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"Agricultural development within the rural-urban continuum"

Towards a Protocol for Double Haploid Production in Pearl Millet using Wide Hybridisation

Likyelesh Gugsa[1], Bettina I.G. Haussmann[1], Jochen Kumlehn[2], Albrecht Melchinger[1]

[1] *University of Hohenheim, Institute of Plant Breeding, Seed Science and Population Genetics, Germany*
[2] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Plant Production, Germany*

Abstract

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important allogamous staple cereal in the semi-arid tropics (sub-Saharan Africa and South Asia). A protocol for doubled haploid (DH) production in pearl millet could facilitate genetic studies as well as crop improvement (via recurrent DH selection) including hybrid breeding. The objectives of the study were to develop a production system for doubled haploid pearl millet through wide crossing and to investigate the haploid induction rate (HIR) of a maize inducer line (RWS). A cytoplasmic male-sterile (CMS) pearl millet (A) line was crossed with regular maize (cv. Gama), inducer maize line (RWS), inducer hybrid (G1F1), sorghum, triticale and a fertile pearl millet (control). Embryo development was triggered by 2,4-D treatment 24 h after pollination and florets/ovaries/embryos were cultured *in vitro*. Simultaneously, grains were obtained from the mother plants of all wide crosses while selfed female plants proved to be fully sterile. Few green and albino plants were regenerated from rescued embryos, however all died after transplanting to soil. Grain setting efficiency varied from 38.1 (regular maize) to 4.3 (RWS) grains per pollinated panicle with a germination percentage of 22 to 50 %. Flow cytometric analysis of the candidate haploid plants revealed haploid induction of 29 out of 146 plants investigated from all crosses with the HIR amounting to 1.6 to 10.5 % in crosses with G1F1 followed by sorghum (6.6 %), triticale (8.0 %), RWS (8.3 %) and Gama. Although no phenotypic markers were observed to identify the haploids, there was retarded endosperm growth in most of the crosses. Haploid panicles exhibited inferior morphological performance such as stunted growth and missing development of floral organs and were completely sterile. The protocol needs to be further investigated with regard to reproducibility before it can be considered useful for breeders. The mechanisms of haploid induction need cytological examination for the insertion of paternal chromosomes and the occurrence of spontaneous chromosome doubling. Morphological markers also need to be developed to facilitate straightforward identification of haploids after wide crossing. Haploids will be subjected to colchicine treatment for DH production.

Keywords: Double haploid, flow cytometry, pearl millet, wide hybridisation

Contact Address: Likyelesh Gugsa, University of Hohenheim, Institute of Plant Breeding, Seed Science and Population Genetics, Fruwirtstr. 21, 70599 Stuttgart, Germany, e-mail: lgugsa@yahoo.com