

Title: Development of efficient embryo-derived regeneration system and optimization of genetic transformation in cumin (*Cuminum cyminum* L.)

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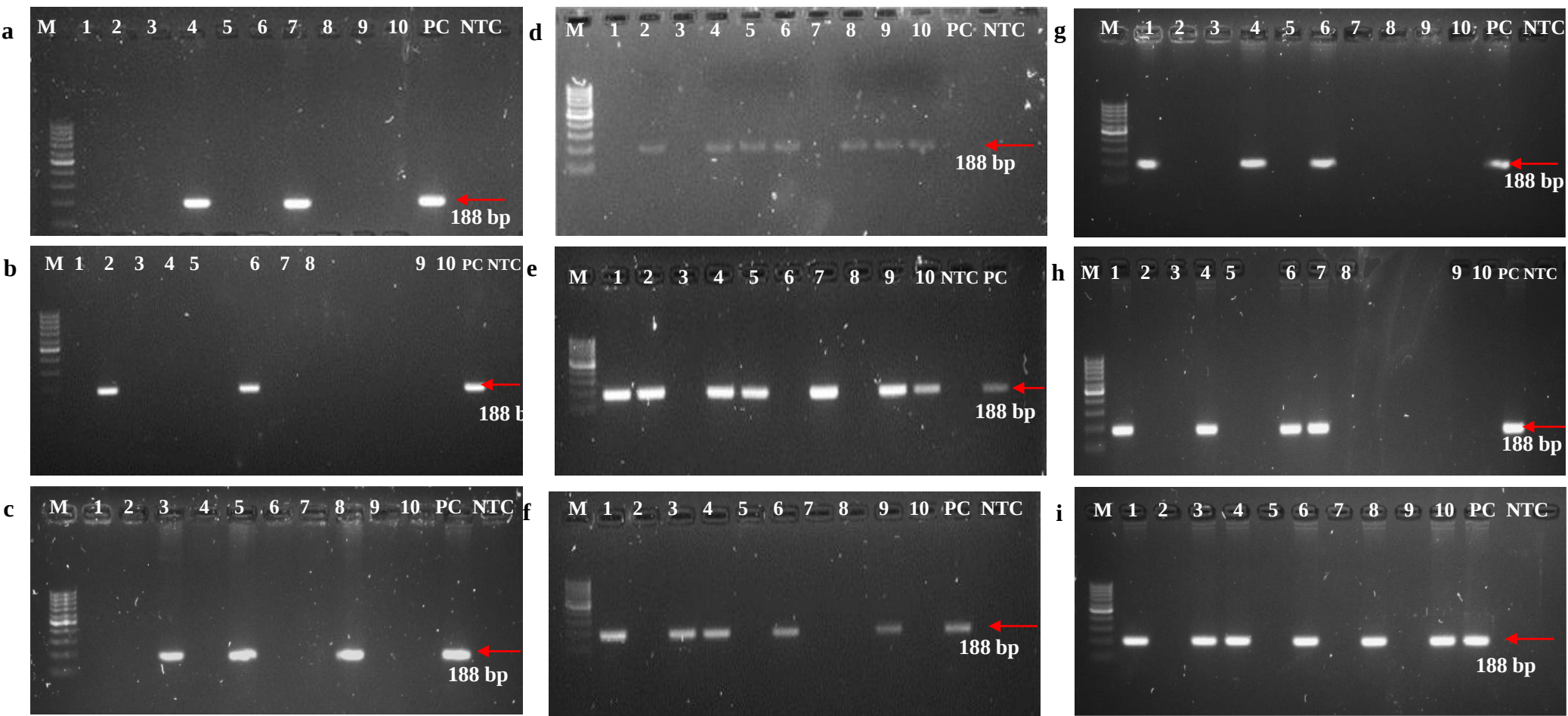
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Abstract:

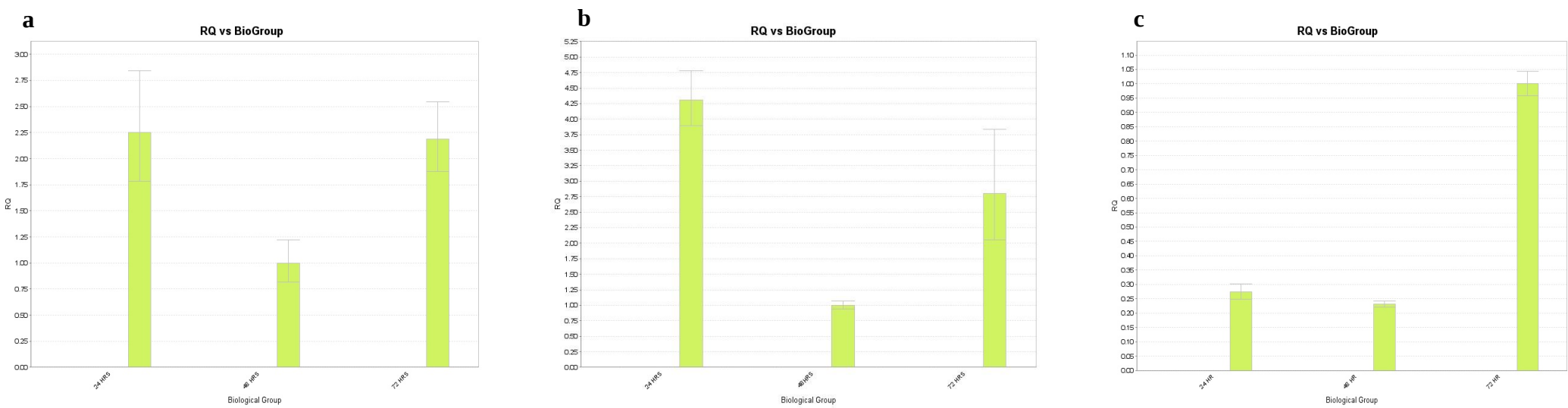
Cumin is an important spice crop with high agronomic and economic importance. A direct regeneration system using embryogenic explants in cumin (*Cuminum cyminum* L.) was established to develop a highly efficient transformation system. Cumin embryos were utilized as an explant which shows higher regeneration efficiency on Gamborg's B₅ media supplemented with 2.0 μM BA+ 0.5 μM NAA. Transformation of pSIM24-eGFP plasmid in cumin was carried out through *Agrobacterium tumefaciens* EHA 105 and gene gun method. The transgenic explants were confirmed for GFP (green fluorescent protein) gene integration through PCR analysis. The *Agrobacterium*-mediated transformed explants showed higher regeneration and transformation efficiency with 0.5 OD₆₀₀ of cell density and 24 hr of co-cultivation compared to 0.4 OD₆₀₀ with 24 hr, 48 hr, and 72 hr co-cultivation time and 0.5 OD₆₀₀ with 48 hr and 72 hr co-cultivation time. It was further confirmed by GFP expression analysis through real-time PCR. Gene gun-mediated transformed explants were cultured on different osmolytes (mannitol, sorbitol, and sucrose) containing media to reduce bombardment stress on explants. Compared to mannitol and sucrose-containing media, transformed explants cultured on sorbitol-containing media showed higher rates of regeneration and transformation. These results were further confirmed by real-time PCR analysis as prominent GFP expression was found in explants cultured on sorbitol-containing media compared to other osmolytes containing media. In the current study, we have developed an efficient transformation system with higher gene expression and regeneration efficiency.

Keywords: Cumin, *Agrobacterium*-mediated transformation, gene gun, Transgenic plant, GFP (green fluorescent protein), osmolytes, real-time PCR

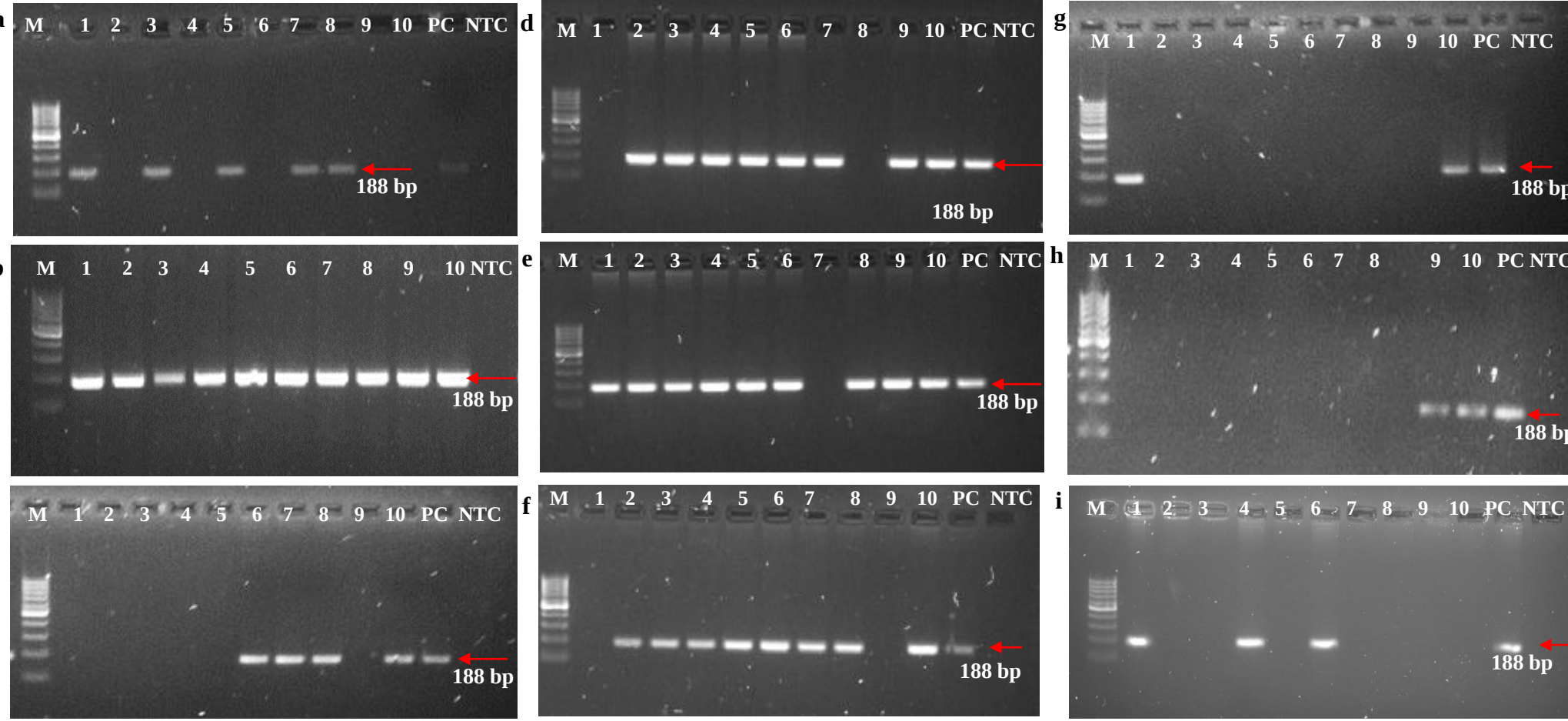
Supplementary fig. S1



Supplementary fig. S2



Supplementary fig. S3



Supplementary fig. S4

