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Short Communication

Use of amino acids and silicon carbide fibers for improving *in vitro* plant regeneration and *Agrobacterium* mediated genetic transformation in Sudan grass [*Sorghum sudanense* (Piper)]

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Immature caryopses (10-14 DAP) of a genotype (SDSL 92115) of Sudan grass [*Sorghum sudanense* (Piper)] were aseptically inoculated on to MS agar media supplemented with 2,4-D (2 mg/l), kinetin (0.5 mg/l) and various concentrations (200, 500 or 1000 mg/l) of proline and/or asparagine. The cultures were incubated in dark at 25 ± 2°C. The first sub-culturing was made to the same medium on which callus was induced. For plant regeneration same basal callusing medium (except for 2,4-D and kinetin) with 2 mg/l IAA and 1 mg/l kinetin was used. Seven to eight weeks old callus from each of the explants was cut into small pieces and put on the regeneration media. The cultures were incubated at 25± 2°C in light (16 hrs photoperiod, 2500-3000 lux) under fluorescent lamp.

For genetic transformation *Agrobacterium* strain LBA 4404 which carried the binary vectors pBI 121 (Fig. 1 a, Clontech, USA) and pTOK 233 (Fig. 1 b, T Komari, Japan) was used. *Agrobacterium* for co-cultivation was prepared as described earlier [1] except for the use of YEM medium in place of YEP medium. Immature embryos were taken in eppendorf tubes containing 5% SCF suspension and 20ul of 7C liquid medium. This mixture was vortexed for 2, 3 or 5 minutes to injure the explants. *Agrobacterium* co-cultivation was done (i) after wounding of explants with SCF and (ii) without wounding of explants with SCF. Co-cultivation and histochemical GUS assay was carried out as described by Singh and Chawla [1]. Fifty immature embryos were also stained to assess the endogenous GUS expression.

Comparable callus induction frequencies, callus growth and friability of the control treatment and the best responding treatment suggested that amino acids had no influence on callus induction frequency (Table 1). Addition of 200 mg/l proline or 200/500/1000 mg/l asparagine or 200 mg/l of each of the two amino acids to the control medium significantly enhanced embryogenic callus induction frequency response. Most of the embryogenic callus induced was compact and friable embryogenic callus was observed only rarely (Fig. 2 A,B). Shoot induction frequency could be significantly improved on supplementing the control medium with 200 mg/l proline (Fig. 2 C) or 200/500/1000 mg/l of asparagine or combination of these two amino acids (200 or 500 mg/l each). These amino acids also

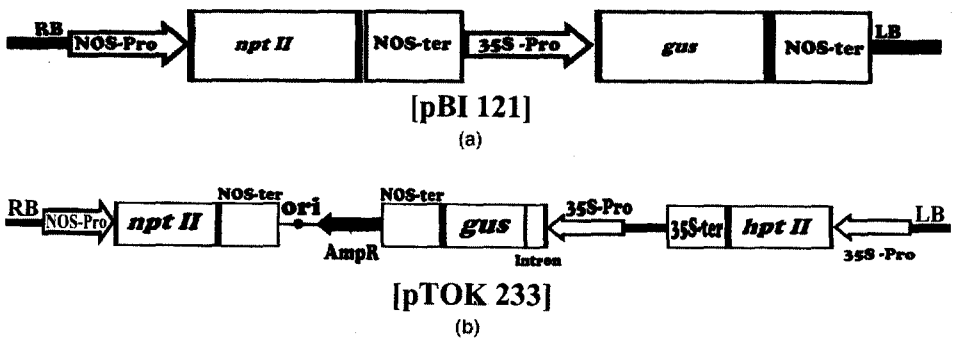

Fig. 1. T-DNA regions of binary vectors: (a) pBI 121 and (b) pTOK 233

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Table 1. Effect of amino acid on *in vitro* callus induction and shoot regeneration from immature embryos of Sudan grass genotype SDSL 92115

Media	Callus induction (%)	Embryogenic callus induction (%)	Shoot induction (%)	No. of shoots/ callus
7C (No Amino Acid)	57.82 (ab)	30.80 (c)	31.36 (ef)	1.36 (d)
8C (200 mg/l Proline)	56.86 (abc)	36.58 (ab)	39.53 (cd)	2.37 (be)
9C (500 mg/l Proline)	55.06 (abed)	23.42 (e)	31.25 (ef)	1.95 (c)
10C (1000 mg/l Proline)	50.42 (defg)	24.64 (de)	35.50 (cde)	2.50 (b)
11C (3000 mg/l Proline)	46.02 (g)	23.06 (e)	33.93 (de)	1.41 (d)
12C (200 mg/l Asparagine)	52.27 (cdef)	35.48 (ab)	47.08 (ab)	3.47 (a)
13C (500 mg/l Asparagine)	60.20 (a)	38.79 (a)	41.58 (be)	3.36 (a)
14C (1000 mg/l Asparagine)	54.53 (bcde)	35.75 (ab)	40.09 (c)	2.12 (be)
15C (3000 mg/l Asparagine)	56.19 (abc)	23.15 (e)	24.73 (g)	0.89 (e)
16C (200 mg/l Proline + 200 mg/l Asparagine)	59.73 (ab)	37.01 (a)	52.73 (a)	3.53 (a)
17C (500 mg/l Proline + 500 mg/l Asparagine)	47.05 (fg)	33.40 (be)	38.67 (cd)	1.05 (de)
18C (1000 mg/l Proline + 1000 mg/l Asparagine)	54.52 (bcde)	30.59 (c)	26.33 (fg)	0.78 (e)
19C (3000 mg/l Proline + 3000 mg/l Asparagine)	49.24 (efg)	27.02 (d)	24.17 (g)	0.89 (e)
	SE of mean	CD at 5%		
Callus induction frequency	1.86	5.33		
Embryogenic callus induction frequency	1.20	3.43		
Shoot induction frequency	2.02	5.79		
No. of shoots/callus	0.16	0.46		

Mean values in the column with different alphabets differ significantly at 5% level of significance

exhibited their stimulatory effect on number of shoots/callus and a significant improvement in shoot regeneration rate was observed when control medium was supplemented with 200/500/1000 mg/l of either proline or asparagine or both (200 mg/l each). Enhancement in the embryogenic callus induction frequency, shoot induction and shoots/callus responses, on the use of amino acid in the culture medium of immature embryo, is in accordance with the findings of Rao *et al.* [2] in sorghum.

It was surprising to find the endogenous GUS expression in 44 per cent of the immature embryos. Pattern of endogenous GUS expression was uniform in all the explants and was characterized by light blue staining of the whole explant. In addition to such a uniform staining of the whole explant, the co-cultivated immature embryos also exhibited the spotted blue pattern of GUS expression (Figs. 2 D,E). The GUS expression frequency of without SCF treatment (9.60%) could almost be doubled with SCF's five minute treatment (17.30 %, Table 2). In both of the vectors five minutes treatment was more effective than two and three minute treatments and control. When both the binary vectors were compared, pBI 121 gave a higher expression frequency of 22.03 per cent as compared to 1.55 per cent of pTOK 233. The occurrence of blue spots in case of pBI 121 was frequent (Fig. 2 D) and on a single embryo a number of spots were observed. In case of pTOK 233, blue spots were present only sparsely (Fig. 2 E). The exact mechanism for SCF mediated genetic transformation is not known. Earlier, Kaeppler *et al.* [3]

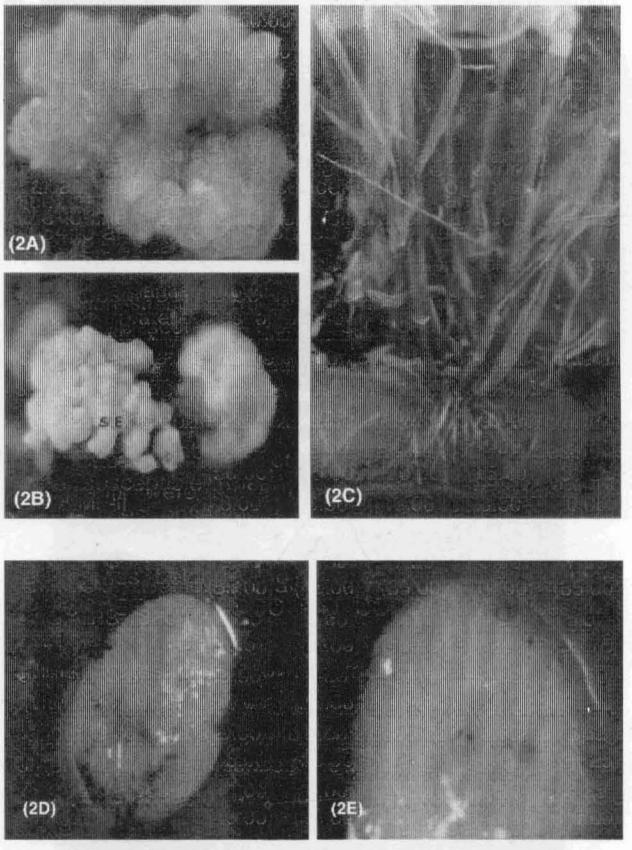

Fig. 2. Compact embryogenic (2 A) and friable embryogenic calli (2 B) with somatic embryo (SE) induced on 200 mg/l Proline and Asparagine supplemented media and profuse plant regeneration from these calli (2 C). GUS expression through pBI 121 (2 D) and pTOK 233 (2E).

Table 2. Effect of silicon carbide fiber treatment on *Agrobacterium* mediated genetic transformation efficiency in Sudan grass genotype SDSL 92115

SCF Treatment		LBA 4404 (pBI 121)	LBA 4404 (pTOK 233)	Mean
Wounding	2 minute	20.00	0.00	10.00
	3 minute	25.00	4.34	14.67
	5 minute	29.60	5.00	17.30
	Mean	24.86	3.11	13.99
No Wounding		19.20	0.00	09.60
Mean		22.03	1.55	11.79

proposed that DNA bound to SCF surface is carried into the penetrated cells and may have the potential to become integrated into the nuclear genome. Later, Thompson *et al.* [4] proposed that the SCFs function as numerous needles and facilitate DNA entry into the cells during vortexing. The common point of two proposals is piercing of cell wall by SCFs, which in the context of the present investigation served as injury to enhance the attachment of *Agrobacterium* to these wounded sites. It has been demonstrated that partial enzymatic digestion for limited cell wall degradation (wounding), in a way that the ability of the cell to undergo differentiation is not impaired, promotes preferential attachment of the *Agrobacterium* at the wound site [5]. The proposal of Mooney and Goodwin [5] that the enhanced attachment of *Agrobacterium* should facilitate transformation, has been found to hold good as injury of explants prior to their co-cultivation with *Agrobacterium* by gold particles (banana) and by SCFs (wheat) has resulted in enhanced transformation efficiencies [1, 6]. The present investigation also supports the observation of these workers.

From the results of present investigation it can be concluded that the efficiency of plant regeneration in this genotype SDSL 92115 of Sudan grass may be significantly improved with the use of proline and asparagine amino acids in the callus induction and plant regeneration media. Our studies also showed that like wheat [1], the efficiency of *Agrobacterium* mediated transformation could be improved in sudan grass also by wounding the explants with SCFs, which are cheap and require no expensive equipment for their use.

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