PLANT REGENERATION OF CYST NEMATODE-RESISTANT GENOTYPES OF SOYBEANS

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ABSTRACT

Soybean, *Glycine max* L. Merr. genotypes susceptible and resistant to soybean cyst nematode, *Heterodera glycines* Ichinohe, were examined for potential use in tissue culture studies. Tissue culture calli were initiated from 5 to 7 mm long immature embryos on MS (Murashige and Skoog) and B5 (Gamborg) media modified with several hormonal concentrations. Best results were obtained with MS medium containing 13.3 μM 6-benzylaminopurine, 0.2 μM naphthalenacetic acid, 5 μM thiamine and 12 μM proline. Cultures were maintained on the MSR (MS + 1.7 μM BAP + 0.2 μM IBA) medium until shoot initiation. Plant regeneration from calli derived from immature embryos by organogenesis was achieved from all the ten genotypes tested. All the PI lines retained their morphogenetic competency after they were maintained on the MSR medium for a year, whereas most of the adapted soybean cultivars lost their ability for regeneration.

INTRODUCTION

REGENERATION of somatic embryos, shoot structures and plants from several species of *Glycine* have been reported. There has been a greater success with the genotypes of *G. soja*, and *G. canescens* in tissue culture than with genotypes of *G. max*[1,2]. Gamborg *et al*[3], described the procedures for the development of somatic embryos in different species of *Glycine* including *G. max*, the cultivated soybeans. Embryoids capable of producing plantlets have been produced on modified Murashige and Skoog (MS) media[4]. Somatic embryos have also been obtained from callus derived from immature embryos and the embryoid development was reported to vary with auxin concentration[5]. Recently, fertile seed producing soybean plants have been regenerated by using modified MS media[6–9].

The purpose of this research was to study plant regeneration in soybean cyst nematode (*Heterodera glycines* Ichinohe) resistant plant introductions (PI) and some of their derivatives and to compare their response to tissue culture after short term and prolonged culturings. This information could be an important tool for a plant breeding program producing soybean cultivars with resistance to cyst nematode.

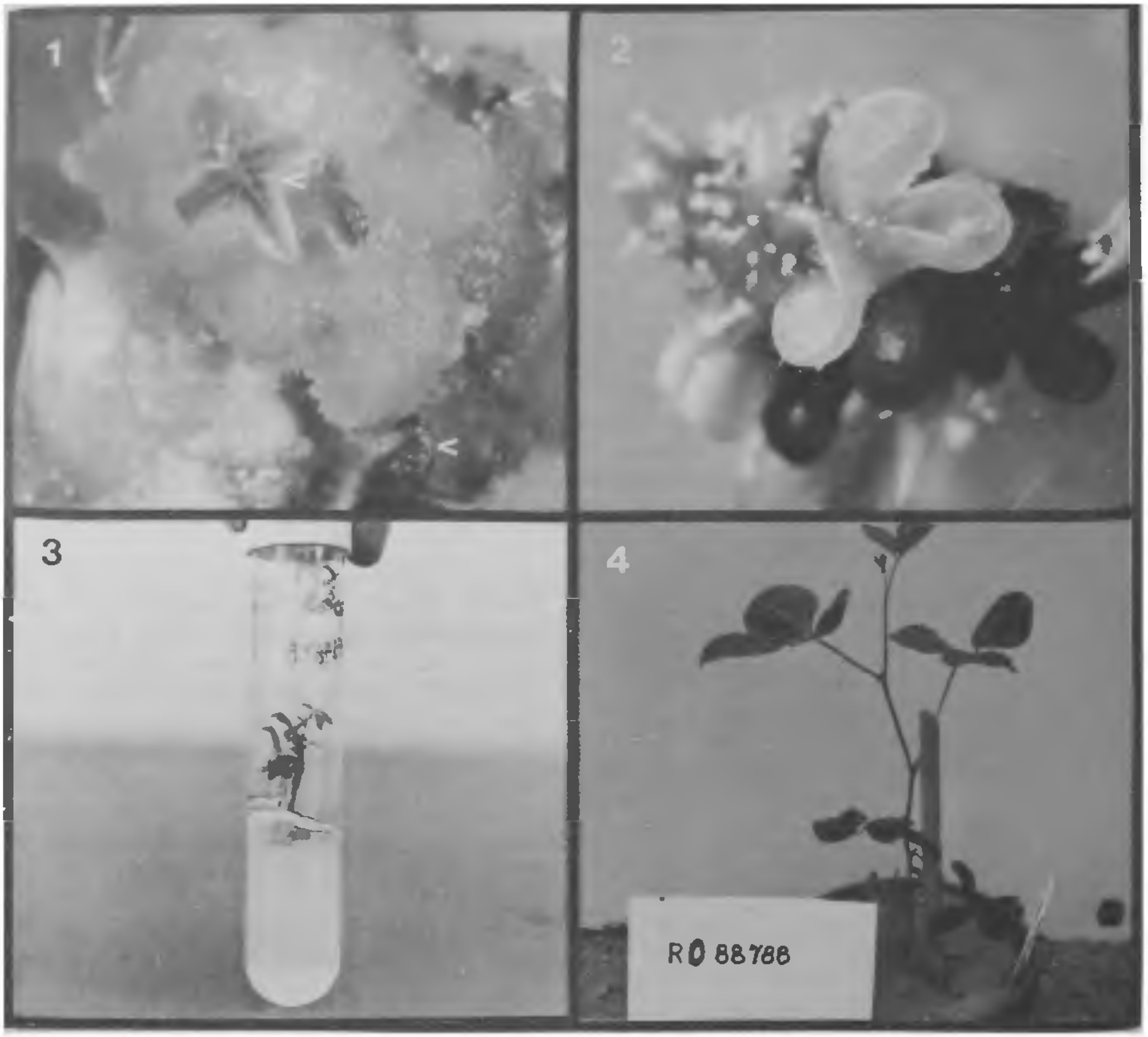
MATERIALS AND METHODS

Soybean (*Glycine max* L. Merr.) genotypes consisting of five plant introductions (PI 17852B, commonly known as Peking, PI 88788, PI 90763, PI 437654 and PI 438489B), and five cultivars (Essex, Lee 74, Pickett 71, Forrest and Bedford) were included in the present study. All the PI lines are resistant to one or more races of soybean cyst nematode[10]. Among the soybean varieties tested, Essex and Lee are susceptible whereas Pickett 71, Forrest and Bedford are resistant to some races of SCN[11]. The plants were grown in pots in the greenhouse and pods with 5–6 mm long immature embryos were collected. The pods were treated with 15% aqueous Clorox (Clorox Company, Oakland,

Table 1 *Per cent regenerative calli formation from immature soybean embryos on MS and B5 media with several hormonal concentrations.*
(Observations taken four weeks after the cultures initiation)

Medium + growth regulators	Regenerative callus initiation (%)
MS + Kinetin 26.2 μM + IBA 0.5 μM	5
MS (min organic*) + 2,3,5 triidobenzoic acid 5.0 μM	0
MS (min organic*) Kinetin 13.3 μM + IBA 0.4 μM (no proline)	0
MS (min organic*) + Kinetin 13.3 μM + IBA 0.4 μM + Proline 12 μM	5
B5 (Gamborg) + kinetin 26.2 μM + IBA 0.5 μM	10
B5 (Gamborg) + kinetin 13.1 μM + IBA 0.25 μM	10
MS + BAP 13.3 μM + NAA 0.2 μM (Barwale *et al*[6])	100

*MS (min organic): i-inositol 18 μM, niacin 0.06 μM, pyridoxine HCL 0.08 μM; thiamine HCL 0.03 μM.



Figures 1–4. 1. Regeneration of soybean by organogenesis. A trifoliate leaf, budding and shoot initiation on regenerative callus; **2.** Adventitious budding and shoots after removal from regenerative callus; **3.** Soybean plantlet in tube on rooting medium, and **4.** Complete regenerated plant in soil in the greenhouse.

CA 94612) solution for 15 min and rinsed twice in sterile distilled water. The immature embryos were removed from the pods under aseptic conditions. Four immature embryos from each genotype were plated in a single 100 × 15 mm disposable petri dish containing 35 ml of solidified medium and replicated five times. For organogenesis, Murashige and Skoog[12] and B5 basic media[3] were used with the modifications of the hormonal concentrations as described in table 1. The media were supplemented with 2.5% sucrose and solidified with 0.64% agar. The pH was adjusted to 5.8 and the media were autoclaved for 15 min at 1.05 kg/cm^2. The plated embryos were placed in the dark at 25°C for four weeks until callus was initiated. The calli were placed on MS basal medium + 1.7 μM BAP + 0.2 μM IBA (MSR) media and maintained by transfer to new media every 3–4 weeks. After shoots appeared on the calli, they were allowed to grow to 1–1.5 cm. At this stage, the shoots were transferred to a hormone-free medium[12] in 25 × 150 mm test tubes for rooting. Rooted shoots were transferred to oasis cubes until the plantlets were hardened. The calli were also cultured for one year on the MSR medium with usual transfers and then tested for their ability to organogenesis.

Table 2 *Description of ten soybean genotypes and their ability to organogenesis*

Genotype	Maturity group*	Plant type	Calli producing shoots (%)	# Shoots/ callus	Calli after 1 year in culture: Calli producing shoots (%)	Calli after 1 year in culture: # Shoots/ callus
Essex	V	D	55	2.3	0	0.0
Lee 74	VI	D	55	2.5	0	0.0
Bedford	V	D	42	2.6	33	2.1
Pickett 71	VI	D	63	2.2	0	0.0
Forrest	V	D	40	2.6	0	0.0
PI 88788	III	I	33	2.8	70	6.9
PI 90763	IV	I	40	3.0	24	2.0
PI 437654	III	I	43	3.1	61	5.4
PI 438489B	IV	I	35	3.0	52	6.1
PI 17852B	IV	D	33	2.7	12	1.3
LSD at 5%			NS	0.3	17	2.9

*Maturity classification based on 0 through *X*, where 0 is very early and *X* is very late. (Soybeans: 1973 Agronomy series 16. Pub. American Society of Agronomy. Madison, WI, USA, (ed.) B. E. Caldwell, D, Determinate; I, Indeterminate.

RESULTS AND DISCUSSION

All genotypes produced some types of callus on every media. A 100% calli formation was observed on MS + 6 BAP 13.3 μM + NAA 0.2 μM medium[6] whereas very little or no calli were produced on other media (table 1). The shoot formation due to organogenesis occurred in all genotypes (figures 1 and 2). The frequency of shoot production by the initial calli ranged from 33 to 63%. The mean number of shoots/callus ranged from 2.2 to 3.1 (table 2). Rooted shoot on hormone-free medium and potted plant are shown in figures 3 and 4. In the calli which were cultured for one year on the maintenance medium the frequencies of shoot production ranged from 0 to 70% whereas the shoots/callus varied from 0 to 6.9%. The calli from the genotypes Essex, Lee 74, Pickett and Forrest lost the ability for morphogenesis; whereas the genotypes, PI 17852B, PI 88788, PI 437654, PI 438489B, PI 90763 and Bedford retained morphogenetic competency after one year in culture.

Morphogenetically competent cultures were obtained only when the immature soybean embryos were used for callus initiation. By using the MS-modified medium[6], we regenerated viable plants from the calli of all the 10 genotypes tested. None of the calli from other media produced any shoots when transferred to MSR media. All five plant introductions retained their ability to regenerate even after a year of culturing on the media, whereas all the cultivated genotypes (cultivars) except Bedford lost their ability to regenerate. It appears from these results that unadapted plant introductions are able to retain their regeneration capability better whereas the adapted cultivars lose their capacity for regeneration after prolonged culturing. Our successful attempt at regenerating soybean plants routinely could be used to accelerate conventional plant breeding procedures to produce cyst-resistant cultivars.

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NEWS

R. D. ASANA MEDAL FOR THE YEAR 1986

Dr Kushal Pal Singh, Assistant Plant Physiologist at Regional Research Station of Himachal Pradesh Krishi Vishva Vidyalaya, Dhaulakuan has been awarded the prestigious R. D. Asana Medal for the year 1986 for his outstanding research contributions in the field of plant physiology by the Indian Society for Plant Physiology, New Delhi.

AWARDS TO IRRI SCIENTISTS

Dr Gurdev S. Khush, IRRI Principal Plant Breeder, was elected a 1987 American Society of Agronomy (ASA) Fellow — the highest recognition the society awards its members.

Dr Benito S. Vergara, IRRI Plant Physiologist, was elected academician of the National Academy of Science and Technology of the Philippines.

Dr N. C. Brady, former IRRI Director General, has been elected to receive the 1987 ASA International Agronomy Award.

WORLD ACADEMY OF ART AND SCIENCE

The World Academy of Art and Science has announced the election of Dr Dennis J. Greenland as Fellow.

Greenland is Deputy Director General of CAB International, U.K. He served as IRRI Deputy Director General from 1979 to 1987.

The Swiss-based World Academy honours individuals from diverse national backgrounds chosen for eminence in the natural and social sciences and the humanities. The Academy promotes a world order in which human dignity is honoured in deed as in word, and expresses concern for the social consequences and policy implications of knowledge.