African Journal of Biotechnology Vol. 8 (9), pp. 1740-1749, 4 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Review

Gene technology and drought: A simple solution for a complex trait?

Philippe Hervé[1,2]* and Rachid Serraj[1]

[1]International Rice Research Institute (IRRI), DAPO Box 7777, Metro Manila, Philippines.
[2]Exelixis Plant Sciences, 16160 SW Upper Boones Ferry Road, Portland, OR 97224, USA.

Accepted 6 February, 2009

The successful use of gene technology for complex crop traits and responses to stress environments remains a challenging approach despite its potential. Stable crop yield in drought prone environments has been one of the most studied complex traits in recent years and transgenic crops with better performance have been repeatedly reported. We reviewed the experimental approach of contrasting case studies that report the enhancement of drought resistance in rice using various strategies. If the overall gene technology method is very similar in the different studies analyzed, the limited number of transgenic lines evaluated remains often a pitfall from a breeding perspective since it does not provide a robust assessment of the strategy. The protocols for plant evaluation and the parameters used to assess stress resistance are very different, which is a major limitation to literature mining. This clearly emphasizes the urgent need to define or redefine the major steps and criteria to meet better crop performance in the field, in particular for less favorable environments. We summarized some of these key parameters and we proposed some enabling solutions that can address crop breeding challenges.

Key words: Transgenic, drought, GM rice, phenotype, screening.

INTRODUCTION

Gene modification (GM) or gene technology is often proposed as a solution for increasing crop yields worldwide, particularly in less-developed areas that are threatened by food insecurity and low crop productivity (Nelson et al., 2007; Zhang, 2007). However the scientific debate over the potential of GM crops in the improvement of crop stress resistance is still highly litigious and the opinions vary from highly optimistic to extremely skeptical (Maris, 2008). While several public and private crop-science companies started to invest heavily in complex genetic traits, such as drought resistance as part of their GM research portfolio, the overall experience of four decades of crop physiological research on stress adaptation highlights the great challenge in translating such research into actual crop yield improvement (Sinclair et al., 2004).

*Corresponding author. E-mail: pherve@exelixis.com.

In a survey of the recent literature using the ISI Web of Science database, we found that the annual number of papers and citations on GM for 'drought tolerance' has been increasing almost exponentially for the last fifteen years, to over 120 papers and 3100 citations in 2007 (Figure 1). These studies were reported in well-reputed international journals and were mainly focused on plant model species such as *Arabidopsis* (365 articles), tobacco (248) and rice (123), and to a lesser extent in maize (66), wheat (38) and barley (29). Most of the rice studies were carried out in five major countries: China (30% of the publications), Japan (18.5%), USA (18.5%), India (17%) and South Korea (11%). While literature mining is becoming more and more critical in this field, it is noteworthy to notice that a large majority of these studies have surprisingly similar scientific hypotheses and common genealogies of the underlying concepts and methodologies. As reported by Passioura (2006), there are hundreds of patents that claim gene inventions and sequences that may improve drought tolerance.

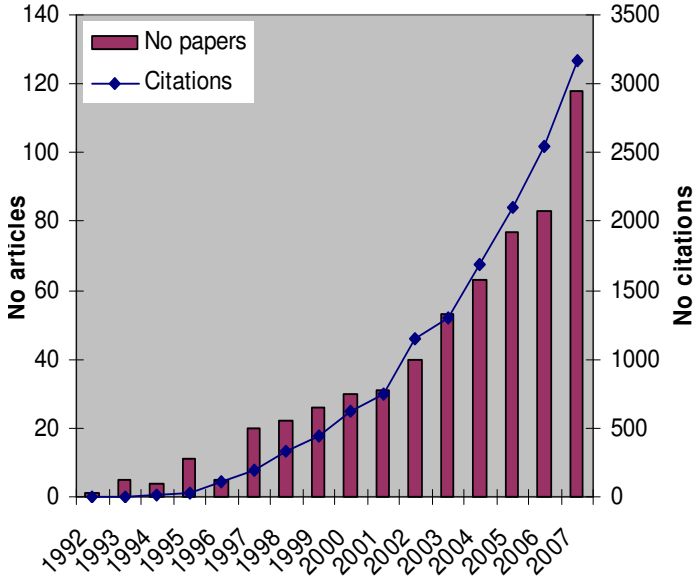

Figure 1. Papers and citations found in the Web of Science database, including 'drought' and 'transgenic' as topic keywords, between 1992 and 2007.

However, most of these studies report the positive effect of genes involved in stress signaling and metabolic pathways using plant evaluation protocols that are generally far away from an agronomic context, with no immediate prospects for producing suitable GM crops that could greatly improve drought adaptation or water productivity in the field.

Because of their high level of integration and multitude of interactions, crop stress responses and adaptation mechanisms are highly complex. At a given time, a single plant must respond to several abiotic and biotic environmental factors while ensuring development and housekeeping functions. At the genetic and molecular levels, this complexity has been illustrated by both the identification of multiple stress-related QTLs (Bernier, 2007; Yue et al., 2006; Zeng et al., 2006) and genome/transcriptome analysis (Bohnert et al., 2006; Kathiresan et al., 2006). Changes in the abundance of the transcripts at the whole genome level confirmed downstream structural genes involved in drought response mechanisms but also unraveled genes encoding regulatory proteins such as transcription factors and protein kinase/phophatases. This last group of genes is assumed to play a key role because they regulate other downstream stress inducible genes or proteins (Yamaguchi-Shinozaki and Shinozaki, 2006). The regulatory genes are contemplated as an entry point of a gene network.

Most of the experiments using gene technology are based on the hypothesis that a higher resistance level can be achieved by adding or modulating the activity of one or a limited number of key components. The over-expression of downstream genes and more recently regulatory genes has been repeatedly reported to enhance drought resistance in plants or crops for the last years. Although some of these experiments aimed at demonstrating a positive effect of a gene, they did barely provide validation of the results at a larger scale or in field conditions so far. In this review, we focused on seven different case studies describing enhanced drought resistance in rice. We did not comment about the scientific strategy used by the authors but critically assessed the methodology, the protocols for plant evaluation and the parameters used. Finally, we attempted to summarize some of the major steps and key criteria to identify better cultivars with enhanced drought resistance using gene technology. The procedures and drought phenotyping methodologies that we recently developed at the International Rice Research Institute (The Philippines) for rice are used as examples. Our overall approach may provide enabling solutions that can address some breeding challenges using biotechnology in particular for developing countries.

SOME LESSONS FROM RECENT LITERATURE

With more than 123 published papers reporting studies on GM rice for drought tolerance, we conducted an in-depth analysis of contrasting case studies based on seven papers. We compared the methodology and results of experiments that described the over-expression in rice of downstream genes encoding the aquaporin RWC3 (Lian et al., 2004), arginine decarboxylase (Capell et al., 2004), superoxide dismutase (Wang et al., 2005), trehalose-6-phosphate synthase/phosphatase (Garg et al., 2002) and the late abundant embryogenesis protein HVA1 (Babu et al., 2004) or transcription factors CBF3/DREB1A (Oh et al., 2005) and NAC1 (Hu et al., 2006). The genes used in these experiments are structural proteins, enzymes and transcription factors and their roles or effects had been previously demonstrated in other species or rice cultivars. We have chosen papers attempting different strategies and it is clear that in all cases, the authors aimed at conferring drought resistance to rice by recombinant DNA technology with prior knowledge of putative gene effects. The overall transformation methodology is summarized in Table 1. The most remarkable features are the diverse gene sources, the use of cDNA driven by an inducible or constitutive promoter, the use of *Agrobacterium tumefaciens* for gene transfer and the use of a japonica cultivar because of its ease for transformation. The vectors and the gene cassettes are commonly and broadly used for crop gene technology. The rice transformation methodology is based on the transformation of callus tissues and selection

Table 1. Transformation methodology.

Article	Lian et al 2004	Capell et al, 2004	Oh et al, 2005	Wang et al, 2005	Garg, 2002	Hu, 2006	Babu et al 2004
Journal	Plant Cell Physiology	PNAS	Plant Physiology	Journal of Plant Physiology	PNAS	PNAS	Plant Science
Gene	RWC3 (aquaporin)	adc (Arginine Decarboxylase)	CBF3 / ABF3	MnSOD (Super Oxyde Dismutase)	OtsA and OtsB	SNAC1	HVA1
Known Function	Aquaporin	Polyamine biosynthesis	Transcription Factor	Reactive oxygene species scavenging	Trehalose biosynthesis	Transcription Factor	LEA protein, membrane
Gene source	rice (?)	Datura	Arabidopsis, Rice	Pea	E Coli	upland rice IRAT109	Barley
DNA	genomic	cDNA	genomic / cDNA	cDNA	genomic	cDNA	cDNA
expression	stress inducible (oxidative stress)	constitutive	constitutive	inducible	inducible	constitutive	constitutive
promoter	SWAP2	maize Ubi-1	maize Ubi-1	SWAP-2	ABRC1-Actin1-HVA22	CaMV 35S	Actin1
Genotype	Zhonghua 11	nd	Nakdong	Zhonghua-11	Pusa Basmati PB-1	Nipponbare	Nipponbare
spp	japonica	nd	japonica	japonica	indica	japonica	japonica
Plasmid	pCambia1301	nd	nd	pCambia1301	pSB11	pCambia1301	nd
Gene Transfer	Agrobacterium	Agrobacterium	Agrobacterium	Agrobacterium	Agrobacterium	Agrobacterium	Biolistic (1996)
Selectable marker	Hygromcyin		bar	Hygromycin	bar	Hygromycin	bar
Analysis of transgenic	PCR		Southern ; RNA blots	PCR		PCR; Northern; Southern	Herbicide, Southern
Number of transgenic plants	14	50	15 / 20	15	29	29	63

with either antibiotic or herbicide agents. Surprisingly, the number of independent primary events reported in these studies is relatively low although the transformation efficiency is high with the used cultivars.

As shown in Table 2, the design of the phenotypic screening is often insufficiently described but the population size is small or unknown, which does not allow a good assessment of the results. As highlighted above, the number of independent events that are reported in these publications for drought resistance evaluation is very low. It is however necessary to assess a significant number of events in order to take into account possible position effect of the transgene. It is well known that the expression level of a gene (or transgene in that case) can be regulated by its position in the genome (structural regulation). Somaclonal drag caused by tissue culture is another important unknown change in the genome that must be taken into consideration since it may influence the overall effect of a transgene. Another common feature is the use of wild type plants as controls except by Hu et al. (2006) who did report the use of isogenic lines. The comparison of transgenic lines versus wild type plants is very common but it does not take into account possible somaclonal drag induced by the transformation protocol. In the case of rice, the re-activation of transposons that may occur during *in vitro* tissue culture may affect the overall plant performance and cannot be neglected. For T1 or T2 population, it is thus more appropriate to compare transgenic lines versus null segregants or isogenic lines as described below.

The phenotypic screening protocols are summarized in Tables 3 and 4. All studies described plant evaluation in green house conditions using pots or hydroponic cultures. Only Hu et al. (2006) did report an extensive screening including paddy field/rain-out shelter and field evaluation. In these studies, drought stress is imposed by water-withholding or replacing water by a PEG solution. As recently discussed by Bhatnagar et al. (2008), the use of PEG in hydroponics can be useful to test certain response of plants under a given osmotic potential (Pilon-Smits et al., 1999), but it offers relatively different conditions than those in the soil where the water reservoir is finite, and dynamics of soil drying is an inherent part of the stress response mechanisms.

It is also noteworthy to indicate that the size of the pots differs greatly from one study to another and that the intensity and the timing of drought treatment is thus very different (data not shown). These studies clearly used different drought treatment to assess the resistance of the transgenic lines and *ad hoc* measurement of drought stress level is barely provided. Sixteen different parameters are monitored but only six of them are used in at

Table 2. Design of the phenotypic screening.

Article	Lian et al 2004	Capell et al, 2004	Oh et al, 2005	Wang et al, 2005	Garg, 2002	Hu, 2006	Babu et al 2004
Number of transgenic lines	14	50	15 / 20	15	22 (29)	29	63
Number of transgenic lines analyzed (T1)	nd			2		5	3
Population size	nd			nd		20 (fertility);6 (mRWC)	
Number of transgenic lines analyzed (T2)		10 (1)				5 (?)	2
Population size		nd				# 70	nd
Number of transgenic lines analyzed (T4)			3 (homozygous)		6 (2 with published data)		
Population size			18 (Fv/Fm) ; 36 (survival rate)		15		
Control	Wild Type	Wild Type	Wild Type	Wild Type	Wild Type	Wild Type	Non Transgenic
						No expression transgenic line	
						isogenic line	

Table 3. Physiological and morphological parameters used in the phenotypic screening.

Article	Lian et al 2004	Capell et al, 2004	Oh et al, 2005	Wang et al, 2005	Garg et al, 2002	Hu et al, 2006	Babu et al 2004
Stage	4 weeks old	8 weeks old	4 weeks old	5 weeks old	5 weeks old	Reproductive Anthesis	6 weeks old
drought prone field							
paddy field - RO shelter							
Soil - Greenhouse							
Hydroponic - Greenhouse							
Relative water Content (RWC)							
Leaf water potential							
Leaf osmotic potential							
minimum RWC							
Photosynthesis rate							
Chlorophyll fluorescence Fv/Fm							
soluble carbohybrates level							
stomata closure							
Transpiration rate							
relative cumulative transpiration							
Leaf rolling							
Root osmotic potential							
spikelet fertility							
Root biomass							
Shoot biomass							
Survival rate (recovery rate)							

Table 4. Comparison of the measurements of parameters used by at least two studies.

Article		Lian et al 2004	Capell et al, 2004	Oh et al, 2005	Wang et al, 2005	Garg et al, 2002	Hu et al, 2006	Babu et al 2004
Stage		4 weeks old	8 weeks old	4 weeks old	5 weeks old	5 weeks old	Reproductive Anthesis	6 weeks old
Relative water Content (RWC)	Transgenic						62% (5days)	92% (28 days)
	Control						50% (5days)	51% (28 days)
Leaf water potential (Mpa)	Transgenic	- 1 (88%)						- 1.6 (62%)
	Control	-1.14						-2.6
Photosynthesis rate (% decrease to initial rate)	Transgenic				86%		75%	
	Control				67%		75%	
Chlorophyll fluorescence Fv/Fm (photooxydative damage)	Transgenic			0.55		0.55		
	Control			0.35		0.3		
Transpiration rate	Transgenic						0.9	
	Control							
relative cumulative transpiration (% initial value)	Transgenic	55%						
	Control	42%						
Survival rate (recovery rate) after re-watering	Transgenic			83 / 58%			50%	
	Control			0 / 8%			10%	

least two studies: relative water content (RWC), leaf water potential, photosynthesis rate, chlorophyll fluorescence, transpiration rate, survival rate or plant recovery rate after re-watering. Important physiological parameters such as yield components, spikelet fertility, or root biomass are however used in only one study. Furthermore, even the simplest visual observation of leaf rolling to monitor the drought stress level is not systematically reported.

As shown in Table 4, there is a similar trend of the different parameters measured under different managed-stress treatments whatever the strategy is. A higher RWC, reduced leaf water potential, higher photosynthesis rate and chlorophyll fluorescence, reduced transpiration rate and higher recovery rate were observed. In each study, the correlation between the over-expression of a gene and a physiological or biochemical response of the plant or plant cell under stress has been established and the authors did conclude about an improved drought resistance in the transgenic lines based on observed parameters. It remains however questionable what significance such reported drought resistance can have on crop performance and yield under stressed and non-stressed conditions. It would also be relevant to assess the same parameters and the performance of all these transgenic lines under similar drought treatment. The next step is an evaluation of the lines under paddy field/field conditions since it is difficult to extrapolate the reported data from pot studies and hydroponics to field performance, in particular for yield and biomass accumulation. A recent comparative approach in rice for a set of genes (Xiao et al. 2009) provide an excellent basis for better assessing the impact selected genes.

Finally, the phenotypic evaluation of the transgenic lines under normal irrigation was not reported in these studies except by Hu et al. (2006). For example, the higher stomatal closure observed under drought in transgenic lines over-expressing SNAC1 is also observed under normal irrigation (Hu et al., 2006) but the authors did report that the photosynthesis rate was however not affected. It is very important to evaluate the transgenic lines under normal irrigation since a better drought resistance may, in some cases, impair the overall performance of a crop under optimal conditions.

Several key issues and questions arise from the above review, including the pertinence of the choice of the tar-

get candidate genes, the transformation protocols, experimental and statistical designs, and the relevance of the screening protocols and criteria for the breeding programs.

EXPERIMENTAL DESIGN AND PRE-SELECTION OF EVENTS

The positive effect of a transgene or a combination of transgenes in a given cultivar does require the evaluation of several primary events. For a complex trait like drought, we recommend the phenotypic evaluation of at least 15 independent single copy events at the T1 generation. Special attention must be given to replication and statistical design, in order to increase trait heritability, the statistical power of the experimental comparison and reduce probabilities of Type 1 and 2 errors. Since the amount of T1 seeds from each primary event is often very limited, it is important to strengthen the first phenotypic screening by more events. The more events show a positive trend under stress conditions the more likely the gene may confer drought resistance to the cultivar.

Current gene technologies for both indica and japonica rice cultivars are very efficient and there is no major technical bottleneck in producing large number of primary events provided that the facilities do not cause any space limitation (Hervé and Kayano, 2006; Hiei and Komari, 2006). Single copy or insert events with at least 100 T1 seeds should be the preferred material for phenotypic evaluation and we argue that expression analysis is not necessarily required at an early stage (T0 plants) because transgene-expression study of primary events does not provide very informative data. We do thus favor systematic phenotypic screening of all single copy events without pre-selection of the events based on the transgene expression. Such expression analysis could rather be done during the second screening in order to establish a possible correlation between the phenotype (stress resistance level) and the expression level of the transgene. Since space for screening is often a major bottleneck, a powerful and relevant molecular screening at an early stage can however include expression analysis of a selected set of genes that are involved in the mechanisms of action being targeted by the transgene. By doing so, the molecular screening allows selecting the events based on the functional drought resistance mechanism induced by the transgene. One must however keep in mind that expression screening at an early stage may be inappropriate if one aims at using drought inducible promoter to drive the expression of the transgene. Finally, it is important to analyze isogenic lines for each event in order to exclude any unknown effect that is not due to the insertion of the transgene. Once it has been demonstrated that the null segregant population does not differ from the wild type during the first screening, only the wild type population can be used as a control in the next generations.

DROUGHT SCREENING CRITERIA

It is essential to link the drought phenotypic screening of GM rice to breeding at a very early step. A comparative phenotypic evaluation of transgenic lines with several breeding lines, both susceptible and resistant, is highly recommended for the first screening. Such a screening would not only allow a precise monitoring of the applied drought stress level but also identify, if any, a competitive advantage of the transgenic lines versus the promising breeding lines. It does obviously require large scale infrastructure but it may speed-up the decision making about the beneficial effect of a gene. Finally, the performance of the events should be assessed under both stress and normal irrigation in order to unravel any off types and any yield penalty in optimal conditions while identifying the best lines under drought stress.

Both the genetic background and the biophysical environments where the GM plants are grown and evaluated will have large impacts on gene expression and plant performance. As it is unlikely that universal ‘drought tolerance’ traits may be identified, it is important to take G x E into account while screening GM plants under drought. Any putative drought resistance trait is unlikely to be important across all water-deficit scenarios. A drought trait that might offer substantial benefit in one weather scenario of developing drought, e.g. early closure of stomata, might well result in a negative response in another scenario (Sinclair and Muchow, 2001). One way to overcome the large G x E limitation is to understand the basic processes accounting for the drought trait and how the mechanism reacts under a range of weather scenarios. Simulation models can provide a way to overcome this limitation by combining mechanistic understanding of a drought trait with a range of weather scenarios. Breeding for specific drought resistance characters can thus be targeted to those geographical regions that would have the highest probability of frequent yield increases. One other possibility to overcome G x E limitation is to adopt a reverse physiology approach, which starts from the measurement of plant performance under drought (Figure 3).

The parameters to be measured during the first phenotypic screenings are obviously important. Because of the inherent technical bottleneck of gene technology (limited amount of seeds, evaluation in confined environment, etc.), it is crucial to monitor the most relevant morphological and physiological parameters. However,

since it is difficult to predict crop yield under drought field conditions from artificial growth conditions (pots and/or hydroponics), one must start with the end in mind, and first evaluate the GM plants for performance under realistic soil drying similar to that occurring in the field. Also, because of the large numbers of transgenic events generated in a high throughput transformation program, it is more efficient to discard a maximum of plants at an early stage. The most robust and integrative selection criteria are biomass accumulation and yield performance. One may want to assess the impact of water deficit on plant growth and non destructive measurements could be the preferred methodology. Parameters such as plant phenology, canopy growth and temperature measurements with imagery, leaf rolling, tillering ability, root biomass, spikelet fertility are relatively simple parameters to be measured for a large number of events and plants. A correct assessment would require two cycles of screening. We argue that the one successful approach would require at least two cycles of large scale screening of events with two cycles of phenotypic evaluation of a limited number of events. It would require a minimum of 2 years to perform such preliminary evaluation. While the large scale screening would demonstrate a gene effect, a more precise phenotyping would allow identifying the most suitable events to be further evaluated in field conditions. As already mentioned, it is essential to include both susceptible and resistant cultivars during the early screening since the application of the drought treatment and re-watering decision would require a visual inspection of well known cultivars. These reference cultivars are the key controls for a linkage of improved lines by gene technology with breeding.

GENE TECHNOLOGY AND REVERSE DROUGHT PHYSIOLOGY

We recently established a new drought screening facility and procedures for transgenic research on drought at IRRI. Due to the bio-safety requirements, it is logistically challenging to perform early drought screening of large populations of transgenic events under field conditions. It is thus necessary to establish a robust and reliable procedure that allows the identification of likely successful transgenic events that can be further evaluated in field conditions. In order to design the screening procedure, the two major considerations were thus 1) a screening facility to mimic field conditions and 2) a screening protocol to realistically demonstrate and validate a gene effect. A containment screen house facility with two independent one meter deep soil beds of equal surface was chosen, which does allow a simultaneous screening under both irrigated and drought conditions (Figure 2A). The drought screening facility is equipped with four main

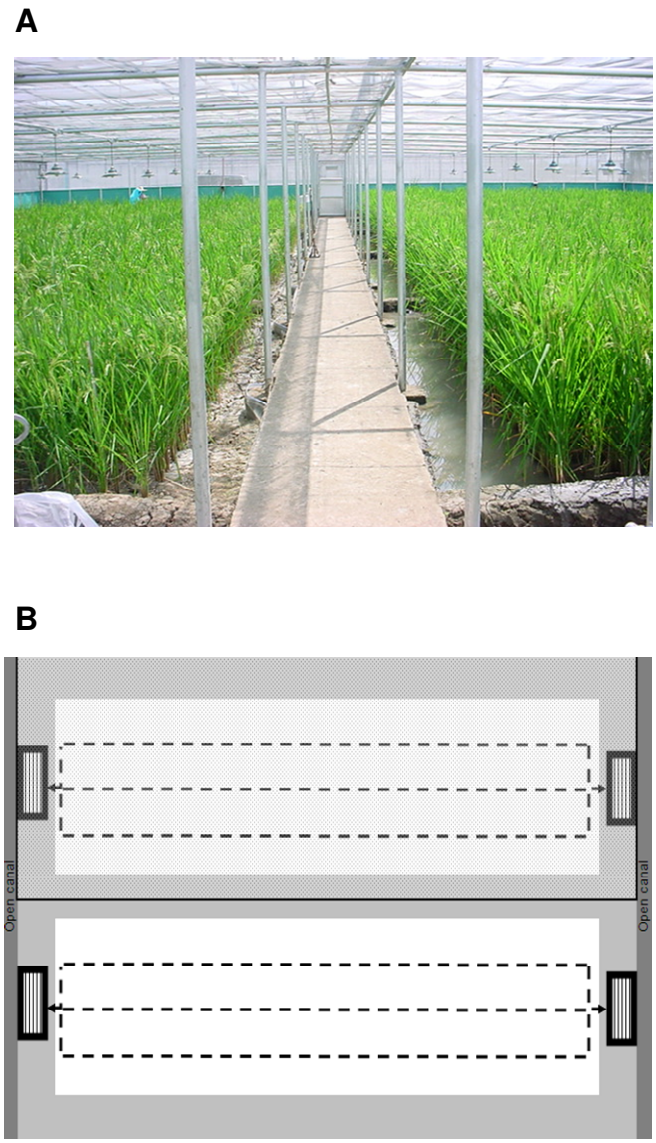

Figure 2. Design of screen house for drought screening of GM rice. (A) General view, with a drought-stressed drained plot covered as rainout shelter (left) and flooded well-watered control (right). (B) Each plot is equipped with three drainage pipes (dashed lines) that are connected at each end of the bed to a sump pit.

systems: (1) a screen house is equipped with a rainout shelter installed only above the bed used for the drought treatment to prevent any rainfall event during the screening. The roof and surrounding screen house divider are made of a double layer of mesh to satisfy bio-safety requirements. Incandescent lamps are installed to provide supplemental lighting if necessary, (2) environmental parameters such as air temperature, relative humidity and vapor pressure deficit (VPD) are monitored continuously through data-loggers, to take into account

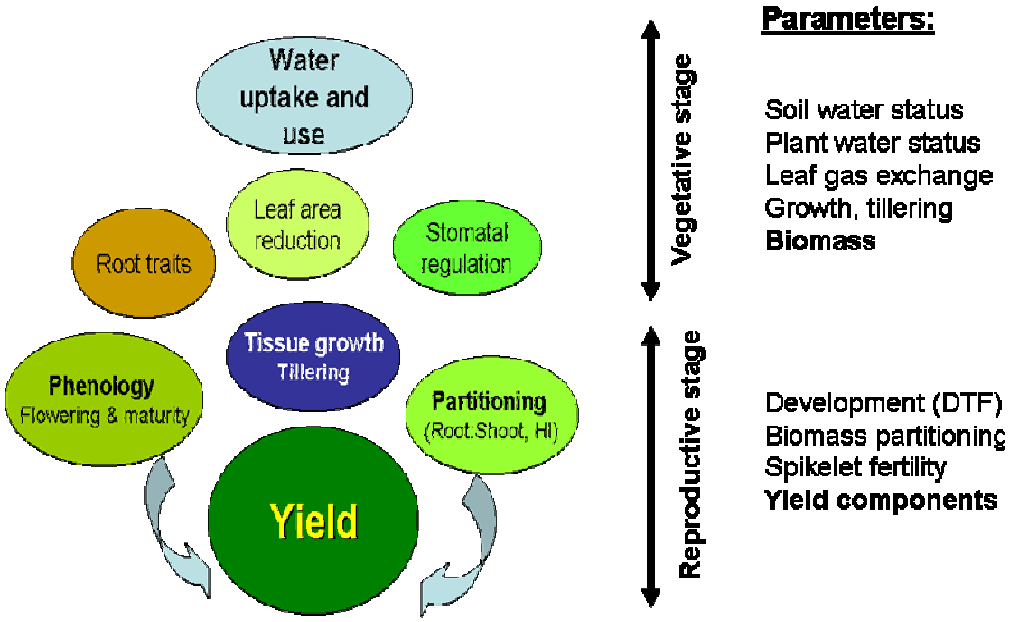

Figure 3. Physiological framework for drought screening: analysis of crop growth and yield components.

the genotype x environment interactions in each screening experiment, (3) a plastic sheet is placed at 1 m depth, surrounding the drought bed, to prevent water seepage and percolation from adjacent flooded plots, (4) each soil bed is equipped with a drainage system consisting of deep pipes at 0.9 m depth that are connected at each end of the bed to a sump pit (Figure 2B). The drainage pipes are wrapped in geotextile fabric and surrounded with small gravels to avoid soil particles from clogging the geotextile. The gravels are placed as a padding to create the needed slope for the pipes. Finally, the pipes are further surrounded with gravel to make at least 4 inches distance between the pipes and the soil layer. This overall design facilitates gravitational flow of the drained water toward the pits at both ends, which allows to gradually reducing the soil moisture of the top 50 cm soil layer.

We established a screening protocol that may facilitate early drought evaluation of large number of events based on an alpha-lattice design with four replications of 17 blocks and five plots each. The material tested in each experiment consists of at least 20 independent single copy events, 5 control varieties including the recipient of the transgene, one drought sensitive variety, two drought resistant varieties and the upland-adapted cultivar Apo that is also used as border plants, and two treatments (irrigated and drought) are evaluated in parallel. The control plot is maintained under flooded conditions and the drought-stressed treatment is imposed by draining the water and gradual soil drying. Periods of managed water deficits are imposed with precise parameters of stress timing, duration and severity. Soil water deficits is imposed a few weeks after transplanting throughout the period bracketing the flowering and grain setting stages, with soil moisture levels decreasing from fully saturated to minimal levels of the Fraction of Transpirable Soil Water (FTSW) (Serraj, personal communication). After the water deficit treatment, plants are generally re-watered and kept under well-watered conditions until physiological maturity.

One of the major pitfalls of drought field screening is the spatial variation of soil moisture due to patchiness of soil draining and drying, which results generally in low heritability and high coefficients of variation. Thus, our drought screening protocol, we used piezometer access tubes that are placed in each drought replication block, to monitor water table levels across the drought bed. Soil moisture profiles are monitored using capacitance probes (Diviner-2000) at different depths in the range from 10 - 70 cm, and by placing 16 soil tensiometers at 15 - 30 cm depth, to monitor the soil water tension in the drought plot, daily throughout the dry-down period.

Plants are continuously monitored for phenology, plant water status and scored for drought stress symptoms. Plant water status parameters (i.e., leaf water potential and relative water content) are measured twice a week during the stress period. For selected sets of transgenic events and their corresponding nulls and checks, leaf gas exchange measurements (photosynthesis, stomatal conductance, Ci and transpiration) are measured using the LiCor6400 photosynthesis system, twice a week during the stress period. At the end of the dry-down experiment,

plants are harvested and evaluated for biomass accumulation and yield components. Additional parameters measured include plant height, tillers and panicles numbers at flowering. Panicles are threshed and total number of spikelets per plant is determined in addition to spikelet sterility percentage. Plant tissue samples are collected during critical periods of the dry-down for various biochemical and hormonal analyses. Finally, to better integrate the physiological phenotyping parameters, we adopted a reverse physiology approach, which consists in focusing the drought evaluation on biomass accumulation and performance under drought (Figure 3), and only investigating the underlying drought response mechanisms in the lines showing promising trends in terms of plant growth and performance under drought.

The first generation of transgenic events (T1) is compared to the control varieties under drought conditions, which allows identifying the best performing events for each gene construct. Isogenic null segregant T1 plants for each event are grown under irrigated conditions to identify any particular off-type lines or yield penalty under optimal conditions. Although it is possible to evaluate null segregants under drought conditions, it may be logistically preferred to optimize the use of the drought plot by increasing the number of events or plants per event to be screened under stress conditions. This first screening allows thus to identify some transgenic lines exhibiting both an optimal performance under optimal or irrigated conditions (no visible negative somaclonal drag in both the transgenic plants and the isogenic null segregant progeny) and the best performance of transgenic plants under stress conditions (possible gene-construct effect). For a subset of selected events from the first screening, the second generation (T2) of transgenic and isogenic null segregants are evaluated in both irrigated and drought conditions. Under drought conditions, the comparison of transgenic plants and their isogenic null segregants for each event allows to validate a gene effect.

The first drought screening experiment using the GM rice screen house facility and approach described above was carried out during the dry season of 2007. We did successfully achieve a gradual reduction of soil moisture and we observed a significant reduction of the soil moisture of the top 50 cm soil layer (data not shown). The drought intensity was sufficient to trigger drought symptoms matching those observed in rain fed lowland field conditions. Our phenotypic data also showed that the calculated yields under irrigated and drought conditions were very similar to the ones obtained in field conditions. For example, the 2007 trials showed that the average yield of rice transformants in the elite indica variety IR64 varied between 9.0 - 30.5 g plant^{-1} and 1.5 - 12.5 g plant^{-1}, under irrigated and drought treatments respectively, corresponding to an equivalent of 1.8 - 6.1 and 0.3 - 2.5 t ha^{-1}. These yield levels were very similar to those observed in non-transgenic lines grown under open field conditions at IRRI during the same year. These data suggest that our design of the screening in a containment facility can precisely mimic open field conditions and can sustain a robust screening procedure.

DROUGHT SURVIVAL VS CROP PERFORMANCE

While substantial research efforts on GM crops for drought has been so far devoted to drought tolerance genes focusing on survival stage under severe stress, several authors did repeatedly demonstrate the little scope this strategy has for crop improvement without further refinement of that approach (Serraj and Sinclair, 2002; Sinclair et al., 2004; Bathnagar et al., 2008). On the other hand, dehydration avoidance strategy is more likely to be relevant as a general approach to relieve agricultural drought and maintain crop performance, before survival drought develops. In rice, long-term multi-location drought studies demonstrated that rain fed lowland rice is mostly a drought avoider. The genotypes that produce higher grain yield under drought are those able to maintain better plant water status around flowering and grain setting. With a few exceptions, studies in rice focused on plant survival and tolerance traits rather than harnessing the dehydration avoidance mechanisms, which may have a better scope for improving rice productivity in the drought-prone rain fed environments.

Finally, there are two general targets for increasing crop yield per se in the drought-prone rain fed environments: (i) increase the overall capacity of plants to produce harvestable yield, and (ii) ameliorate the resistance to abiotic stresses. The main challenge for deploying successful gene technologies for stress environments is not different than other breeding approaches and is to what extent any improvement for a target environment does compromise the yield potential of the crop. Farmers are more interested in crop performance and yield stability than in drought tolerance per se. Therefore, it is crucial to measure systematically the variations in biomass production and yield components that result from gene modification.

CONCLUDING REMARKS

Skepticism about the potential of improving complex traits by gene technology may come from the methodology such research has been conducted or reported so far. In this short note, we reviewed the recent literature, and analyzed seven different case studies describing enhanced drought resistance in rice by over-expressing a

single gene. Although each paper provided supportive evidences that the transgenic lines did show better growth under drought treatment, the overall criticism of these studies is the low number of events and a lack of convincing data reporting the evaluation of transgenic material. This is likely due to a lack of linkage with breeding and to major technical bottlenecks. It is thus urgent to fully integrate gene technology within breeding programs and link it with proper physiological dissection and to assess the transgenic lines like any other cultivars. It is also necessary to fasten the introgression or stacking of any trait to the most promising advanced breeding lines or stress-sensitive rice mega-varieties. There has been a substantial amount of research devoted to improve drought resistance using transgenic plants, especially in the private sector, and this will contribute to sustaining yield of rice breeding lines under adverse environments. However, the bottleneck has remained the screening procedure. We suggest an early screening in deep soil bed in a containment facility that can offer the advantage of mimicking field conditions leading to a robust pre-selection of events, based on biomass accumulation and performance under both well-watered and drought conditions. This can then undergo multiple locations field trials, which would provide the ultimate validation of any improved variety. A key take home message is that one must deploy gene technology like any other breeding approach and it is critical to assess to what extent any improvement for a target environment does compromise the yield potential of the crop across a range of environments. By doing so, gene technology will successfully and efficiently become part of a simple solution to a complex trait.

ACKNOWLEDGEMENTS

The author would like to thank Dr Bas Bouman, Ms Olivyn Angeles and Mr Rolly Torres for their advice in designing the drainage system and establishment of the screen house for the drought evaluation described in this paper.

REFERENCES

Chandra Babu R, Zhang J, Blum A, Hod THD, Wue R, Nguyen HT (2004). *HVA1*, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection, Plant Sci. 166: 855-864

Bernier J, Kumar A, Venuprasad R, Spaner D, Atlin GN (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Sci. 47: 507-518.

Bhatnagar-Mathur P, Vadez V, Sharma KK (2008). Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep. 27: 411-424.

Bohnert HJ, Gong Q, Li P, Ma S (2006). Unraveling abiotic stress tolerance mechanisms – getting genomics going, Curr. Opin. Plant Biol. 9: 180-188.

Capell T, Bassie L, Christou P (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. PNAS 101(26): 9909-9914.

Garg AK, Kim JK, Owens TG, Ranwala AP, Choi YD, Kochian LV, Wu RJ (2002). Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses, PNAS 99(25): 15898-15903.

Hervé P, Kayano T (2006) Japonica rice varieties (*Oryza sativa* Nipponbare and others), pp 213-222. In *Agrobacterium* Protocols, Second Edition, Volume I, Wang K (Ed), Humana Press, ISBN 1-59745-130-4

Hiei Y, Komari T (2006) Improved protocols for transformation of indica rice mediated by *Agrobacterium tumefaciens*, Plant Cell Tissue Organ Cult. 85: 271-283

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. PNAS 103(35): 12987-12992.

Kathiresan A, Lafitte HR, Chen J, Mansueto L, Bruskiewich R, Bennett J (2006). Gene expression microarrays and their application in drought stress research, Field Crops Res. 97: 101-110.

Lian HL, Yu X, Ye Q, Ding XS, Kitagawa Y, Kwak S-S, Su W-A, Tang Z-C (2004). The Role of Aquaporin RWC3 in drought avoidance in rice. Plant Cell Physiol. 45(4): 481-489.

Marris E (2008). More crop per drop. Nature 452: 273-277.

Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG,. Hinchey BS, Kumimoto RW, Maszle DR, Canales RD, Krolikowski KA, Dotson SB, Gutterson N, Ratcliffe OJ, Heard JE (2007). Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. PNAS 104 (42): 16450-16455.

Oh S-J, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK (2005). Arabidopsis CBF3/DREB1A and ABF3 in Transgenic Rice Increased Tolerance to Abiotic Stress without Stunting Growth, Plant Physiol, 138: 341-351.

Passioura J (2006). Increasing crop productivity when water is scarce - from breeding to field management. Agric. Water Manage. 80: 176-196.

Pilon-Smits EAH, Terry N, Sears T, van Dun K (1999) Enhanced drought resistance in fructan-producing sugar beet. Plant Physiol Biochem 37: 313-317.

Serraj R, Sinclair TR (2002). Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25: 333-341.

Sinclair TR, Purcell LC, Sneller CH (2004). Crop transformation and the challenge to increase yield potential. Trends Plant Sci. 9 (2): 70-75.

Sinclair TR, Muchow RC (2001). System analysis of plant traits to increase grain yield on limited water supplies. Agron. J. 93: 263-270.

Wang F-Z, Wanga Q-B, Kwon S-Y, Kwak S-S, Su W-A (2005). Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide Dismutase, J. Plant Physiol. 162: 465-472

Xiao BZ, Chen X, Xiang CB, Tang N, Zhang QF, Xiong LZ (2009) Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. Mol. Plant 2(1): 73-83.

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional Regulatory Networks in Cellular Responses and Tolerance to Dehydration and Cold Stresses. Annu. Rev. Plant Biol., 57:781–803

Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K, Jin D, Xing Y, Zhang Q (2006) Genetic Basis of Drought Resistance at reproductive Stage in Rice: Separation of Drought Tolerance From Drought Avoidance, Genetics 172: 1213-1228.

Zeng H, Zhong Y, Luo L (2006) Drought tolerance genes in rice, Funct. Integr. Genomics 6: 338-341.

Zhang Q (2007). Strategies for developing Green Super Rice. PNAS 104(42): 16402-16409.