Produzione ed utilizzo dei terreni colturali per tessuti vegetali *in vitro*

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Duchefa Biochemie bv produce circa 500 differenti tipi di terreni colturali. Oltre a circa 50 terreni colturali citati in letteratura sono prodotti altri 400 terreni colturali specifici su richiesta di clienti quali università, istituti di ricerca e aziende di micropropagazione in tutto il mondo. Le quantità prodotte possono variare tra un minimo di 1 kg fino a lotti di 560 kg di terreno colturale. Negli ultimi tempi la formulazione di terreni colturali per tessuti vegetali sta variando rapidamente a motivo dell'ingresso in produzione di aziende *Plant Biotech* e l'impiego dei bioreattori *TIS*. Le aziende Biotech necessitano di terreni colturali ben definiti e precisi in grandi quantità perché siano garantite le alte produzioni finali di metaboliti secondari mediate da cellule *GMO*. Le aziende di micropropagazione richiedono specifici terreni colturali per riuscire a produrre nel tempo giusto la specie e le quantità di piantine di cui i clienti abbisognano. L'incremento dell'impiego della tecnologia *Temporary Immersion System* e dei bioreattori sta evolvendo la produzione di terreni colturali differenti dai sistemi tradizionali, rispetto agli impieghi usuali di sali e modifica delle loro rispettive concentrazioni.

Parole chiave: Plant Biotech, Temporary Immersion System, GMO.

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Trait stacking in African rice using multiplex genome editing

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Rice is the second most cultivated cereal worldwide: a staple food for half of the world's population. The genus *Oryza* comprises two domesticated species: *Oryza sativa* and *O. glaberrima*, known as Asian and African rice. Plant breeding has produced high-yielding Asian varieties, however these frequently have greater demand for agricultural inputs and are more susceptible to biotic and abiotic stresses. In contrast, African rice has been scarcely improved for yield, but is known for its resistance to pests, drought and nutrient deficiencies, and is well suited to harsh agricultural environments. Our lab was the first to set up an efficient method to transform African rice: we were able to induce formation of callus from seeds, to infect cells with *Agrobacterium* and to obtain regenerated plants. The aim of this project is to transfer the knowledge gained in the recent years on the molecular mechanisms influencing rice yield of *Oryza sativa* to African rice, using genome editing with CRISPR_Cas9 to stack traits of interest, but meantime keeping the genetic background of this highly adaptive species intact. In the first instance we targeted the *htd1* gene (*high tillering dwarf1*), since the knock out gives a clear phenotype of a bushy-dwarf plant. Secondly, we employed the well-known Green Revolution gene *GA20ox* (a.k.a., *semi dwarf1*), the product of which catalyses the last step of active GAs biosynthesis. Knockout results indicate that reduced height and higher panicle/leaf ratio occurred in plants. Lastly, we tested multiple knock-out constructs, selecting three genes known to influence seed weight and panicle architecture in Asian rice, namely *gs3*, *gw2* and *gn1a*. *Gs3* encodes for a transmembrane protein, thought to be negative regulator of grains growth (length and width); *gw2* negatively regulates cell division in spikelet hulls; while *gn1a* is a cytokinin-degrading enzyme that was found to be lowly expressed in high yielding varieties. To date out of about 80 transformed plants we observed multiple dual and triple gene disruptions after sequencing of the targeted loci. Preliminary observations of mutant plants demonstrate reduced seed size for the *htd1* mutants while an increased grain size in plants with two or more gene knockouts in the agronomical traits.

Key words: *Oryza glaberrima*, yield improvement, crispr cas9, plant tissue culture.

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