

Competence of Tropical Maize Lines to *Agrobacterium*-Mediated Transformation and the Expression of Maize Poly (ADP-Ribose) Polymerase (*PARP2*) Gene under Drought Stress.

Joshua Kiilu muli

Abstract

Drought stress affects many maize growing regions in sub-Saharan Africa. As population increases, the gap between maize supply and demand increases. There is, therefore, need to develop maize lines that are tolerant to drought as well as amiable to *Agrobacterium*-mediated transformation. This study aimed to assess the competence of tropical maize lines after co-cultivation on Yeats Extract Peptone (YEP) media to *Agrobacterium*-mediated transformation and determine the relative expression of *PARP2* gene under drought during development. Tropical inbred maize lines CML 144, CML 216, A04, and E04 as well as *Agrobacterium* strain EHA101 harboring vector pTF102 containing the *GUS* reporter gene were used in this study. The ability of immature maize embryos to form embryogenic calli was determined after pre-culturing these embryos on YEP and Murashige and Skoog (MS) media. Transient *GUS* assay was used to evaluate the competence of the inbred maize lines to *Agrobacterium*-mediated transformation using YEP as co-cultivation media or YEP that was supplemented with growth regulator 2,4-D (YEP+2,4-D), Cysteine (YEP+CYS), Proline (YEP+PRO) or in combination (YEP+ALL). In all cases, MS media was used as the control. The optimum *Agrobacterium* concentration for infection of immature maize embryos before co-cultivation was determined. The physiological response of tropical maize lines to drought stress was evaluated and the expression of the *PARP2* gene at different drought levels determined by Polymerase chain reaction (PCR). The data collected was analysed using ANOVA at 95% confidence interval with SAS statistical computer software (version 9.1.3). Separation of means was carried out using Tukey's pairwise comparison at 5% probability level. Callus formation frequency and regeneration were genotype dependent ($P=0.0001$). Immature embryos from the four inbred maize lines exhibited high transient *GUS* expression when co-cultivated with *Agrobacterium* on YEP (12.31%), YEP+PRO (13.75%) and YEP+ALL (8.68%) media than when cocultivated on MS media (6.76%). Co cultivation in YEP+2,4-D and YEP+CYS, however, resulted in lower transient *GUS* expression than on MS media. *Agrobacterium tumefaciens* at a concentration of 0.07 (OD660) gave the highest transient *GUS* expression (20.90%) while higher concentrations of 0.2 and 0.8 resulted in low transient *GUS* expression (9.17% and 12.22%), suggesting that YEP media is superior to MS media in enhancing the competence of immature embryos to *Agrobacterium*-mediated transformation. Thus YEP media is proposed as an alternative media in in *Agrobacterium*-mediated transformation protocols. Growth rate, fresh weights of seedlings and dry weights of seedlings was low in seedlings subjected to severe drought stress compared to seedlings subjected to moderate drought and unstressed conditions. Severe and moderate drought stresses induced the expression of maize *PARP2* gene, suggesting that deregulation of maize *PARP2* gene is likely to improve the ability of tropical maize to resist severe drought stress condition.