Table 7. Comparison between observed forecast ISMR and sum of forecast AIMR

Year	Observed forecast ISMR *A* (mm)	Sum of forecast AIMR (June–Sept.) *B* (mm)	*A/B*
1995	944	864	1.1
1996	991	792	1.3
1997	886	756	1.2
1998	889	808	1.1
1999	861	1044	0.8
2000	793	656	1.2

observed values; and σ_p and σ_o are the standard deviation (SD) of the predicted and observed values respectively.

Table 5 summarizes the hindcast skill of a 2-layer CN for 12 months for 74 hindcasts.

While the hindcast skill for AIMR is poor when compared to ISMR, it is encouraging to note that average error is still smaller than the SD of the data in most cases. We record here, therefore, our experimental forecast for 12 months for the year 1999 and 2000 (Table 6).

We would like to emphasize that these forecasts are purely experimental, and not meant for any operational use. The success of the previous years is to be accepted with usual and necessary caution. In case of AIMR, there are greater uncertainties as the forecast (or hindcast) skill is somewhat inversely proportional to the SD of the data. More importantly, the present forecasts of AIMR are like a pattern-forecast for the entire period from 1995 to 2000, since the observed data in this case was employed only until 1994. These monthly forecasts, on the other hand, will put much more stringent tests on the NN forecasts. For example, the sum of AIMR for June to September should also show a good agreement with forecast for ISMR, so that an organized hierarchy of NN forecast systems can be developed. In the present case, this condition was tested and verified. As an example, Table 7 shows a comparison between observed ISMR along with forecast ISMR for 1999 and 2000 and the sum of forecast AIMR for June to September for 1995 to 2000.

Even moderate success in AIMR can have enormous impact in areas like agricultural planning. For example, both for 1998 and 1999, and especially for 2000, the low value of forecast ISMR is due to significant deficit in the AIMR for September. Indeed, the ISMR for 2000 is seen to be peaked in essentially two months, July and August. These features have practical and significant implications for issues like crop choice, irrigation planning and sowing schedule. For the same reason, however, a very thorough and objective evaluation of the forecast skill is necessary. We hope that the results published in this article will provide a definite step in this direction.

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ACKNOWLEDGEMENTS. G.C., V.S., K.V.S. and M.K.N. thank Dr R. N. Singh, Scientist-in-Charge, C-MMACS for permission and encouraging support to carry out this project work at C-MMACS.

Received 21 November 1998; revised accepted 8 March 1999

Use of silicon carbide fibers for *Agrobacterium*-mediated transformation in wheat

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Silicon carbide fibers have been used for causing wounds in the immature wheat embryos for *Agrobacterium*-mediated genetic transformation. Immature embryos were put in a 5% silicon carbide fiber (SCF) suspension and vortexed for 2 or 3 min followed by co-cultivation with *Agrobacterium tumefaciens* strain LBA4404. The strain harboured the binary vectors pBI121 and pTOK233 which contained selectable marker genes and *gus* as the reporter gene. *Agrobacterium*-infected explants were stained for GUS activity. Without wounding the GUS expression was observed in 2.4% of the embryo, while 33.3% of the embryos showed GUS expression after wounding with SCF for 2 min. The binary vector pBI121 showed better response than pTOK233 for GUS expression. We propose that SCFs can be used for wounding to improve frequency of transformation by disarmed *Agrobacterium* strains.

TRANSFORMATION studies were conducted on wheat with the *Agrobacterium* strain LBA4404 which carried the binary vectors pBI121 (Clonetech, USA) and pTOK233 (Yuko Hiei, Japan). The T-DNA of pBI121 contained the *gus* reporter gene controlled by 35S promoter and *npt*II selectable marker gene controlled by the nopaline synthase (NOS) promoter. The binary vector pTOK233 contained the *gus* reporter gene controlled by CaMV35S promoter, which had an intron in the N-terminal region of

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the coding sequence. The genes for selectable markers were hygromycin phosphotransferase (*hpt*II) controlled by the 35S promoter conferring resistance to hygromycin and *npt*II controlled by the NOS promoter conferring resistance to kanamycin. SCFs were obtained from Advanced Composite Materials Corp. USA, whose size averaged 0.6 µm in diameter and 10–80 µm in length. SCFs were used for effecting wounds in the immature embryo explants for *Agrobacterium*-mediated transformation.

The strain LBA4404 was grown on YEP medium plates (yeast extract, 10 g/l; peptone, 10 g/l; sodium chloride, 5 g/l; pH 7.0). For binary vector pBI121, YEP medium with 50 mg/l kanamycin, 50 mg/l streptomycin and 50 mg/l rifampicin was used while for pTOK233, YEP medium with 50 mg/l kanamycin, 100 mg/l streptomycin and 50 mg/l hygromycin was used. *Agrobacterium* strains were grown in petri plates or liquid medium at 28°C for 2 days in the dark. *Agrobacterium* suspension for co-cultivation experiments was prepared by picking a single colony from the YEP media plates and inoculated in 5 ml of liquid YEP medium containing acetosyringone and antibiotics as per the binary vectors. The O.D. of the culture was measured at A-600 nm. The culture was diluted or concentrated to bring its value to 0.1 O.D. For co-cultivation, the *Agrobacterium* cell suspension was centrifuged at 5000 rpm for 10 min, supernatant removed and diluted to a final concentration in MSE1 medium (MS^1 basic salts supplemented with 2 mg/l 2,4-D, 100 mg/l glutamine, 200 mg/l casein hydrolysate and 100 µM acetosyringone).

Immature embryos (1 to 1.5 mm diameter) of two Indian varieties, UP2338 and PBW226 were used as explants for co-cultivation with *Agrobacterium*. Twelve immature embryos were taken in eppendorf tubes containing 5% SCF suspension (2.5 mg dissolved in 50 µl of sterile distilled water) and 20 µl of MSE1 liquid medium. This mixture was vortexed (Genei vortex, Bangalore) for 2 to 3 min to injure the explants. *Agrobacterium* co-cultivation was done: (i) after wounding of explants with SCF; and (ii) without wounding with SCF. Both the explants were transferred to tubes containing acetosyringone-induced *Agrobacterium* culture and were incubated at 25°C for 2 h on a platform shaker at 100 rpm. The explants were then blot dried on a sterile filter paper and then transferred to MSE1 medium plates without selection agent in the dark at 25°C for 3 days. After co-cultivation, the explants were transferred to MSEC medium (MSE1 with 400 mg/l cefotaxime) for 3 days. The explants were then transferred to a selection medium of MSK121 (MSEC with 100 mg/l kanamycin) or MSK233 (MSEC with 100 mg/l kanamycin and 100 mg/l hygromycin). Histochemical GUS assay was performed on explants taken from selection media, i.e. after 6 to 10 days of co-cultivation. X-Gluc stain was prepared by dissolving 30 mg X-Gluc in 500 µl DMSO and then 10 ml solution is made up with 5 mM each of potassium ferricyanide and potassium ferrocyanide, 0.1 M phosphate buffer (pH 7.0) and 0.3% Triton X-100. The co-cultivated embryos were put in wells of ELISA plates with X-Gluc stain and incubated at 37°C for 36 h.

The GUS expression frequency in the explants without SCF treatment in both the varieties with both the vectors was very low (2.4%) whereas with SCF treatment the overall frequency was 20.7% (Table 1, Figure 1). Wounding of embryos with SCF for 2 min was more effective (33.3%) than treatment with SCF for 3 min (12.5%) in both the varieties with both the binary vectors. GUS activity was reproducibly observed when maize cell suspension cultures were vortexed for 60 s on vortex Genei II along with plasmid DNA[2]. High transient expression of *gus* gene was observed when dry embryos of wheat were vortexed in a suspension of SCFs and vector

Table 1. GUS expression in immature embryos with and without wounding with silicon carbide fibers followed by co-cultivation with *Agrobacterium tumefaciens* strains carrying two different binary vectors

		Wounding with SCF								
		2 min			3 min			Without wounding with SCF		
Variety	*Agrobacterium* strain (binary vector)	No. treated	No. stained	No. GUS positive	No. treated	No. stained	No. GUS positive	No. treated	No. stained	No. GUS positive
UP2338	LBA4404 (pBI121)	20	6	6 (100)	20	6	0 (0)	60	11	0 (0)
	LBA4404 (pTOK233)	16	5	0 (0)	41	11	3 (27.2)	70	11	0 (0)
PBW226	LBA4404 (pBI121)	20	5	0 (0)	20	6	0 (0)	55	7	1 (14.2)
	LBA4404 (pTOK233)	16	5	1 (20)	33	9	1 (11.1)	66	12	0 (0)
	Mean	72	21	7 (33.3)	114	32	4 (12.5)	251	41	1 (2.4)

Values in parentheses indicate percentage of GUS expression.

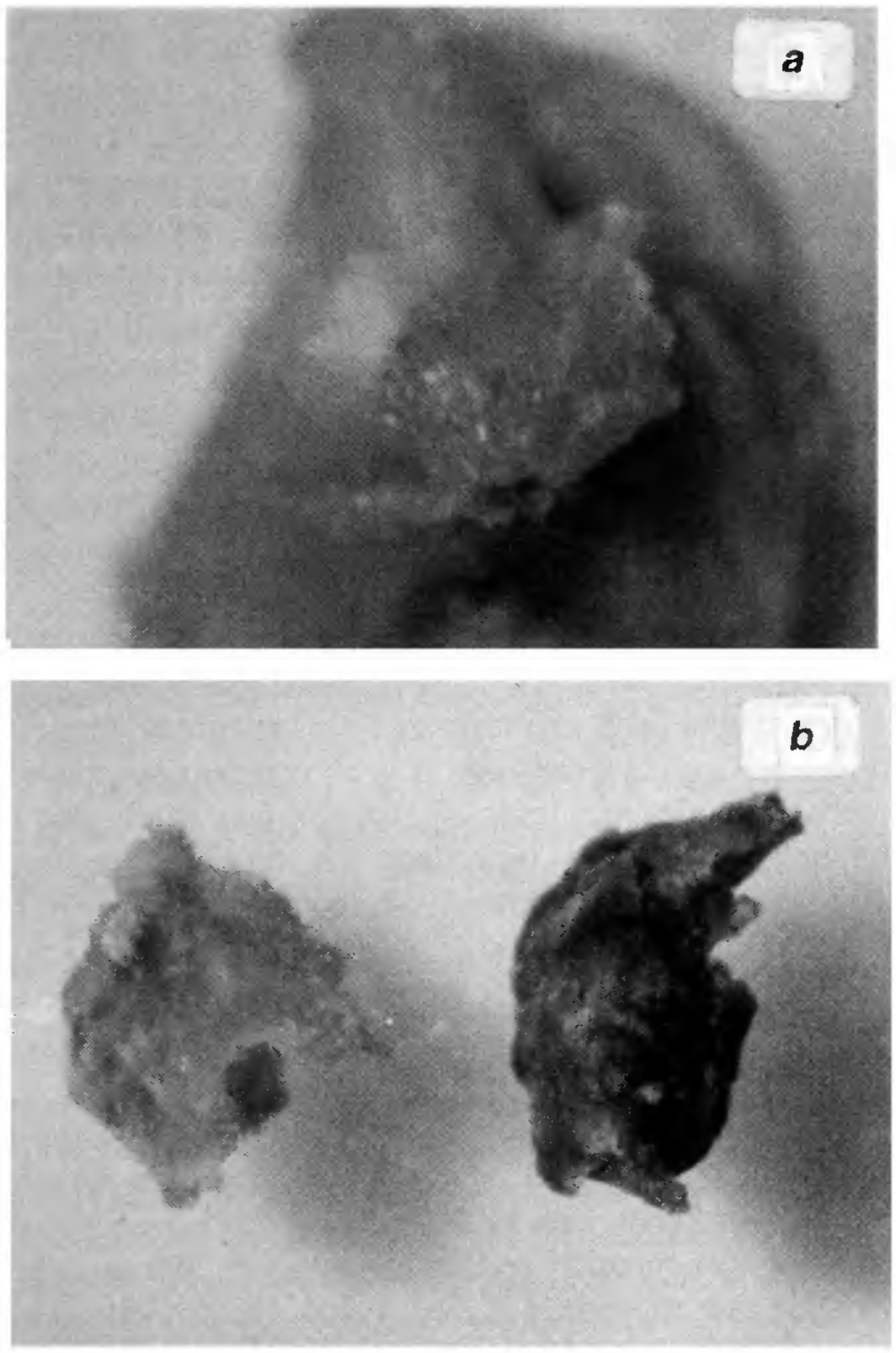

Figure 1. GUS expression in embryo explants, *a*, without wounding with silicon carbide fibers and after 10 days of co-cultivation with pBI121 (15 ×); *b*, after wounding with silicon carbide fibers for 2 min and after 10 days of co-cultivation with pBI121 (right) and control (left) (8 ×).

DNA for 10–30 min[3]. The exact mechanism for SCF-mediated transformation is not known. Silicon carbide has great intrinsic hardness and fractures to give sharp cutting edges[4]. It has been observed in maize cell suspension cultures that SCF pierces the walls of cells during vortexing and along with the fibers the DNA adhering to it might enter the cells[5]. But later reports suggest that SCFs do not carry the DNA into the treated cells but function as numerous needles facilitating DNA delivery into the cells during the mixing process[2]. In our experiments also, SCF only created injury to the cells, which facilitated *Agrobacterium*-mediated transformation. Serik and coworkers[3] reported that GUS expression appeared in the form of small patches in SCF-treated mature embryos in a direct delivery approach. In the present study also GUS expression was observed as patches on the embryo surface. It seems that SCF whiskers have created injury on the immature embryos which resulted in good infection.

On comparison, binary vector pBI121 was found to give better transformation frequencies than pTOK233. When both the varieties were considered UP2338 was found to be better with pBI121 while PBW226 responded only when co-cultivated with pTOK233 vector.

Generally, in monocots, co-cultivation with *Agrobacterium* requires wounding of the tissue for the infection process to take place. Wounding by bombardment with naked gold particles using a particle gun followed by *Agrobacterium* co-cultivation has been used[6]. But this technique is expensive and needs skillful handling. Workers have used SCFs for delivery of DNA as a physical direct method of transformation in *Nicotiana*[7], maize[2,5] and wheat[3].

Preculture of rice embryos for 2–5 days followed by co-cultivation with *Agrobacterium* for 2–3 days and then transfer to a medium with antibiotic selection agent resulted in efficient production of transgenic plants[8]. High GUS expression frequency in wheat has been observed with the addition of Silwet surfactant in the inoculation medium[9]. Our studies showed that the efficiency of *Agrobacterium*-mediated transformation can be improved by using SCF for wounding. This can be considered as a practical alternative requiring no expensive equipment or consumables, especially in laboratories which have no access to sophisticated equipment.

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ACKNOWLEDGEMENTS. We thank Dr Yuko Hiei, Japan Tobacco Inc, Japan, for providing the binary vector pTOK233 and Dr Foroughi-Wehr, Gruenbach, Germany, for providing silicon carbide fibers. N.S. is grateful to ICAR for fellowship assistance during this study.

Received 10 September 1998; revised accepted 3 March 1999