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Presenter Information

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Over-expression of tricarboxylic acid metabolism genes in forage crops *Neonotonia wightii* and *Brachiaria brizantha* leads to ectopic root development

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Key words : aluminum tolerance, *Brachiaria brizantha*, organic acid, perennial soybean, transgenic plant

Introduction Acid soils comprise approximately half of the total agriculturally viable surface of the planet and its main limitation is caused by aluminum toxicity that leads to impaired root growth, thus decreasing water and nutrient absorption by the plants. Recently, it has been demonstrated that anion secretion by the roots is an important mechanism of Al tolerance. In the present work, it has been stably introduced and over-expressed genes coding for a citrate and malate synthase genes in perennial soybean (*N. wightii*) and palisadegrass (*B. brizantha*), respectively.

Material and methods *N. wightii* was transformed by co-cultivation of zygotic embryo apices with *A. tumefaciens* LBA4404 p35S-MiCS and *B. brizantha*, with co-cultivation of scutellum with *A. tumefaciens* EHA101 pIG121-TaCIC (ZmUbi pro) and biolistics of zygotic embryos. Transgenic plants were screened by antibiotic resistance (kanamycin and hygromycin for *N. wightii* and *B. brizantha*, respectively) histochemical GUS and gene-specific PCR and molecularly analyzed by semi-quantitative PCR.

Results In perennial soybean, the transformation efficiency was of approximately 1.4% (Table 1, Figure 1). *B. brizantha* transgenic plants were obtained by biolistics (0.23% of efficiency) and *A. tumefaciens* co-culture of scutellum-derived calli (0.7%)(Table 2, Figure 2).

Table 1 *Neonotonia wightii* genetic transformation.

experim. number	Co-cultured embryos	Kan ^R embryos
1	50	1
2	50	2
3	30	1
4	86	2
5	50	0
6	50	0
7	74	0
8	52	1
9	71	1
10	76	2
11	70	0
12	81	1
13	50	0
TOTAL	790	11

Table 2 *Brachiaria brizantha* genetic transformation.

experim. number	n. embryos	hyg ^R embryos
Biolistics		
1	72	0
2	72	0
3	72	0
4	72	1
5	144	1
6	72	0
7	72	0
8	72	0
9	144	0
10	72	0
<i>A. tumefaciens</i> EHA105		
1	219	0
2	154	1
3	87	0
4	120	0
5	231	1
6	71	0
7	82	1
8	56	2
TOTAL	1884	7

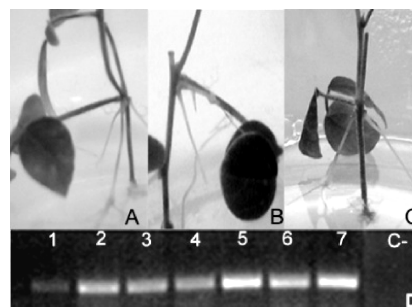


Figure 1 Ectopic root formation in transgenic *N. wightii* plants. (A), (B), (C) ectopic root formation from plant node, petiole and stem, respectively. (D) semi-quantitative PCR of MiMIC expression. The numbers above the lanes correspond to plant number, C-negative control. Lane 2 corresponds to the plant shown in A, lane 5 represents the plant shown in B and lane six corresponds to plant C.

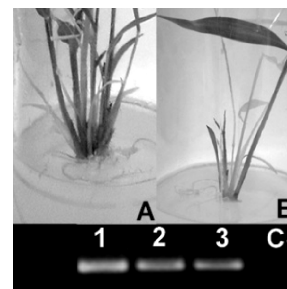


Figure 2 Ectopic root formation in transgenic *B. brizantha* plants. (A), (B) ectopic root formation. (C) semi-quantitative PCR of TaMIC expression. The numbers above the lanes correspond to plant number, C-negative control. Lane 1 corresponds to the plant shown in A and lane 3 represents the plant shown in B.

Conclusion Transgene expression analysis in transgenic *N. wightii* and *B. brizantha* has demonstrated an association between transgene expression and ectopic root development.