Stable integration and expression of wasabi defensin gene in ‘‘Egusi’’ melon (*Colocynthis citrullus* L.) confers resistance to Fusarium wilt and Alternaria leaf spot

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Abstract

Production of ‘‘Egusi’’ melon (*Colocynthis citrullus* L.) in West Africa is limited by fungal diseases, such as Alternaria leaf spot and Fusarium wilt. In order to engineer ‘‘Egusi’’ resistant to these diseases, cotyledonary explants of two ‘‘Egusi’’ genotypes, ‘Ejagham’ and NHC1- 130, were transformed with *Agrobacterium tumefaciens* strain EHA101 harbouring wasabi defensin gene (isolated from *Wasabia japonica* L.) in a binary vector pEKH1. After co-cultivation for 3 days, infected explants were transferred to MS medium containing 100 mgl-l kanamycin to select transformed tissues. After 3 weeks of culture, adventitious shoots appeared directly along the edges of the explants. As much as 19 out of 52 (36.5%) and 25 out of 71 (35.2%) of the explants in genotype NHC1-130 and ‘Ejagham’, respectively, formed shoots after 6 weeks of culture. As much as 74% (14 out of 19) of the shoots regenerated in genotype NHC1-130 and 72% (18 out of 25) of those produced in genotype ‘Ejagham’ were transgenic. A DNA fragment corresponding to the wasabi defensin gene or the selection marker nptII was amplified by PCR from the genomic DNA of all regenerated plant clones rooted on hormone-free MS medium under the same selection pressure, suggesting their transgenic nature. Southern blot analysis confirmed successful integration of 1–5 copies of the transgene. RT-PCR, northern and western blot analyses revealed that wasabi defensin gene was expressed in transgenic lines. Transgenic lines showed increased levels of resistance to *Alternaria solani*, which causes Alternaria leaf spot and *Fusarium oxysporum*, which causes Fusarium wilt, as compared to that of untransformed plants.