in short supply due to out-migration of the rural labor force or where higher-paying job opportunities exist locally (de Brauw, 2007). While DSR systems reduce requirements for water and labor, most of the modern high yielding rice varieties have been bred for puddled transplanted systems and show yield reductions ranging between 20-50% when grown under DSR. It is therefore crucial to improve the adaptation of high-yielding lowland varieties to DSR through the identification and inclusion of key traits.

Whether changes in rice production systems towards AWD and DSR are driven by shortages of water and labor or by the need to mitigate greenhouse gas emissions, the move away from the traditional paddy system of rice will increase exposure of the rice crop to effects of climate change. In addition to abiotic stresses like drought or heat, rice will need to more efficiently compete with weeds. Since these challenges are driven by changes in the production system, they are better defined in space and time compared to random drought or heat events and therefore offer rather well-defined challenges that can be addressed in rice breeding.

**1.3 Challenges in adapting rice to DSR and AWD**

Lowland rice breeding in Asia has typically been conducted following the traditional transplanted puddled rice (TPP) system where seedlings raised in a dense and protected nursery are transplanted into flooded fields. Rice varieties bred for this TPP system fail to achieve targeted yields in the DSR establishment (Sagare et al., 2020) because direct seeded rice seedlings have to overcome a host of challenges, which vary across the plants’ developmental phases and require specific adaptive traits (Table 1). At the earliest stage, the need to germinate under anaerobic conditions if fields are not perfectly levelled (Figure 2) or heavy rains cause temporary flooding is crucial (Jeong et al., 2020). Thus, rapid and uniform emergence is increasingly important under DSR compared to the seedling nursery in TPP. To facilitate seedling establishment, DSR- adapted varieties require early root and shoot vigor (Singh et al., 2017), efficient nutrient uptake, drought tolerance, and increased weed competitiveness (Figure 2) (Rao et al., 2007). Past the seedling establishment stage, nematode-, herbicide- and lodging resistance are key traits (Sagare et al., 2020).

Challenges in adapting rice to AWD are related to the alternate irrigation pattern that changes soil moisture dynamics, affecting water but also nutrient availability (Price et al., 2013). For the efficient acquisition of spatiotemporally dynamic edaphic resources, an ideal root system would show a high degree of developmental plasticity, allowing the plant to adjust root system development in response to changing nutrient and water availability during wetting/drying cycles.

The lack of varieties adapted to conditions imposed by DSR or AWD forbears the widespread adoption of such water-saving technologies by farmers (Carrijo et al., 2017; Sriphirom et al., 2019). In the following sections we explore the traits needed to adapt elite rice breeding lines to DSR and AWD systems, to what extent donors, loci or major genes have been identified and how breeding systems are being modified to produce varieties better adapted to DSR and AWD.

**2. Optimizing rice for DSR and AWD – new breeding targets**

**Early root vigor**

Early root vigor is the genetic capability of a plant to develop, extend, and proliferate the root system fast and early (Palta & Watt, 2009). Early root vigor, mostly seminal and their lateral roots, facilitates exploring a greater soil volume. This allows the plants to optimize water and nutrient uptake (Wang, Thorup-Kristensen, Jensen, & Magid, 2016), outcompete weeds (Singh et al., 2017), and provide root anchorage for lodging resistance (Shah et al., 2019). This trait is important in the plant’s yield under DSR, as the adaptation to direct-seeding- or wetting-drying cycles that starts after two weeks of planting- underlies in the seedlings' early root vigor to establish the root system at an early stage (Mahender et al., 2015; Ishfaq et al., 2020). A deeper rooting has also been shown to increase rice yield during drought (Uga et al. 2013).

**Plastic root development**

The ability to respond to changing external conditions by phenotypic adaptations is called plasticity (Suralta et al., 2018). During the drying cycle in AWD soil water saturation decreases, affecting not only water uptake but also the availability of immobile nutrients like phosphorus, iron, manganese and zinc (Wang, Thorup-Kristensen, Jensen, & Magid, 2016) or nutrients transported by diffusion such as nitrate (Dechorgnat et al., 2011). Root plasticity allows plants to adjust the root system in accordance to these limiting edaphic resources (Sandhu et al., 2016; Suralta et al., 2018). For example, the Nipponbare-Kasalath derived chromosome segment substitution line 50 demonstrates plasticity in both lateral root branching (Kano, Inukai, Kitano, & Yamauchi, 2011) and total root length (Tran et al., 2015) under water deficit conditions with positive effects on water uptake, photosynthesis, and dry matter production (Kano, Inukai, Kitano, & Yamauchi, 2011; Tran et al., 2015). Similarly, the IR64-based introgression line, YTH183, exhibits plasticity in terms of root elongation and branching following re-watering after drought (Kano-Nakata et al., 2013), resulting in improved water uptake and shoot dry matter production (Kano-Nakata et al., 2013).

We propose that DSR- or AWD-adaptable rice varieties require plasticity for the two lateral root classes present in rice: (a) L-type lateral root elongation leading to a deeper and wider root system and (b) S-type lateral root generation for efficient acquisition of fluctuating resources. Because, when water is available at field capacity, S-type lateral roots (Figure 3) largely contribute to acquisition of water and nutrients like P that are available in the topsoil (de Bauw et al., 2020). As the soil profile dries, L-type lateral roots contribute more to the total water uptake (Watanabe et al., 2020; de Bauw et al., 2020). Therefore, the DSR- or AWD-adaptable varieties need the genetic potential to elongate the L-type roots to scavenge resources in deeper layers during the dry period, and either have the ability to maintain functional S-type roots in the dry topsoil or to initiate the de novo production of S-type roots on newly formed axile roots in the topsoil upon re-watering (Figure 3). In contrast to drought stress, water is resupplied in AWD before water limitations cause severe stress. To improve water and nutrient uptake in AWD it would therefore be advantageous to maintain as much of the root system in a functional state as possible. This contrasts with severe drought stress where remobilization of nutrients from dying roots and reinvestment in newly formed axile roots would be beneficial upon water resupply (A. Henry, J. Kant, unpublished/personal communication).

The root system complexity is increased by root hairs, which are present on all rice root types (Nestler et al. 2016). In addition to taking up water and nutrients root hairs also contribute to C exudation into the rhizosphere and favor larger rhizosheath formation (Holz et al. 2018). This excreted mucilage, surrounding the root hair shaft and proving higher conductivity with the soil, leads to a higher moisture level in a drying soil and could therefore provide the ability to take up water and nutrients longer (Carminati et al. 2017). Therefore, longer root hairs support nutrient and water uptake efficiency even if the differences may seem small (Nestler & Wissuwa 2016) and DSR- or AWD-adaptable rice varieties should include the ability to form longer root hairs.

**3. Trait discovery**

In the previous sections we highlighted the need to include several adaptive traits (see Table 1) in breeding material adapted to DSR/AWD. Improvements in each of these traits will increase rice productivity and yield, with rapid emergence, drought tolerance, lodging resistance, and nematode tolerance being among the most crucial ones.

In the last two decades, a significant effort has been made to develop rice varieties tolerant to various abiotic stresses. This began with the development of submergence tolerant rice varieties with the *Sub1* gene and extended to other abiotic stresses such as salinity, nutrient deficiency and drought. In case of drought, multiple major QTLs such as *qDTY12.1, qDTY3.1, qDTY2.2, qDTY6.1*and *qDTY3.2* were identified (Bernier et al. 2007, Venuprasad et al. 2008, Dixit et al. 2012, Dixit et al. 2014a, Dixit et al. 2014b, Dixit et al. 2015). These QTLs were identified in mapping populations using landraces evolved in drought prone conditions and high yielding popular varieties such as Swarna, TDK1, Vandana and IR64. These major QTLs are therefore utilized to combine drought tolerance of these landraces with high yield, phenotypic acceptability and grain quality of the popular rice varieties. It was noted that most of the high yielding rice varieties developed in the post green revolution era were susceptible to drought and that tight linkages of QTLs for drought tolerance with undesirable traits such as tall plant height, short maturity period and yield penalty existed (Vikram et al. 2015). Similar is the case of anaerobic germination where major QTLs have been detected in tight linkage with the gene conferring the undesired red pericarp color (Septiningsih et al. 2013, Ghosal et al. 2019).  Breaking such negative linkages using modern breeding techniques is essential if such stress tolerance QTLs are to be transferred to elite cultivars for commercial use.

*3.1 QTL for seedling vigor*

Continued from the research on abiotic stress tolerance, studies have specifically targeted adaptation to direct seeding with donors for crop establishment traits such as early and uniform emergence, anaerobic germination and early vigor having been identified and QTL mapped (Dixit et al. 2015, Ghosal et al. 2019, Ghosal et al. 2020, Septiningsih et al. 2013) (supplementary Table S1). QTL hotspots for early vigor (EV) have been identified in multiple stress environments under DSR both in the field and glasshouse experiment (Singh et al, 2017). One of the characteristics important for seedling vigor is the ability of the seedling to mobilize reserves stored in the seed until the seedling becomes photoautotrophic (Pritchard et al. 2002). Seed reserve utilization efficiency (SRUE) is particularly important in facilitating wet DSR where pre-germinated seeds are sown in the puddled field (Ali et al., 2020). QTLs identified for this trait are *qSRUE4.3* and *qWMSR7.1* (Cheng et al., 2013). Another important trait affecting stand establishment in DSR is rapid shoot elongation to ensure oxygen supply to the root system under flooded conditions and two QTLs (*qHES8* and *qHES9*) showed positive contributions to shoot elongation (Cordero-Lara et al. 2016). Superior seed vigor allows the plant to have a head start against weed and to have a high grain yield potential (Cheng et al., 2013).

While seedling development relies on carbohydrates and nutrients stored in the seed during the initial stages it has been shown that phosphate (P) uptake via roots commences as early as 3 days after germination and that this process is not limited by P transporter activity but by root size (Julia at al. 2018). It has furthermore been observed that genotypic differences exist in how fast seedlings become independent of their seed-P reserves and that this is associated with rapid root development (Pariasca-Tanaka et al. 2015). Such early root development may be of little importance for transplanted rice as roots are typically damaged during the transplanting process but will be critically important in DSR where early root development lays the foundation for a root system that efficiently explores the soil for nutrients and water.

Wang et al. (2018) screened a diversity panel for seedling root development under P deficient conditions and identified a QTL (*qTIPS-11*) for root tip number on chromosome 11. A glycosyl hydrolase was identified as a candidate gene and gene bank accessions with the positive allele of this gene increased lateral root number by more than 30%, presumably because the hydrolase softened root cortical cell layers and thus facilitated lateral root tip emergence and elongation and nutrient uptake (Wang et al., 2018). Additional loci associated with the acquisition of nutrients P, N, K and Na have been mapped and are summarized in supplementary Table S1.

*3.2 QTL for water use efficiency and drought tolerance*

While many of the seedling vigor, and especially seedling root vigor traits should improve water uptake in addition to nutrient uptake, additional loci would be needed to improve water efficiency in more mature plants during AWD. Three strategies have been highlighted in breeding for water-use efficiency: (1) maximize the passage of water through the crop while minimizing evaporation from soil surfaces; (2) gain more carbon per unit transpired water (transpiration efficiency); (3) optimize the partitioning of biomass to sinks (harvest index) (Condon et al. 2004).

A large number of QTL associated with root development at deeper layers has been identified in studies conducted under more severe drought stress (Sabar et al. 2019). Additionally, a large number of QTL for yield under drought have been identified (Lanceras, et al. 2004; Grondin et al., 2018; Yadav et al. 2019) (supplementary Table S1). One such QTL (*qDTY3.2*) has been shown to have a major role in water uptake through reduced root growth near the surface but increased root elongation under drought conditions (Grondin et al., 2018). However, as we pointed out earlier, the fluctuating water level under AWD will put limits to what extent a concentration of root biomass at depth can be a target trait for AWD-adapted rice. Further studies are clearly needed to clarify this point and to assess to what extent QTL for root plasticity may be sought and utilized instead.

Transpiration efficiency has been evaluated using carbon isotope discrimination (∆13C) techniques and stomatal conductance and canopy temperature measurements. A novel QTL *qCTd11* associated with high stomatal conductance, leaf photosynthesis and lower canopy temperature was mapped on chromosome 11 (Fukada et al., 2018) whereas (Melandri et al., 2019) identified a reduced canopy temperature QTL (*qCT1*) on chromosome 1 with the advantage that this QTL appears fixed in high-yielding genotypes of short plant height.

*3.3 Traits for the control of weeds and nematode or lodging resistance*

Weed control in transplanted flooded rice was largely achieved by the combination of suppressing weed growth by maintaining flooded conditions and by providing rice plants with a head start as transplanted 3-4 week old seedlings were able to out-compete any newly germinating weeds. These advantages no longer exist in DSR and managing weed infestations has consequently become a major problem in DSR (Rao et al., 2007). Since DSR is a response to labor shortages it is unlikely that manual weeding will be the main solution and application of herbicides or developing rice varieties that more rapidly close their canopy without having had a substantial head start needs to be considered. Progress in identifying loci associated with this ability has been slower than for other traits discussed above and while some seedling vigor QTL discussed above are candidates, searching for loci increasing leaf photosynthesis may offer a solution. A QTL for increased leaf nitrogen content (*qLNC4*) was positively associated with the rate of photosynthesis per leaf area (Hirotsu et al., 2017) and may offer opportunities to accelerate canopy closure.

An alternative strategy is the development of herbicide-resistant rice varieties. Map-based cloning revealed a gene (*CYP72A31*) conferring tolerance to acetolactate synthase-inhibiting herbicides (Saika et al., 2014), however, tolerance was achieved by overexpression of *CYP72A31*. In other studies multiple-herbicide tolerance to bensulfuron methyl (BM) and glufosinate herbicides has been achieved through the over-expression of *AHAS* (*Acetohydroxyacid synthase*) (Fartyal et al., 2018). Naturally occurring variants that can be explored in MAS seem to be not available.

The root-knot nematode *Meloidogyne graminicola* has rarely occurred in lowland rice in the past because continuous flooding has kept nematode populations low (Galeng-Lawilao, et al. 2018). However, nematode damage and ensuing seedling mortality can lead to severe yield losses when rice is continuously cropped under aerobic conditions. Thus, a shift to DSR and AWD is likely leading to increasing incidences of nematode damage. Several nematode resistance QTL (*qYR5.1*, *qYR11.1, qGR4.1, qGR8.1*) have been identified (Table S1) (Galeng-Lawilao et al. 2018) of which some are specifically related to reduce galling and lower J2 (second-stage juveniles) reproduction (Galeng-Lawilao et al., 2020).

Lodging is an old problem that has primarily been solved by developing semi-dwarf varieties with the *sd1* gene (Peng & Khush, 2003). Apart from lodging due to a weakness of the stem it appears that varieties grown under DSR are more prone to lodging as a result of insufficient root anchorage in the ground. This is likely caused by the shallow root system typical of paddy rice varieties, which had not been a problem when seedlings were transplanted at depths of 3-5 cm but would provide insufficient anchorage when seeds are broadcast onto the soil surface in DSR. This problem is being addressed with QTL associated with culm diameter and culm strength (Yadav et al. 2017) and addition lodging resistance QTL have been identified from the wild relative *O. longistaminata* (Long et al., 2020). However, novel QTL addressing the root anchorage problem through modifications in root architecture are needed to better prevent lodging under DSR conditions.

**4. Phenotyping supporting trait identification and breeding**

The measurable and observable traits of a plant constitute its phenotype, which can be adjusted constantly as a result of interactions between genotype and environment. Phenotyping is therefore necessary to confirm the purpose of a genetic variant depending on the given conditions. Choosing controlled environment conditions for phenotyping ensures accurate and reproducible conditions being imposed and allows the screening of larger numbers of lines under such uniform conditions (Poorter et al. 2016). QTL identification is therefore often conducted under such conditions. However, phenotypic measurements from controlled conditions may not correlate well with field observation as genotype x environment (G x E) interactions are expected to play an important role, especially in the adaptation to climate change and water management. Their validation in the field over different sites, years and agronomic practices is therefore essential (Sadras et al. 2020).

We have listed several target traits for the development of new rice varieties adapted to DSR or AWD (Table 1) and these differ in complexity in terms of phenotyping. Adaptation to aerobic or anaerobic germination as well as uniform emergence lend themselves to controlled environment phenotyping sterile germination well plates because potentially important environmental effects (temperature, water depth etc.) are well known and can be controlled easily. At higher throughput field screens are equally suitable (Figure 4 A-B). Similarly, phenotyping of the potential early seedling vigor is suitable for controlled environment phenotyping, whereas later stage seedling vigor or crop establishment is more dependent on the ability of seedlings to establish a root system and acquire nutrients and water from soil, factors that are more prone to environmental (soil) variation. Phenotyping in soil, either in pots or the field, may therefore be preferable and standardized high-throughput phenotyping desirable. Non-destructive, precise phenotyping has recently been published using a non-destructive imaging technique (Anandan et al., 2020). Three images (top and two side views) are taken using a digital SLR camera and processed using color-based image segmentation to estimate the whole-plant area (WPA; Figure 4C). For larger plants, plant height data can be estimated based on the canopy height model (CHM), which is initially extracted from digital surface models (DSMs) (Kawamura et al., 2020).

Increasingly unmanned aerial vehicles (UAV) are being tested and employed in field phenotyping to obtain data of key traits rapidly and non-destructively (Figure 4 D-F). Depending on the mounted camera or detector, information in visible light (e.g. measurement of greenness, higher indicating more chlorophyll content), near-infrared (crop biomass; Figure 4 E-F), thermal (canopy temperature, with a lower indicating more transpiration and thus more water availability), or combinations of different wavelengths can be measured. At later growth stages the estimation of above-ground biomass (Devia et al., 2019) and grain yield are feasible (Duan et al., 2019). Breeding for DSR and AWD may particularly benefit from UAV-based phenotyping for rapid canopy closure and adaptation to water deficits as detected by low canopy temperatures at the end of drying periods under AWD. Another application for sensors is GHG detection with non-dispersive infrared gas sensors (Dinh et al. 2016) offering much faster e.g. methane detection compared to the manual closed chamber methods often used in fields (Lesmeister & Koschorreck 2017).

Several root traits, including root plasticity, are advantageous for DSR and AWD-adapted varieties as discussed above. Root phenotyping usually involves extracting roots from the soil (shovelomics) or working in rhizotrons and obtaining images of extracted roots (Tracy et al. 2020). For image-based root phenotyping several software applications are available that extract root architectural information (root types, number of roots, branching intensity, angle, etc.) and can be measured semi-automated (Das et al. 2015; Anand & York 2020). Unfortunately, the entire process remains labor and time consuming and may therefore only be employed to identify suitable parents/donors with contrasting but complementary root traits.

With most of the high-throughput phenotypic data relying on images, image processing tools and their automation are key to moving these applications from research to applied plant breeding. Early tools were the open source software ImageJ (<http://imagej.nih.gov/ij>) which has been popular for measuring medium to high-throughput phenotypic images (Schneider, Rasband & Eliceiri, 2012). Plug-ins and macros for ImageJ have been published, with HTPheno (<http://htpheno.ipk-gatersleben.de/>) processing plant top and side view images to estimate height, width, and shoot area (Hartmann et al., 2011). More specific and high-throughput leaf phenotyping can be done with Leaf Analyzer (<http://leafanalyser.openillusionist.org.uk/>) (Weight et al., 2008), semi-automated and landmark-free with Leaf Processor (<http://gips.group.shef.ac.uk/resources.html>) (Backhaus et al., 2010), and automated leaf shape and size analysis and correlation to biomass and plant damage with LAMINA (<http://lamina.sourceforge.net/>) (Bylesjö et al., 2008). An application combining the analysis of plant shape (height, area, volume), color and fluorescence with large scale data management tools can be found at IAP (<http://sourceforge.net/projects/iapg2p/>) (Klukas, Chen & Pape, 2014).

As image-based phenotyping produces ever larger datasets, possibly in the TB range, data handling, storage and sharing becomes an increasingly complex issue. Data repositories such as Germinate (<https://germinateplatform.github.io/get-germinate/>) offer some solutions. They enable availability to project partners and, at a set time point, to the general public. In order to find applications in plant breeding outside the realm of breeding research or large breeding centers/companies that can maintain GIS and bioinformatic support teams in-house, user-friendliness of applications remains a major bottleneck. Image-based segmentation algorithms that can be run on a state-of-the-art smartphones (Müller-Linow et al., 2019) offers solutions and could be key in putting these technologies in the hands of researchers, breeders, and farmers alike.

**5. Breeding high-yielding rice varieties adapted to DSR/AWD**

**5.1 From conventional to marker-assisted breeding for DSR and AWD**

Enhancing yield and abiotic stress tolerance has always been a target in rice breeding but methods employed to achieve this goal have changed over time. Conventional breeding approaches such as pedigree and backcross breeding have been used extensively to develop high yielding stress tolerant rice varieties with acceptable eating quality. The outline of a pedigree breeding process shown in supplementary Figure S1 highlights that different targets traits had been selected for at different stages in the breeding process and that selection was mostly based on field phenotyping. It should be noted that where wild relatives or donors with many undesirable traits had to be used, advanced backcross lines needed to be developed to transfer the trait of interest into a phenotypically acceptable background to ease further breeding activities. Varieties adapted to various stress-prone environments were successfully developed through this process (supplementary Table S2) and this included developing/testing varieties for DSR and AWD.

*5.1.1 Marker assisted breeding*

QTL mapping and trait development activities have allowed the identification of genomic regions that can be transferred through marker assisted breeding as a way to improve existing cultivars for one or more traits. In relation to abiotic stresses, one of the key early successes in terms of marker assisted breeding came from the development of submergence tolerant cultivars using the *Sub1* gene. The first rice cultivar developed through systematic marker-assisted backcrossing approach was Swarna-*Sub1* and a systematic pipeline using foreground, recombinant and background markers in a sequential manner to develop NILs of Swarna with the *Sub1* gene is described by Neeraja et al. 2007. The advantages of MAS over conventional selection are two-fold and largely due to shortening the product development time. It is possible to recover 99% of the recipient genome at BC4 with MAS while this process will only be achieved at BC6 with conventional backcross breeding. In addition to requiring fewer generations of backcrosses, the use of markers diagnostic of the phenotype is reducing the need for costly and time-consuming phenotyping.

Other large effect QTLs (*qDTY12.1* and others; supplementary Table S3) have been utilized in a similar fashion to improve the yield of cultivars Vandana, Swarna, IR64, Sabitri and TDK1 under drought. It was interesting to note that a stepwise MAS strategy to pyramid QTLs *qDTY2.2, qDTY3.1, qDTY12.1*showed that combining two QTLs (*qDTY3.1* + *qDTY2.2* or *qDTY3.1 + qDTY12.1*) resulted in better performance compared to three-QTL combinations (Shamsudin et al., 2016). Some of these QTLs have been combined with *Sub1* to develop breeding lines tolerant of both drought and submergence (Dixit et al. 2017, Sandhu et al. 2019, Swamy et al. 2013).

Additional traits of relevance for developing climate-resilient varieties being brought into elite germplasm are those related to anaerobic germination, flowering time, early and uniform emergence, stem strength, etc. It should be mentioned that in several cases donors of such positive alleles were wild relatives of *Oryza sativa*. For instance, drought tolerance was introgressed through MABC from *O. rufipogon* (Zhang et al., 2006) while *O. longistaminata* and *O. rufipogon* accessions are being explored as sources of novel alleles for drought-related traits such as leaf elongation, stomatal conductance and membrane stability.

Products developed through MAS have shown wider adaptation to the target population of environments and higher stability of yield compared to the recipient parents, while maintaining the positive attributes of the recipient variety that matches farmer and consumer preferences. Both aspects have facilitated their release and adoption as varieties for stress prone areas (supplementary Table S3). QTLs such as *Sub1*, *AG1* and *AG2* are important to ensure adaptation of DSR breeding lines to floods at various stages in areas such as river deltas and basins and have been introgressed into elite lines. The products of these MAS programs will have an edge over general breeding populations for DSR related traits and are being tested for performance under DSR with encouraging results. However, at this point no variety has specifically been released for use in DSR and further improvements in seedling vigor, weed competitiveness and other traits are needed.

**5.2 Population improvement approaches**

Making continuous genetic gains in terms of yield in the face of changing climatic conditions and varying local stresses is key to successful crop improvement. Cobb et al. (2019a) have highlighted the key elements of a cutting-edge breeding program designed based on the framework of the ‘genetic gain equation’, also commonly known as the ‘breeder’s equation’. Genetic gain (ΔG) is described as the predicted change in the trait mean value over time due to selection within a population (Moose and Mumm, 2008) and the equation relates to four key factors: The degree of phenotypic variation present (σp), the narrow sense heritability of the trait (*h2*), the selection intensity (*i*) and the time necessary to complete a cycle of selection (L). The equation can thus be described as:

$$∆G=\frac{h^{2} i σ\_{p }}{L}$$

The selection response is directly proportional to *h2, i* and *σp* (particularly, on *h2*) and inversely proportional to *L*. The rate of genetic gain thus depends on increasing the values in the numerator of the equation while reducing the cycle time (*L*). The following section briefly describes the key components of a breeding program aimed at improving genetic gain in less favorable environments, building upon the resources created over the past two decades.

*The elite core breeding panel:* Conventional and marker assisted breeding approaches above led to the development of multiple breeding lines with high yield potential and tolerance to abiotic stresses, resistance to pests and diseases, preferred grain quality, and improved adaptation to specific target environments and management practices such as DSR and AWD. While only a small portion of these lines has been released as varieties, others represent valuable breeding material for the introgression of major genes and QTLs. Considering that donors used in introgressing these loci are from a rather diverse set of landraces, it is likely that the genetic diversity among such breeding lines is not a limiting factor once they are assembled into a core elite breeding panel. At the same time, these lines are selected specifically (either phenotypically or using markers) to ensure high proportion of the genetic makeup of elite breeding material. These breeding materials can be composed of the top 10%-20% of the breeding lines developed with high yield, tolerance to various abiotic stresses and presence of major gene targets. These materials thus bring both the aspects and lead to relatively higher amount of genetic variance while maintaining high proportion of elite genetics and yield similar to breeding programs that have traditionally targeted grain yield as primary target. Genotypic fingerprinting is done in a systematic effort to avoid selecting breeding lines and MAS products with overly high genetic similarities for the core panel.

The development of a closed pool of elite parents with the required traits is a must to build upon the work done so far and to ensure continued genetic gains through population development strategies such as genomic selection. Once the progeny is in the crossing block, the utilization of the original pool is minimized, however, as new requirements arise, addition to this pool are made through systematic inclusions of improved versions of the current elite breeding material. For example, a new disease tolerance gene can be added to the most recent elite material and the NILs can be included to the core panel to further strengthen the breeding pool and enhance the presence of positive alleles through systematic elite X elite crossing. Further, the elite core panel can be sequenced and mid density genotyping can be done for the subsequent progenies. Combining these two through suitable imputation processes can be a powerful tool for genomic selection for generations to come.

After phenotypic and genotypic characterization, the presence of major genes and QTLs for tolerance to biotic and abiotic stresses as well as the presence of critical gaps in terms of major genes would be identified. Based on this information planning of crosses and initiating systematic MAS to address gap traits would follow. In cases, where new stresses, new variants of existing stresses or new traits arise as critical, QTL mapping can be taken up with the most advance breeding material as recipient if evidence of major causal loci exists. A systematic approach to integrating the MAS approach into main-stream breeding has been outlined by Cobb et al. 2019b, emphasizing the use of most recent elite breeding lines or released varieties as recipients. Using such a panel of elite lines as a starting point can jump start a population breeding program while building upon the progress made through conventional breeding and MAS.

Population improvement has been successfully practiced in rice at CIAT using a recessive nuclear male-sterility gene to facilitate outcrossing of elite breeding lines (Grenier et al., 2015). Recurrent selection is expected to increase the frequency of alleles controlling complex traits and this process has been aided by genomic selection. At the end of each cycle, the selected best individuals are also used for the development of improved varieties through conventional pedigree breeding.

**5.3 The breeding strategy for increased genetic gain**

A diagrammatic representation of breeding methodology for using population improvement approaches is presented in **Figure 5**. This representation is modelled after the OneRice Breeding strategy of the International Rice Research Institute (IRRI). The approach combines Elite X Elite crossing, rapid generation advance, MAS for major genes, genomic selection for small additive effect alleles and multi-location testing for clear characterization of breeding material for Genotype X Environment X Management (GXEXM). The combination of these aspects brings together elite parents with key target traits to generate a set of related breeding lines that are genotyped to develop a genomic relationship matrix. RGA reduces the overall cycle time thus improving the rate of genetic gain over time. The approach allows rapid development of a large number of breeding lines and ease of genotyping with low density genome wide markers. At the same time, markers for major genes are also used to identify the frequency of known large effect QTLs/ genes. A subset of these breeding lines is then tested in the target environment to generate phenotype data sets that are combined with the genotypic information and used to predict the best parents out of the breeding pool for the next breeding cycle. The process allows for selection for major genes as well as small effect alleles simultaneously and leads to the development of better adopted individuals with each breeding cycle. At the same time, breeding lines identified in multi-location testing are advanced for further testing and release as products. The steps involved in the breeding strategy presented in figure 5 are as follows: Product concepts that specify trait requirements and their threshold level based on target environments are identified and selection indices are developed. These are used to select parents from the core panel of elite lines to develop the next generation of elite breeding lines using rapid generation advance (RGA). A representative subset of these lines ensuring the presence of all breeding families is selected and screened in the target environments and under controlled phenotyping setups to generate phenotypic data. Breeding value of all elite lines is predicted using these datasets and parents for the next breeding cycle are identified. The population size and selection intensity at each of these levels is determined by the target traits, the frequency of lines possessing all or most of the traits and the overall performance of the breeding lines in the multi-location testing. Increasing population size and selection intensity increases the genetic gain but raises costs of the program. Technologies that reduce per line cost are thus critical in optimizing selection intensity (Cobb et al. 2019a)*.*

Representative lines from LST tested in multi-location trial (MLT) 1 to identify the best lines for advancement as products. The selection criteria are determined by the product concepts and trait threshold level determined in them. A second testing MLT 2 is conducted to confirm the test results and identify the best breeding lines for nominations for variety release.

The methods described above allow systematic flow of tolerance and adaptation traits into a breeding program without disturbing the progress made in the past. The strategy also provides clear connecting points for trait discovery, marker assisted selection and breeding and maintains a clear workflow. Implementing pipelines with clear deliverables and advancement criteria at each stage ensures the advancement of the most robust and high-quality products at each level. The outcomes from activities conducted on developing varieties for tolerance to drought and for suitability to DSR and AWD can be aligned systematically in the framework described above to ensure continuous genetic gains.

**5.4 Future of innovative breeding in rice**

In addition to the various conventional breeding methods described above, it can be expected that genome editing will become an important tool for applied plant breeding in the future. CRISPR-Cas9 has emerged as the classic novel technique for genome editing for rice improvement. It has gained more patronage compared to other genome-editing tools such as zinc finger nucleases and transcriptional activator like effector nucleases (TALENs) because of its higher efficiency in simultaneous editing of multiple target genes (Liu et al., 2017). Genome editing studies to develop rice adapted to DSR/AWD conditions are limited. Attempts have been made to improve plant growth and stress response using edited ABA receptor genes (*pyrabactin resistance 1-like;* (*PYL1*, 4 and 6)) (Miao et al., 2018). As genome editing tools will continue to improve and new approaches are being suggested, f.e. through vector-based RNA delivery causing trait modification in agronomic practice without heritable genome editing (Torti et al. 2021), we expect novel solutions to emerge that may allow the pursuit of breeding targets for which currently no satisfactory solution exists, such as targeting a reduction in methane emissions, or increasing photosynthetic efficiency.

In terms of direct seeded rice, traits that are controlled by major genes can make the first set of targets for genome editing. These may include crop establishment related traits such as anaerobic germination and early and uniform emergence. Further, known targets to enhance tolerance to predominant biotic stresses such as blast, nematodes and brown spots can be edited to make rapid progress. The technology can also be used to create variation for rare traits such as herbicide resistance. Mutations are being used to create variation at present but a more targeted approach using genome editing can be used to develop lines with differential response to key herbicides. Further, trait development studies can benefit from genome editing in validation of key target genes identified through expression studies.

**6. Conclusions and outlook**

The adoption of AWD and DSR management practices has gained momentum over the past decade and adapting high-yielding rice varieties to these water saving practices is a rather recent breeding objective, especially in Asia. However, breeding programs for drought tolerance in upland and rainfed lowland rice has been in place for decades and evaluating elite breeding lines from these programs for performance under AWD and DSR may provide initial improvements. Longer term solutions will require the development of a focused variety development pipeline for AWD and DSR systems. This process has been initiated at international and national levels. For some of the most important traits, drought tolerance, anaerobic germination and early vigor, QTL have been identified and elite lines carrying these QTL are being assembled into core panels. Further characterization of the core panels will allow the development of better understanding of phenotypic and genetic variance of the traits in breeding populations. Pre-breeding efforts need to be initiated for traits that have extremely low or no variance in the elite breeding populations. Missing traits with reasonable variance in the elite breeding population can be handled through population improvement approaches using genomic predictions.

The inclusion of relevant major genes and QTL for obvious traits of relevance is of importance but adaptation to new production environments is likely governed by a host of minor loci and yet to be discovered traits and QTL. Genomic selection offers opportunities to more rapidly shift allele frequencies at such loci in the desired direction, especially since these may not have been selected for in breeding for the traditional transplanted and fully flooded rice system. A major challenge in these efforts are the numerous variations existing within water saving practices. DSR can be implemented under aerobic conditions throughout or with various levels of flooding during the crop cycle as DSR and AWD may be combined. Sowing may be done through drilling by machine or by hand through broadcasting of seed into dry, wet or even puddled soil. Methods continue to evolve and so will breeding objectives, which necessitates clear product concepts with associated phenotyping protocols. There is limited information available in terms of the genetic correlation for yield under transplanted, dry direct seeded and puddled direct seeded systems. This ranges from medium to low depending on the growing environments and seasons. Further understanding of this aspect for other critical traits still remains to be understood. Studies that will characterize the GXEXM aspects are thus needed as the first step to breed better varieties for these systems. Multi-location testing networks generating high-quality data through rigorous testing of breeding lines under their systems is thus a critical step to understand these aspects and divide the target environments into clusters of mega-environments. Genomic predictions can then be used to choose the right parents for each of these clusters for maximum gains. Trait indices combining key targets may also be used to make gains for multiple traits at the same time.

One remaining challenge is the identification of additional traits and related screening methods specifically needed for DSR and AWD. We highlighted the potential importance of root plasticity for which traits need to be dissected phenotypically and genetically. A shift to more aerobic growth conditions furthermore offers opportunities to address the low nitrogen use efficiency typically associated with flooded rice production. Likewise, reducing methane emission via exploring variation for plant traits potentially reducing methanogenesis or subsequent venting through the plant. While this may not be of high priority in terms of food security or improving farm income, efforts to reduce the environmental footprint of rice are likely to gain importance. Reducing GHG emissions through breeding would provide positive feedback loops in relation to lessening anticipated negative effects of climate change on rice production.

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**Figure Captions**

**Figure 1**. Methane gas emission from traditional flooded rice cultivation. (a) Flooded water cuts off oxygen supply to the rhizosphere creating an anaerobic environment. Anaerobic decomposition of the soil organic matters produces methane gas that is released into the atmosphere through diffusion, ebullition and plant root and stem aerenchyma (adapted from Hasan, 2013); (b) the drying cycle in the Alternate Wetting and Drying technology allows influx of oxygen into the soil creating an aerobic environment. This aeration reduces the production of methane, however, under aerobic conditions a different GHG, nitrous oxide N2O, is formed and released into the atmosphere. Losses of N under flooded conditions are mainly in the form of leaching, runoff and ammonia volatilization.

**Figure 2**. Commonly used crop establishment methods in Rice with their land preparation, ecologies and water management. (Source: Ladha et al. 2009). Manual transplanting into puddled field with a low level of standing water (a); direct seeding by broadcasting (b) or drum seeding (c) into puddled fields; machine row seeding into moist (d) or dry (e) soil; drought stress causes poor germination and seedling establishment in dry DSR (f); and high weed infestation (g) in dry DSR. Sources:

(a) <https://agriculturalinformation4u.blogspot.com/2016/01/advantages-and-disadvantages-of.html>; (b) [www.newindianexpress.com/states/odisha/2020/jul/01/surplus-rains-bring-cheer-to-paddy-growers-in-odisha-2163800.html](http://www.newindianexpress.com/states/odisha/2020/jul/01/surplus-rains-bring-cheer-to-paddy-growers-in-odisha-2163800.html); (c) [www.officialgazette.gov.ph/2016/02/16/direct-seeded-rice](http://www.officialgazette.gov.ph/2016/02/16/direct-seeded-rice)[/](https://www.officialgazette.gov.ph/2016/02/16/direct-seeded-rice/); (d) [www.mdpi.com/2071-1050/11/22/6499/html](http://www.mdpi.com/2071-1050/11/22/6499/html); (e) Shashank Singh, Research gate; (f and g) https://link.springer.com/article/10.1007/s13593-017-0466-2/figures/1

**Figure 3**. Illustration of the effect of fluctuating soil moisture in a rice field. The blue wave-like line represents the water level in the field variation due to the alternate wetting and drying over time. The lateral roots (LRs) may die off over the drying period, but upon rewatering (A-C) *de novo* crown roots (red arrow) are formed which elongate and produce L-type and S-type LRs within a few days (D-F). The new LRs bring more resource acquisition-efficiency when the top soil is re-watered (note that S-type roots emerge at densities of up to 15 cm-1). (A, D) overview images, (D,E) blow ups, (C, F) scanned single crown roots.

**Figure 4**. Phenotyping for the ability to germinate under anaerobic conditions (a). Seeds were sown into 5 cm of standing water. Number of seedlings germinated and seedling biomass are evaluated (b) and used in QTL mapping and early generation breeding line selection (Ghosal et al. 2019). Imaging techniques are used at the seedling stage, capturing the top and two side views of seedlings to derive early vigor parameters (c) (Anandan et al., 2020). Phenotyping by unmanned aerial vehicle (UAV) of a genomic selection panel und DSR at Santa Rosa station, CIAT (d). Using multispectral near-infrared (NIR) imagery (e – f) can estimate crop biomass (NDVI) at various stages during crop development (Devia et al. 2019). UAV based screening allows differentiation of genotypes for groundcover, drought tolerance (e), nitrogen utilization efficiency (f).

**Figure 5**. Breeding methodology for population improvement integrating elite X elite crosses, rapid generation advance (RGA), marker assisted selection (MAS), multi-location testing, genomic predictions and recycling of parents to develop breeding lines for sustaining continued genetic gain in rice (Adapted from the OneRice breeding strategy developed at IRRI).

**Table Captions**

**Table 1.** Commonly used crop establishment methods in Rice with their land preparation, ecologies and water management. (Source: Ladha et al. 2009)**.**

**Table 2.** Desirable traits for the DSR and AWD adaptable rice varieties

**Supplementary material**

**Supplementary Figure S1.** Flow chart describing pedigree selection process for developing drought tolerant rice varieties suitable for rainfed lowland and upland conditions. (Source Dixit et al. 2014).

**Supplementary Table S1.** Abiotic stress tolerance traits and their QTL

**Supplementary Table S2.** Abiotic stress tolerant rice varieties developed at IRRI using conventional breeding approaches.

**Supplementary Table S3.** Rice varieties tolerant to one or more abiotic stresses developed using marker assisted selection.