

CASE STUDIES: MARKER-AIDED SELECTION

CASE STUDY 1: Marker-aided selection (MAS) for drought tolerance and disease resistance in pearl millet in India



Dr SK Gupta explaining about ICRISAT's pearl millet research.Credit: ICRISAT

Pearl millet (*Pennisetum glaucum*) is extremely important as a major grain staple and as animal feed in some of the driest areas of Asia and Africa. Grown primarily by smallholder farmers in marginal areas under rain-fed conditions, improving yields and resistance to drought and disease through modern breeding is important to millions of livelihoods. New **hybrids** have been developed that offer higher yields, but also greater vulnerability to certain diseases. In India, pearl millet is grown on 9 million hectares of which 70% are planted with hybrid cultivars. Since pearl millet hybrids first reached farmers' fields in India in the late 1960s, each of the

hybrid varieties grown have been attacked by a downy mildew plant disease that can result in up to 80% crop loss.³

From 1999-2002, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) with the John Innes Centre and the Plant Sciences Research Programme of the Department for International Development (DFID) developed biotechnological solutions to help reduce the incidence of this disease in hybrid cultivars. After mapping the genomic regions of pearl millet that control downy mildew resistance, straw yield potential, and grain and straw yield under drought stress conditions, breeders used conventional breeding and marker-aided selection (MAS) to transfer several genomic regions with improved downy mildew resistance to 2 parental lines of popular hybrid millet. MAS was then used to derive 2 new varieties - ICMR 01004 and ICMR 01007 - with 2 different gene blocks for downy mildew resistance. In trials, these varieties have had grain and straw yields equal to or better than their parent lines whilst showing a vast improvement in their resistance to downy mildew. ⁴

CASE STUDY 2: Deepwater rice, International Rice Research Institute (IRRI)

The use of marker-aided selection (MAS) was key to the development of submergence-tolerant rice, a potentially revolutionary rice variety able to withstand submergence in water for a number of weeks.⁵ Rice in Asia is typically grown in standing water, but deep flooding for more than a couple of days is detrimental to crop growth and viability. Deep flooding affects more than 25% of global rice-producing land, a proportion expected to rise as a result of global warming. Flash flooding can submerge rice plants, often at the seedling stage, for several weeks.



A deepwater rice field at Gazipur near Dhaka. Credit : Md Johir Uddin







Deepwater rice is known for its ability to elongate its internodes. These have hollow structures and function as snorkels to allow gas exchange with the atmosphere to prevent drowning. In 2009, a pair of genes responsible was identified by a team at the Nagoya University in Japan.⁶ They were named SNORKEL1 and SNORKEL2. Under deep-water conditions, ethylene – a plant hormone – accumulates in the plant and induces expression of the two genes. Their products then trigger remarkable internode elongation via growth hormones, causing the rice plant to grow by up to eight metres in the presence of rising water levels.

Another gene *Sub1A* with a similar function has also been discovered by the International Rice Research Institute (IRRI) in the Philippines.⁷ The resulting rice was named Scuba rice. It responds to ethylene by limiting the elongation of the internodes. This conserves carbohydrates so permitting regrowth when the flood recedes.⁸ The rice becomes dormant during the flooding then continues growing once floodwaters recede.

There is potential to utilise both sets of genes so that high-yielding rice varieties can withstand both flooding that is deep and quick, where *Submergence* genes are appropriate, and floodwaters that climb in a progressive and prolonged fashion, for which *Snorkel* genes are better suited.⁹

MAS-based breeding is already underway. Markers for the *Sub1* locus have now been used to integrate *Sub1A* from IRRI deep-water rice into a widely grown Indian variety, *Swarna*. The resulting crosses when grown in the field in the Philippines exhibited the submergence tolerance, but the yields, plant height, harvest index and grain quality remained the same. New submergence-tolerant varieties are now being produced in this way in Laos, Bangladesh and India, and in Thailand where a submergence-tolerant jasmine rice is being bred.¹⁰ In one farmer's fields during IRRIs Indian field trials, 95% to 98% of the scuba rice plants recovered while only 10% to 12% of the traditional varieties survived. Within 1 year of its release, scuba rice was adopted by more than 100,000 Indian farmers.¹¹ As of 2012, 3 million farmers were using the new variety, Swarna-Sub1.

CASE STUDY 3: Bringing Marker-aided selection to West Africa for improved cowpea



Genetic diversity of cowpea seed. Credit: J. Ehlers, UC Riverside.

Cowpea is one of the most drought tolerant of all grain legume crops and it serves as a major source of protein for millions in sub-Saharan Africa. Similar to other 'minor' food crops little investment has been directed towards the improvement of the cowpea. Yet, the cowpea is vulnerable to the parasitic weed *Striga gesneroides*. With no chemical options for disease control, one feasible solution is improved breeding.

In order to identify genes for cowpea resistance, marker-aided selection (MAS) can infinitely speed up the process compared to conventional breeding and varietal selection.

The Kirkhouse Trust, a UK-based charity, is helping to bring MAS technology to West African cowpea breeders. With support from the Trust, a consortium of cowpea breeders has been formed across the region, all provided with equipment, chemical reagents and





training. Progress has been uneven, attributed in part to a lack of trained personnel, but Burkina Faso has succeeding in building a functional laboratory.

The major focus of the Trust has been to support *in situ* programmes, but it has also invested in a cowpea genome sequencing programme, the foundation for designing genetic markers. Junior and senior breeding staff have been **trained** through a series of 3 to 6 month courses, supported by visits from researchers from the University of Virginia, regional workshops, and working visits for technicians to the Ouagadougou laboratory.¹²





¹ Department for International Development (DFID) 2002, *Project: Use of molecular markers to improve terminal drought tolerance in pearl millet*, Available from: http://r4d.dfid.gov.uk/Project/2155/ [8 July 2015].

² Raney, T (ed) 2004, 'What is agricultural biotechnology?' The state of food and agriculture 2003-2004: Agricultural Biotechnology – meeting the needs of the poor? Food and Agricultural Organisation of the United Nations (FAO).

³ Howarth, CJ & Yadav, RS 2002, *Sucessful marker assisted selection for drought tolerance and disease resistance in pearl millet*, UK Department for International Development, London, and Centre for Arid Zone Studies, University of Wales, Bangor.

⁴ Raney, T (ed) 2004, 'What is agricultural biotechnology?' The state of food and agriculture 2003-2004: Agricultural Biotechnology – meeting the needs of the poor? Food and Agricultural Organisation of the United Nations (FAO).

⁵ Voesenek, L& Bailey-Serres, J 2009, 'Plant biology: Genetics of high-rise rice' Nature, vol. 460 pp. 959-960.

⁶ Hattori, Y, Nagai, K, Furukawa, S, Xian-Jun, S, Kawano, R, Sakakibara, H, Wu, J, Matsumoto, T, Yoshimura, A, Kitano, H, Matsuoka, M, Mori, H & Ashikari, M 2009, The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water' *Nature*, vol. 406, pp. 1026-1030.

⁷ Xu, k, Xu, X, Fukao, T, Canlus, P, Maghirang-Rodriguez, R, Heuer, R, Ismail, AM, Bailey-Serres, J, Ronald, PC & Mackill, DJ 2006, 'Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice' *Nature*, vol. 442, pp. 705-708.

⁸ Voesenek, L& Bailey-Serres, J 2009, 'Plant biology: Genetics of high-rise rice' Nature, vol. 460 pp. 959-960.

⁹ Dolgin, E 2009, 'The resistant rice of the future: Cross-breeding could create rice varieties that can survive flooding and fungi' 20 August 2009. *Nature News*, Available from: http://www.nature.com/news/2009/090820/full/news.2009.841.html [7 July 2015].

¹⁰ Siangliw, M, Toojinda, T, Tragoonrung, S & Vanavichit, A 2003, '<u>Thai jasmine rice carrying QTLch9</u> (SubQTL) is submergence tolerant' *Annals of Botany*, vol. 91, pp. 255-261.

¹¹ Department for International Development (DFID) 2010, Case Study: *Sowing the seeds of scuba rice*, Available from: https://www.gov.uk/government/case-studies/sowing-the-seeds-of-scuba-rice [7 July 2015].

¹² Koebner, R 2010, 'Making a mark for Cowpea' *New Agriculturalist,* Available from: http://www.new-ag.info/en/developments/devltem.php?a=1732 [7 July 2015].