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Kenyatta University

Improvement in Inheritance of somatic embryogenesis and plantlet regeneration in tropical Maize lines from friable Callus

[1]John Muoma, [2]Omwoyo Ombori, [1] Jesse Machuka
[1] Department of Biochemistry and Biotechnology, Kenyatta University, P.O Box 43844-00100 Nairobi, Kenya
[2] Departments of Plant and Microbial Science, Kenyatta University, P.O Box 43844-00100 Nairobi, Kenya

Recalcitrance to regeneration and transformation of tropical maize has slowed down the potential research in improvement of variety, quality and capability of tropical maize to withstand abiotic and biotic stresses. Plant Transformation laboratory (PTL) in Kenyatta University, Kenya has developed a regeneration system for tropical maize inbred lines important to Kenyan breeders using 1-2 mm in size of immature zygotic embryos as explants. This system has proved to be highly genotype-dependent. In this study, inbred, single cross hybrid and backcross generations developed from crossing maize inbred CML216 with a commercially important inbred maize lines TL08 demonstrated genetic effects on somatic embryogenesis and plant regeneration when immature zygotic embryos were cultured on N6 medium according to Frame et al 2002. Additive gene effects were more important in the crosses than dominant gene effects causing a 50% increase in somatic embryogenesis when maternal inbred was CML216 and TL08 as the paternal (Table 1) and up to 33.3% increase in the regeneration frequency (Table 2) in single cross hybrids relative to inbreds. In backcross generations of the four crosses, maternal and/or paternal effects were significant in the frequency of somatic embryos formed by the F_1 three weeks after culture as well as in the frequency of plants regenerated per embryo, nine weeks after culture. Analysis of genetic variances suggests that crosses with CML216 as maternal donor with TL08 as pollen donor had up to 64% increase in somatic embryogenesis. Regeneration in all experiments was independent of the crossing pattern with the inbred poor rooting pattern resulting in poor acclimatization of the regenerants. TL08 and CML216 had a low regeneration frequency of 15% and 24% respectively (Table 2). Hybrid vigor was exhibited by the high regeneration efficiency of between 60-63% and 55-61% of the regenerated plants in case of the single cross hybrid and the back cross generation respectively with the exception of (CML216 x TL08) X TL08 and (TL08 X CML216) X CML216, which had a regeneration efficiency of 32% and 44% respectively (Table 2). The experiments were done with CML216 as control for the F_1 as it had consistently shown a high percentage of somatic embryogenesis of up to 97%.

In conclusion, the results from the two tropical inbred lines and their crosses indicated that regeneration is genetically controlled by nuclear genes in maize. Segregation for somatic embryogenesis in the cross between CML216 and recalcitrant but commercially important inbred TL08 could be accounted for by a small number of genes as a large proportion of genotypic variation for the formation of type 1 and type 2 callus may be due to additive gene effects. The effective understanding of inheritance of somatic embryogenesis of tropical maize is a very important process for any future work on maize transformation. This will provide a sure way of deciding which tropical maize lines can be used for gene transfer with maximum success. The current transformation frequencies of 50% can be further improved to 75% as a result of 50 %

increase in the number of immature zygotic embryos forming somatic embryos. Previous transformation efficiencies at PTL at Kenyatta University, Kenya of 5-10% can equally be improved to up to 15%. Overall these results will provide a sure way of regeneration of *Agrobacterium* mediated transformed events with high number of putative transformed events.

Table 1. Callus induction from immature embryos of two tropical inbred lines TL08 and CML216 and their single cross hybrid and back cross generation

Genotype	Number of embryo cultured	Calli forming somatic embryos	Somatic embryo induction frequency (%)
CML216	200	194	97
TL08	200	81	40
CML216 x TL08	150	96	64
TL08 X CML216	190	97	51
(CML216 x TL08) X CML216	120	91	76
(TL08 X CML216) X CML216	100	73	73
(CML216 x TL08) X TL08	250	118	47
(TL08 X CML216) X TL08	150	66	44

Table 2. Regeneration of tropical inbred lines TL08 and CML216 and their single cross hybrid and back cross generation

Genotype	Calli forming somatic embryos	Regenerants	Regeneration frequency (%)	Acclimatized plants	Regeneration efficiency (%)
CML216	90	50	55	22	24
TL08	50	38	76	8	15
CML216 x TL08	45	44	99	27	60
TL08 X CML216	60	46	76	38	63
(CML216 x TL08) X CML216	55	40	73	34	61
(TL08 X CML216) X CML216	60	35	58	26	44
(CML216 x TL08) X TL08	40	37	92	13	32
(TL08 X CML216) X TL08	75	61	81	39	55