20 Gene Flow as a Source of Adaptation of Durum Wheat to Changing Climate Conditions

Double Gradient Selection Technique

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INTRODUCTION

Fluctuating environments are recurring scenarios in dryland agriculture with detrimental effects to crop production such as durum wheat crop that could lead to losses in yield. The dryland Mediterranean type of environment in particular vis-à-vis climate is characterized by low and highly erratic annual rainfall varying from 200 to 800 mm, with usually a poor rainfall distribution coupled with intermittent periods of drought and temperature extremes (cold and heat) that can occur at any stage of durum wheat plant development (Nachit et al. 1992a, 1992b).

These environmental fluctuations and stresses are expected to amplify as climate models show relatively consistent predictions that the Mediterranean basin will become hotter and drier over the next century with annual precipitation likely to decrease by 4%–27% and 3–5°C increase in temperature (IPCC 2007, Habash et al. 2009). The assessment of the fifth report remains in agreement with the later projections including the probable increase in extreme weather events.

Because of limitation of irrigation on a large scale for durum wheat commercial production, the only applicable alternative is the improvement of drought tolerance and yield stability through genetics and plant breeding supported by an understanding of the environment fluctuations, stress physiology and the use of molecular markers.

This chapter gives a broad review of the durum wheat research program, with emphasis on the challenges involved, particularly drought research to cope with water limitations and environmental fluctuations. Overall, the chapter focuses on strategies to breed for fluctuating environment in the context climate changing conditions.

STRATEGY OF ADAPTATION

The ICARDA dryland durum wheat-breeding program was initiated in 1977 in northern Syria. The ICARDA main research station (Tel Hadya) and its related research sites (Latakia, Terbol, Kfardan, and Breda) are located in dryland area where agriculture originated and evolved across different environmental climatic gradients over millennia. This region, known as the Fertile Crescent harbors, as a result, has a wealth of wheat landraces along with their wild relatives spanning these different agro-ecological zones, from lowland plains to highland plateaus, and from favorable to stressed environmental conditions (Nachit 1992, 1998a). There are thousands of landraces that were grown over long periods and are still found along with their related wheat *Triticum* parental species (Nachit and Elouafi 2004). These genetic resources grow under drought, cold, and heat as well as under multiple biotic stresses with the highest virulence of disease races and insect biotypes along the gradient environmental conditions.

The combinations of these abiotic and biotic stresses made the breeding work in dryland, such as West Asia and North Africa (WANA), both complex and challenging. Thus these wheat landraces and their wild relatives have been strategically incorporated in germplasm renewal continuously to buffer the environmental change and fluctuating conditions (Nachit and Ouassou 1988, Nachit 1992). This sustained gene flow from landraces and wild relatives to the improved varieties has been adopted strategically to enable durum wheat crop to withstand environmental fluctuations, abiotic stresses, and recurrent biotic virulence.

Modeling of gene flow has shown that not only does it enhance genetic variation, and thus the capacity to adapt, but it could also increase directly the population's adaptation as it introduces alleles that are newly adaptive in the recipient population (Shaw and Etterson 2012). Gene flow may involve a large number of networked genes that have been found to be involved with traits, such as those associated with water-use efficiency (WUE). The expression of some genes may increase or decrease the expression of other genes, thus forming a complex network of interactions (Liebovitch et al. 2006). There are a number of such complex networks of genes that involve genotype–environment interactions and also epistatic interactions between genes regulating variation for traits (Cooper et al. 2000, Dennis 2002). The interaction and functioning among these networks of genes could be predicted if these networks were understood and appropriately quantified (van Oosterom et al. 2004). The models of gene networks are based on the expression of gene i as a level of mRNA, with value X, among N genes, represented by a connection matrix M. The relationship of a new value X' at a subsequent time with the original X is given as

$$X' = MX$$

The crosses that have been made with the sustained gene flow have led to the development of germplasm with essentially durum genotypes performing well under stressed environments with abiotic constraints such as drought and temperature extremes.

The assessment of the extent of genetic variation created and acquired was based on the use of trait measurements such as carbon isotopic composition ($\delta^{13}C$), which is a useful surrogate for integrated plant WUE. Negative $\delta^{13}C$ is indicative of lower WUE. The definition of $\delta^{13}C$, expressed in per thousand units (‰), is given as follows:

$$\delta^{13}C = \left[\left(R_{\text{sample}} / R_{\text{standard}}\right) - 1\right] \times 1000 \tag{20.1}$$

where R is the ratio between carbon 13 (^{13}C) and carbon 12 (^{12}C) isotopes.

The $^{13}C/^{12}C$ ratio of plant biomass is used as a natural indicator of photosynthetic WUE to measure the variation of $^{13}C/^{12}C$ in plant tissue with respect to the atmospheric source of carbon during growth (Farquhar et al. 1989, Nachit 1998b, Xu et al. 2004).

In C3 plant species, there is discrimination against ^{13}C, in comparison to the more abundant ^{12}C in the atmosphere during photosynthetic carbon capture. Genetic variation in transpiration efficiency has been found to be negatively correlated in C3 species (Xu et al. 2004).

TARGETED ENVIRONMENT

Before developing a selection procedure for tolerance to abiotic stresses, it is crucial to determine the frequency of occurrence of a particular stress and its timing in relation to crop development in each agroecological zone. Results of our earlier selection work under contrasting environments show that genotypes selected under favorable conditions do not necessarily do so under less favorable conditions and vice versa. It may be difficult to select for genotypes with a high-yield potential in dryland environments, but it is far more difficult to select for moisture-stress tolerance under high-input environments (Nachit 1992). However, it appears that selection only under extreme environmental conditions (too favorable or too dry) is not an efficient way to identify cultivars for Mediterranean drylands, which are characterized by a high year-to-year variability and by an unpredictable alternation between favorable and less favorable seasons. Breeding cultivars that combine yielding ability with stress tolerance and yield stability are therefore a prerequisite to the adaptation to the Mediterranean dryland conditions (Nachit 1989).

In our selection strategy, all early segregating populations are subjected to the stresses encountered in the Mediterranean dryland, for example, drought, cold, heat, rusts, *Septoria tritici*, root rot, Hessian fly, and stem sawfly, with the aim of identifying the populations that do particularly well in certain environments and are not sensitive to the stresses of other environments (Nachit 1996). The pedigree method of selection is used to select individual plants from the populations that were selected across sites/environments. As for the bulk method, it is more extensively used to select among populations across sites during the winter and summer testing. This method presents the double advantage of testing more crosses at several sites/environments. The promising bulked segregating populations are tested in several agroecological zones of the Mediterranean region in collaboration with national programs in the region. With this selection procedure, it is possible to identify at early stages the populations that combine productivity and stability with tolerance to biotic and abiotic stresses. Disease resistance requires constant surveillance, as it can be broken by new races, as in the case of rusts.

DOUBLE GRADIENT SELECTION TECHNIQUE

Dryland productivity can fluctuate from null to 6 tons per hectare. This fluctuation may also be accompanied by variations in attacks by biotic stresses. In relatively favorable rainfall seasons, attacks by rusts and *Septorai tritici* are a threat to the crop, whereas, in the dry seasons, Hessian fly and wheat stem sawfly are the major biotic constraints with devastating effects when the environment is conducive for these diseases to thrive. Thus, the basis of our breeding work is to select durum wheat populations and advanced lines with resistance to abiotic and biotic stresses, and to test for yield stability and productivity under Mediterranean dryland conditions. The cornerstone of this strategy is the introgression of resistance genes from landraces and wild relatives to durum advanced genotypes and the utilization of contrasting and representing environments in the Mediterranean region. A double gradient selection technique (DGST) was developed in the 1980s. The DGST sites are representatives of abiotic and biotic stresses in WANA for temperature extremes, varying from cold to hot, and for water regimes, varying from severe drought to irrigated conditions. The DGST reflects the environmental fluctuations encountered in the Mediterranean drylands.

The DGST employs five environments that are extensively used during the various phases of selection in segregating populations and testing of advanced lines. The DGST approach has its origin from practice, and later in the program its conceptual framework has been established. Under the DGST, the environments/sites used were as follows:

1. Tel Hadya, the main research station at ICARDA-HQ, located in Syria at 36°01′ N latitude, 36°56′ E longitude, and 284 masl. It has a Mediterranean continental climate with an average annual precipitation of 335 mm.
2. Breda station (Br), located also in Syria at 35°56′ N latitude, 37°10′ E longitude, and 300 masl, with clay soil, and an average annual precipitation of 260 mm.

The Breda station is characterized by drought and harsh continental climatic conditions, and it provides a dry site with cold winters and high natural infestation of wheat stem sawfly.

3. Latakia station, located at 35°32′ N latitude, 35°46′ E longitude, and 7 masl, with an annual average rainfall of 784 mm, mild winters, and severe disease pressure. Latakia is a high rainfall site used to test for resistance to diseases under natural and artificial infestation, particularly to *S. tritici* and BYDV.
4. Terbol station, located in the Bekaa valley in Lebanon at 33°33′ N latitude, 35°59′ E longitude, and 890 masl. It is characterized by cold winters but favorable growing conditions, and it has a fine clay soil and an average annual precipitation of 524 mm. It is used during the winter season to test for yield potential and resistance to yellow rust and cold, and during the summer to screen for resistance to heat and stem and leaf rusts.
5. Kfardan (Kf) station, located also in Lebanon in the Bekaa valley at 34°01′ N latitude, 36°06′ E longitude, and 1080 masl and characterized by extreme temperature fluctuations. It has a fine clay soil and an average annual precipitation of 402 mm.

Further, with the DGST at the Tel Hadya main station, six effectively different environments were created (Nachit et al. 1995). Realizing that an important part of our work, particularly in the early stage, had to be done on a research station, we developed a stress-screening technique using simulated environments (Nachit 1983, Nachit and Ketata 1986). The same germplasm is thus subjected to different stresses (cold, drought, terminal stress, and heat) according to date of sowing. Thus, at the Tel Hadya station, durum wheat germplasm is sown at these times and conditions:

- Mid-October (early sowing) with supplemental irrigation (including rainfall of 450 mm) to simulate a crop cycle with long duration and favorable growing conditions. The early sowing conditions subject the plants to cold damage (winterkill) during the tillering stage, to frost during the anthesis stage, and to attacks of yellow rust and *S. tritici*.
- Normal date of sowing, which here presents the plants with a typical Mediterranean continental dryland condition.
- Early April (late sowing) to simulate a short growing season, subjecting the plants to terminal heat and drought stresses, particularly during the grain-filling stage. Late sowing conditions increase the attacks by aphids, barley yellow dwarf virus (BYDV), and Hessian fly.
- Early July to mid-October (summer sowing) to test for high temperature conditions during all crop-growth stages. The summer sowing conditions subject the durum wheat plants to extreme high temperatures and sirocco winds.
- Under irrigated conditions for high-yield potential selection.
- After rotation with vetch to induce dryland-favorable conditions and slow release of nitrogen, mobilized from the vetch root decomposition.

Overall, the testing sites/environments of the DSGT provide two interacting selection gradients for rainfall and temperature regimes, and the environmental conditions of the DGST encompass the main abiotic and biotic stresses that prevail in the Mediterranean dryland.

USE OF LANDRACES TO BROADEN THE GENETIC BASE FOR DROUGHT RESISTANCE IN DURUM

In terms of genetic variation, the Mediterranean durum landraces were found to possess also desirable traits lacking in other materials, such as resistance to drought and cold, early growth vigor, long peduncle, and high fertile tillering. Our results on the use of durum wheat landraces in the hybridization program show that substantial progress can be achieved in developing improved cultivars for dry areas. Several genotypes were developed for rainfed conditions with reduced height (80–100 cm). It was clearly demonstrated that grain yield in dryland does not correlate with the height of plant.

Furthermore, the knowledge of physiological mechanisms involved in drought tolerance is a prerequisite to increasing durum dryland productivity. High relative water content and high capacity of osmotic adjustment were identified as important traits for drought tolerance (Habash et al. 2014). Selection for several morpho-physiological traits related to drought tolerance has been performed in populations issued from crosses between durum wheat and its wild relatives (Nachit 1996). Crosses initiated in the mid-1980s at our program (Nachit 1996) are now generating several advanced lines with better performance under environments with abiotic constraints such as drought and temperature extremes (heat and drought).

GENOMIC TECHNOLOGIES DISSECT SYSTEM RESPONSES TO WATER STRESS

Genomic tools and “omic” technologies are increasingly employed in screening for genes and metabolic pathways responsive to water stress to help identify key molecular signatures for stress resistance in adapted material. It is hoped that such tools could complement other genetic and physiological approaches to gain a better insight into mechanisms defining resistance to water stress and to identify genes that could be used as markers in molecular genetic efforts. A recent study applied transcriptome technologies to RILs from the Lahn × Cham1 mapping population known to segregate for yield stability and drought resistance established in extensive field trials. The study designed a unique water stress time transient that enabled a suite of physiological, biochemical, and molecular measurements to be applied (Habash et al. 2014). This enabled the identification of one RIL (#2219) as having constitutively higher stomatal conductance, photosynthesis, transpiration, abscisic acid content, and enhanced osmotic adjustment at equivalent leaf water compared to parents, thus defining a possible physiological strategy for high-yield stability under water stress. Various statistical models were applied to the extensive flag leaf transcriptome datasets and uncovered:

1. Global and similar trends of early changes in regulatory pathways, reconfiguration of primary and secondary metabolism, and lowered expression of transcripts in photosynthesis in all lines subjected to water stress.
2. Statistically significant differences in a large number of genes among the genotypes, in terms of gene expression magnitude and profile under stress, with a high number belonging to regulatory pathways.
3. Constitutive differences in a large number of genes between the genotypes, demonstrating the uniqueness of each line transcriptome.
4. A high level of structure in the transcriptome response to water stress in each wheat line, suggesting genome-wide co-ordination of transcription.

Applying a systems-based analytical framework, in terms of biological robustness theory, the findings suggest that each durum line transcriptome responded to water stress in a genome-specific manner, which may contribute to an overall different strategy of resistance to water stress integrated over levels of function and time. Such studies produce large dataset that can be used in meta-analysis to identify fundamental features of plant responses to water stress and most immediately can be exploited in the search for new molecular markers and breeding strategies for drought resistance in durum wheat.

IMPLICATIONS OF THE DGST APPROACH VIS-À-VIS DURUM WHEAT IMPROVEMENT

During the past 10 years, the contribution made by the stress-tolerant and productive durum wheat genotypes developed by ICARDA is reflected in the significant production increase, from less than 1–3.4 million tons in some areas, where the new varieties were adopted, without any significant increase in the cropped area (1.2 mha). However, with unrelenting population growth in most countries such as WANA region where nearly all of countries are experiencing food deficits, the major challenge for researchers is to increase food production, mainly wheat.

Over the past three decades, ICARDA has developed durum wheat cultivars with high tolerance to drought and WUE, such as Cham1, Cham3, Cham5, and Cham7. These cultivars were released in some areas where they have contributed significantly to increase durum wheat production. For example, in Syria, these cultivars also have a high standard of grain quality. Through the enhanced production of durum grain and good quality, Syria not only achieved self-sufficiency in durum production, but it rose to become the third largest exporter of durum wheat in the world. This achievement (Figure 20.1) was mainly because of variety improvement, which has also prompted the farmers to use improved agricultural practices.

FUTURE WORK

The increase in environmental fluctuation as a result of climate change will negatively affect yield and increase yield variation from year to year. To develop a germplasm with

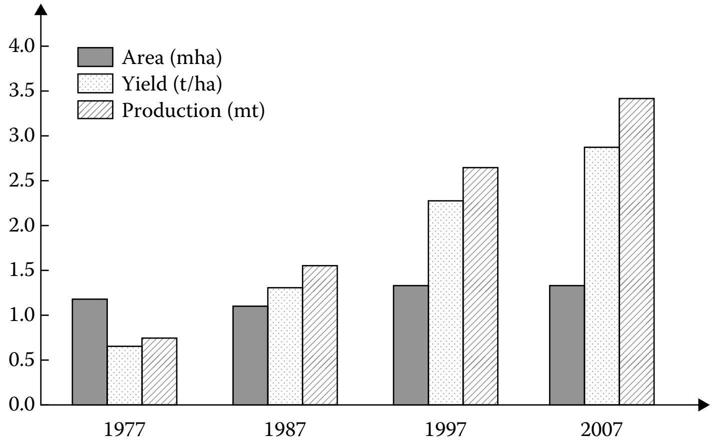

FIGURE 20.1 Area (*left bars*), grain yield (*middle bars*), and production (*right bars*) of the major varieties of durum wheat in Syria over the past 30 years (Haurani/Gezira in 1977, Haurani/Cham1 in 1987, Cham3/Cham1 in 1997, and Cham3/Cham5 in 2007).

buffering ability in durum wheat against these environmental and climatic changes constraints, more gene introgression will be carried out to improve wheat cultivars through the use of landraces and *Triticum* wild relatives. Gene flow from landraces and wild relatives to the improved varieties has been adopted to enable the durum crop to withstand environmental fluctuations, abiotic stresses, and recurrent biotic virulence.

The use of modeling of gene flow is of paramount importance to broaden the genetic base of wheat and to capture complex traits based on quantitative models for predicting functional gene networks.

Conceptual mathematical frameworks will also continue to be used to seek ways to speed up the process of the DGST approach as part of the strategy. The process will involve modeling dynamics that capture complexity such as the predator–prey–predator model to study the infection of a plant when a virus (the initial predator) attacks the plant cells (the initial prey). In such models, the goal is the recovery of solutions of the various inverse problems, each of which highlights a specific feature of the underlying predator–prey–predator dynamics.

The future work will continue to involve capturing complex traits along with modeling *in silico* of gene flow and gene introgression of useful genes in anticipation to buffer the environmental fluctuations and tolerance to stresses.

REFERENCES

Cooper, M., D.W. Podlich, and S.C. Chapman. 2000. Computer simulation linked to gene information databases as a strategic research tool to evaluate molecular approaches for genetic improvement of crops. In *Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments*, A strategic planning workshop held at CIMMYT, El Batan, Mexico, June 21–25, 1999, eds. J.-M. Ribaut and D. Poland, 192–166. Mexico, DF, Mexico: CIMMYT.

Dennis, C. 2002. Gene regulation: The brave new world of RNA. *Nature* 418:122–124.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 40:503–537.

Habash, D.Z., M. Baudo, M. Hindle, S.J. Powers, M. Defoin-Platel, R. Mitchell, M. Saqi, C. Rawlings, K. Latiri, J.L. Araus, A. Abdulkader, R. Tuberosa, D.W. Lawlor, and M. Nachit. 2014. Systems responses to progressive water stress in durum wheat. *PLoS One* 29:9(9).

Habash D.Z., Z. Kehel, and M. Nachit. 2009. Genomic approaches for designing durum wheat ready for climate change with a focus on drought. *J Exp Bot* 60(10):2805–15.

IPCC. 2007. Climate change 2007: The physical science basis. In *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller. Cambridge, UK, New York: Cambridge University Press.

Kehel, Z., A. Garcia-Ferrer, and M. Nachit. 2013. Using Bayesian and Eigen approaches to study spatial genetic structure of Moroccan and Syrian durum wheat landraces. *Am J Mol Biol* 3:17–31. doi:10.4236/ajmb.2013.31003.

Liebovitch, L.S., V.K. Jirsa, and L.A. Shehadeh. 2006. Structure of genetic regulatory networks: Evidence for scale free networks. In *Complexus mundi: Emergent patterns in nature*, ed. M. Novak, 1–8. Singapore: World Scientific.

Nachit, M.M. 1983. Use of planting dates to select stress tolerant and yield stable genotypes for the rainfed Mediterranean environment. *Rachis* 3:15–17.

Nachit, M.M. 1989. Moisture stress tolerance in durum wheat (*T. turgidum* var. L. *durum*) under Mediterranean dryland conditions. In *Proceedings of the 12th EUCARPIA congress: Science for plant breeding*, February 27–March 4, 1989, Göttingen, Germany, 2.

Nachit, M.M. 1992. Durum breeding for Mediterranean drylands of North Africa and West Asia. In *Durum wheats: Challenges and opportunities*. Wheat Special Report, No. 9, 14–27. Mexico, DF, Mexico: CIMMYT.

Nachit, M.M. 1996. Durum wheat breeding. In *Cereal Improvement Program Annual report*, 84–123. Aleppo, Syria: ICARDA 010/1500.

Nachit, M.M. 1998a. Durum breeding research to improve dry-land productivity in the Mediterranean region. In *SEWANA Durum Research Network*, eds. M.M. Nachit, M. Baum, E. Porceddu, P. Monneveux, and E. Picard, 1–15. Aleppo, Syria: ICARDA.

Nachit, M.M. 1998b. Association of grain yield in dryland and carbon isotope discrimination with molecular markers in durum (*Triticum turgidum* L. var. *durum*). In *Proceedings of 9th International Wheat Genetics Symposium*, Saskatoon, ed. A.E. Slinkard, 218–223. Saskatchewan, Canada.

Nachit, M.M., M. Baum, A. Impiglia, and H. Ketata. 1995. Studies on some grain quality traits in durum wheat grown in Mediterranean environments. In *Durum wheat quality in the Mediterranean region, Proceedings of the seminar held in Zaragoza, Spain*, November 17–19, 1993, *Options Méditerranéenes*, eds. N.D. Fonzo, F. Kaan, and M. Nachit, Series A, 22:181–187. Zaragoza, Spain: CIHEAM. om.ciheam.org/article.php?IDPDF=95605369.

Nachit, M.M. and I. Elouafi. 2004. Durum wheat adaptation in the Mediterranean dryland: Breeding, stress physiology, and molecular markers. In *Challenges and strategies for dryland agriculture*, eds. S.C. Rao and J. Ryan, CSSA Special Publication no. 32, 203–218. Madison, WI: Crop Science of America and American Society of Agronomy.

Nachit, M.M. and H. Ketata. 1986. Breeding strategy for improving in Mediterranean rainfed conditions. In *Proceedings of the International Wheat Conference*, Rabat, Morocco, May 2–9, 1983.

Nachit, M.M. and A. Ouassou. 1988. Association of yield potential, drought tolerance and stability of yield in *Triticum turgidum* var. *durum*. In *Proceedings of the 7th International Wheat Genetics Symposium*, eds. T.E. Miller and R.M.D. Koebner, 867–870, Cambridge: Institute of Plant Science Research.

Nachit, M.M., M.E. Sorrells, R.W. Zobel, H.G. Gauch, W.R. Coffman, and R.A. Fischer. 1992a. Association of morpho-physiological traits with grain yield and components of genotype-environment interaction in durum wheat. Pt. 1. *J Genet Breed* 46:363–368.

Nachit, M.M., M.E. Sorrells, R.W. Zobel, H.G. Gauch, W.R. Coffman, and R.A. Fischer. 1992b. Association of morpho-physiological traits with grain yield and components of genotype-environment interaction in durum wheat. Pt. 2. *J Genet Breed* 46:369–372.

Shaw, R.G. and J.R. Etterson. 2012. Rapid climate change and the rate of adaptation: Insight from experimental quantitative genetics. *New Phytol* 195(4):752–765.

van Oosterom, E., G. Hammer, and S. Chapman. 2004. Can transition to flowering be modelled dynamically from the gene level? In *New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress*, eds. T. Fischer, N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell, and D. Lloyd, 647. Gosford, Australia: The Regional Institute. http://www.cropscience.org.au/icsc2004/poster/3/2/1/647_vanoosteromej.htm.

Welch, S.M., Z. Dong, and J.L. Roe. 2004. Modelling gene networks controlling transition to flowering in *Arabidopsis*. In *New directions for a diverse planet: Proceedings for the 4th International Crop Science Congress*, eds. T. Fischer, N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell, and D. Lloyd, 158. Gosford, Australia: The Regional Institute. http://www.cropscience.org.au/icsc2004/symposia/3/2/158_welchsm.htm.

Welch, S.M., J.L. Roe, and Z. Dong. 2003. A genetic neural network model of flowering time control in *Arabidopsis thaliana*. *Agron J* 95:71–81.

Xu, Y., J.R. Coburn, B.E. Gollands et al. 2004. *Mapping quantitative trait loci for water use efficiency in rice*. Paper presented at the International Plant and Animal Genome XII Conference, January 10–14, 2004, San Diego, CA.