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Bringing high-throughput techniques to orphan crop of Africa: Highlights from the Tef TILLING Project[1]

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Summary

Orphan- or understudied-crops are mostly staple food crops in developing world. They are broadly classified under cereals, legumes, root crops, fruits and vegetables. These under-researched crops contribute to the diet of a large portion of resource-poor consumers and at the same time generate income for small-holder farmers in developing countries, particularly in Africa. In addition, they perform better than major crops of the world under extreme soil and climatic conditions. However, orphan crops are not without problems. Due to lack of scientific investigation, most of them produce low yields while others have a variety of toxins that affect the health of consumers. Here, we present some highlights on the status and future perspectives of the Tef Biotechnology Project that employs modern improvement technique in order to genetically improve tef (*Eragrostis tef*), one of the most important orphan crop in Africa. A reverse genetics approach known as TILLING (Targeting Induced Local Lesions IN Genome) is implemented in order to tackle lodging, the major yield limiting factor in tef.

Introduction

Orphan crops are also known as underutilized-, lost- or disadvantaged-crops. Most of these understudied crops are staple food crops in developing world. Some of the most important orphan crops belong to cereals [e.g., finger millet (*Eleusine coracana*) and tef (*Eragrostis tef*)], legumes [cowpea (*Vigna unguiculata*), and bambara groundnut (*Vigna subterranea*)], root crops [cassava (*Manihot esculenta),* and yam (*Dioscorea sp*.)], fruit crops [banana and plantain (*Musa* spp. L)], and many vegetables. These crops grow better than major crops such as maize and wheat under extreme environmental conditions. However, due to lack of genetic improvement, these crops produce inferior yield in terms of both quality and quantity. The major bottlenecks affecting the productivity of orphan crops are genetic traits such as low yield (for example, in finger millet and tef), poor in nutrition [cassava and enset (*Ensete ventricosum*)], and production of toxic substances [cassava and grass pea (*Lathyrus sativus*)]. Modern crop breeding utilizes techniques such as Marker-Assisted Breeding, and reverse genetics approach known as TILLING (Targeting Induced Local Lesions IN Genome). The application of these techniques to the understudied crops is important in order to boost productivity and feed the largely underfed and undernourished population of Africa. TILLING is a recently developed improvement method but proved to efficient in the detection useful mutations in crops such as wheat (Slade *et al*. 2004), barley (Caldwell *et al*. 2004), maize (Till *et al*. 2004), rice (Till *et al*. 2007), and sorghum (Xin *et al*. 2008). Detailed procedure of TILLING is indicated in Fig. 1.

The Tef Biotechnology Project

The Tef Biotechnology Project, hosted by the Institute of Plant Sciences, University of Bern in Switzerland, focuses on boosting the productivity of an orphan crop tef through developing lodging tolerant cultivars. Tef is ecologically and agronomically versatile crop. The crop is preferred both by farmers and consumers because farmers benefit since tef is tolerant to drought, water-logging, and pests particularly against storage pests. Consumers prefer tef not only because it makes good quality bread but also free of gluten (Spaenij-Dekking *et al*. 2005) for which many people are allergic.

However, tef is one of the low yielding crop in the world. Lodging is considered as the major yield limiting factor. The causes of lodging in tef are related to the standing power of the plant (morphological) and environment (fertilizer, weather). Tef has a tall and tender stem that is susceptible to lodging caused by wind and rain. In addition, when optimum amount of fertilizer is applied to increase the yield, high incidence of lodging occurs. As a consequence, the yield from the crop is severely reduced in terms of total grain yield and quality. The lodged plant also poses a great problem for harvesting. Crops such as wheat and rice were significantly affected by lodging prior to Green revolution that occurred in 1960's in Asia and Latin America. The development of improved seeds that produce plants with semi-dwarf stature increased the lodging resistance and responsiveness towards the fertilizer application, resulting in boosting the yield per unit area. The key genes played significant role during Green Revolution contain the mutated Reduced height-1 (Rht-B1 and Rht-D1) gene in wheat (Peng *et al.* 1999) and *semi-dwarf* (*sd-1*) in rice (Sasaki *et al*. 2002, Spielmeyer *et al*. 2002).

The Tef Biotechnology Project implements TILLING. We have generated over 10 000 M_1 and 6000 M_2 tef populations mutagenized by ethyl-methanesulphonate (EMS), a chemical known to induce point mutations. Leaf samples were collected from 2-3 weeks old M_2 seedlings and the seeds of M_3 lines were harvested. DNA isolation was made from M_2 samples. Currently we are screening the mutagenized population for two genes known to influence plant height in related crop species. These are HTD1 (HIGH-TILLERING DWARF1, Zou *et al.*, 2006) and DWARF4 (Sakamoto *et al.*, 2006). Based on sequence information, each of these genes is represented by two copies having high homology in the exon region but divergent in the intron region. The divergent region is used to design copy specific labelled primers in order to amplify only one copy at a time.

The major problem we have faced in the implementation of TILLING is related to the polyploid nature of the plant. Tef is an allotetraploid with 2n=4x=40. Since each gene is represented at least in two copies, PCR amplification and detection of a single copy is difficult. In order to tackle this problem, we first identify how many copies of a gene are present in the genome. Then, we design primer(s) that can preferentially amplify one copy at a time. Similar techniques were applied for polyploidy potato (Elias *et al*. 2009).

In a separate experiment, in order to visually identify semi-dwarf lines from the mutagenized population, we grew about 8 plants each from 4000 M_2 families for about four weeks in the greenhouse where major environmental factors such as light, temperature and humidity are properly regulated. Families having at least two semi-dwarf plants were considered as candidates and promoted to the next level of evaluation. Ten candidates inherit the semi-dwarf trait to the next generation. These candidates are currently being investigated for fertility, productivity and other characters. Seeds of the best line were sent to the Ethiopian national agricultural research for further field tests and introgression to high yielding and adaptable lines.

Conclusion and perspectives

We implement TILLING, a non-transgenic high-throughput technique in order to change the stature of tef, one of the most important but poor yielding African crop. The major goal of our project is to boost the yield of tef through developing semi-dwarf and lodging tolerant cultivars. Currently we have completed optimization steps and ready to begin the high-throughput screening for selected genes known to alter plant height in crops such as rice. Several semi-dwarf candidates obtained from separate screening are being evaluated in Bern and Ethiopia.

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References

Caldwell DG, McCallum N, Shaw P, Muehlbauer GJ, Marshall DF, Waugh R. 2004. A structured mutant population for forwar and reverse genetics in Barley (*Hordeum vulgare* L.). *Plant Journal* 40:143-150.

Elias R, Till BJ, Mba C, -Safadi B. 2009. Optimizing TILLING and Ecotilling techniques for potato (*Solanum tuberosum* L). *BMC Res Notes* 2:141.

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar Christou P, Snape Jw, Gale MD and Harberd NP. 1999. Green revolution genes encode mutant gibberellin response modulators. *Nature* 400: 258-261.

Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S, Ueguc -Tanaka M, Mizutani M, Sakata K, Takatsuto S, Yoshida S, Tanaka H, Kitano H, Matsuoka M. 2006. Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. *Nature Biotechnology* 24:105-109.

Sasaki A, Ashikari M, Ueguc -Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M. 2002. Green revolution: a mutant gibberellin-synthesis gene in rice. *Nature* 416:701-702.

Slade AJ, Fuersternberg SI, Loeffler D, Steine MN, and Faciotti D. 200 A reverse genetic, nontransgenic approach to heat crop improvement by TILLING. *Nat. Biotechnol* 23:75-81.

Spae -Dekking L, KooyWinkelaar Y, and Koning F. 2005. The Ethiopian cereal tef in celiac disease. *The New England Journal of Medicine* 353:1748-1749.

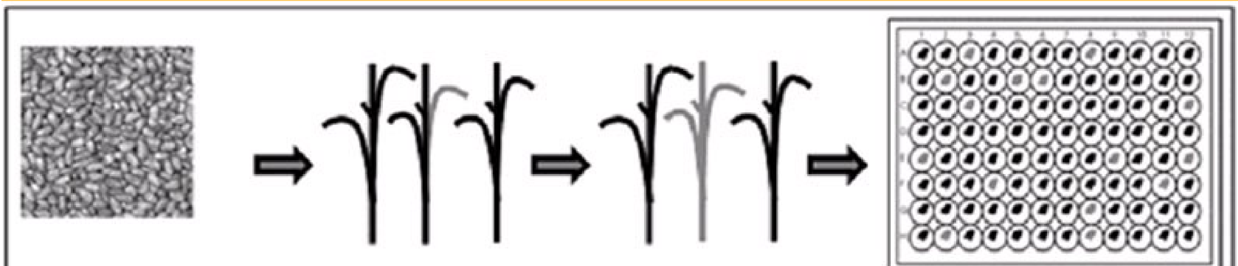
Spielmeyer W, Ellis MH, Chandler PM. 2002Semidwarf (sd-1), “green revolution” rice, contains a defective gibberellin 20-oxidase gene. *PNAS* 99:9043-9048.

Till BJ, Cooper J, Tai TH, Colowit P, Greene EA, Henikoff S, Comai L. 2007. Discovery of chemically induced mutations in ric by TILLING. *BMC Plant Biol*. 7:19.

Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, Young K, Bowers E, Codomo CA, Enns LC, Odden AR, Greene E Comai L, and Heinkoff JG. 2004. Discovery of induced point mutations in maize genes by TILLING. *BMC Plant Biol*. 4:12.

Xin Z, Wang ML, Barkley NA, Burow G, Franks C, Pederson G, Burke J. 2008. Applying genotyping (TILLING) an phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. *BMC Plant Biology* 8:103.

Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, Zhu L. 2006. The rice HIG-TILLERING DWARF1 encoding an ortholog of Arabidopsis MAX3 is required for negative regulation of the outgrowth of axillary buds. *Plant Journal* 48:687-698.



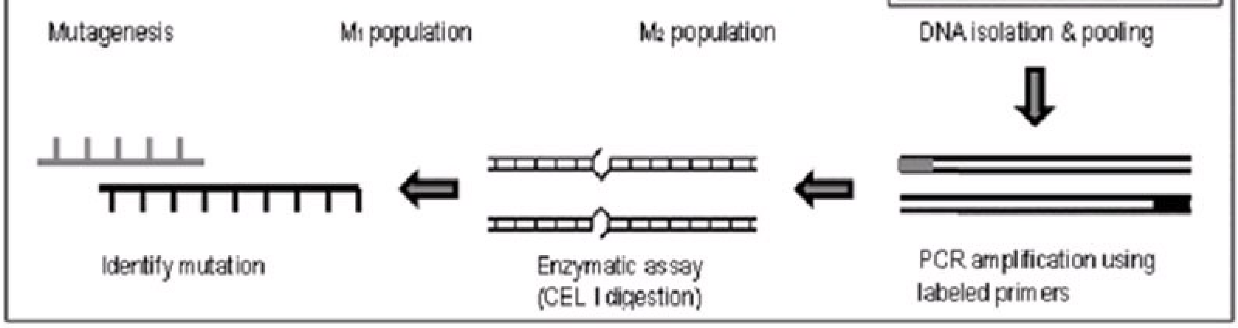


Fig. 1. The TILLING method.