Increase in straw and grain weights has led to non-significant changes in harvest index.

Assimilation and conductance rates and internal CO_2 (Ci) levels were higher and A/gs values were lower in the material (Table 2) developed. Germination (%), root and shoot lengths and seedling vigour were greater (Table 3). The inhibitory effect of PEG was less pronounced in the somaclonal material.

Assimilation rates determine yield[18,19]. Stomatal conductance is the rate-limiting step in photosynthesis[20]. Here, the somaclonal plants exhibited greater 'A' and gs rates, resulting in enhanced Ci and reduced A/gs values, showing favourable changes in gas exchange parameters. These along with greater total leaf area indicate greater canopy photosynthesis, i.e. enhanced capacity and size of source, culminating in enhanced yield potential, TDM and seed yield, probably by partitioning dry matter more towards reproductive sinks.

The uniformity noticed at present is the result of single plant selection, unlike in previous cases[8,9,10,12], where a population of tissue culture-derived plants was assessed. Greater germination per cent and seedling vigour at all levels of PEG stress, suggest better stress tolerance capacity of somaclonal seeds. The enhanced root length of these, contributing to the seedling vigour, indicates a more elaborate water harvesting mechanism, which is of significance in a rainfed (upland) cultivar like Halubbalu. The present results and previous report on submergence-tolerant somaclones[13] indicate the usefulness of the *in vitro* selection technique in developing rice lines suitable for contrasting situations. Hence, the trials are underway for field evaluation of this promising line developed by *in vitro* selection.

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Received 1 October 1992; revised accepted 12 May 1993

Cryopreservation of penaeid prawn embryos

T. Subramoniam and S. Samuel Newton
Department of Zoology, University of Madras, Guindy Campus, Madras 600 025, India

The cryopreservation of penaeid prawn embryos has not been successfully carried out so far although standard protocols for several mammalian embryos are available. Penaeid prawn embryos develop in the sea water medium and hence the methodology developed for mammalian embryos, whose development takes place in the intra-uterine environment, cannot be applied for prawn embryo cryopreservation. We have successfully frozen, for the first time, the nauplii of the penaeid prawn *P. indicus* to -30°C and -40°C using liquid nitrogen vapour. In order to develop the freezing protocol for these embryos we have considered the following principal factors: cryoprotectant toxicity, addition and dilution of cryoprotectant, equilibration time, freezing and thawing rates. The response of the morula stage embryos (2 h after spawning) and nauplii to several permeating cryoprotectants were studied in detail. The hatch percentage was used as an index to evaluate survival of cryoprotectant-treated morulae and direct observation of morphology and motility was used to assess survival of cryoprotectant-treated as well as frozen nauplii. Our initial attempt to vitrify the embryos was not successful.

CRYOGENIC storage of mammalian embryos and gametes has been widely used for *in vitro* fertilization (IVF) and embryo transfer technology. Though well known and popular at IVF clinics[1] and cattle breeding research stations[2] it is only recently that the application potentials of cryopreservation have been realized in the aquaculture industry. The cryopreservation of embryos of the penaeid prawn *Penaeus indicus* assumes much importance as it will help in ameliorating the problem of seed scarcity faced by the aquaculture industry during the lean season. The cryopreservation protocol

devised for *P. indicus* embryos can be extended to the embryos of other penaeid species.

Morulae and nauplii were exposed to seven permeating cryoprotectants, glycerol, ethylene glycol, dimethyl sulphoxide (DMSO), propylene glycol, formamide, acetamide and methanol at concentrations ranging from 5 to 20% v/v. The cryoprotectants were diluted in filtered, UV-treated sea water and added in 1 or 3 serial additions. As our preliminary studies on cryoprotectant toxicity showed that the cryoprotectants were more toxic at higher temperatures, all the addition and dilution procedures were carried out at 15°C unless otherwise mentioned.

Glycerol, a widely used cryoprotectant[3], and formamide were extremely toxic to morulae resulting in high mortality at concentrations above 5% v/v. Acetamide, propylene glycol, methanol and DMSO were relatively less toxic, but ethylene glycol was the least toxic. The embryos could be safely exposed, without significant drop in hatch percentage, to ethylene glycol concentrations $\leq 15\%$ v/v for 15 min. Equilibration time (period of exposure to cryoprotectant) was an important factor with regard to cryoprotectant toxicity as there was a notable fall in survival in all cases when the equilibration time was extended beyond 15 min. In comparison to the morula stage embryos, the nauplii were tolerant to much higher concentrations of cryoprotectant. With the exception of formamide, the nauplii tolerated all the above mentioned cryoprotectants in concentrations up to 25% v/v.

Since the prime prerequisite for vitrification is the ability of the biological sample to withstand high concentrations of cryoprotectant[4], nauplii were used for cryoprotectant toxicity studies in vitrification experiments. Accordingly, the vitrification medium was arrived at by taking into consideration individual properties of certain permeating and non-permeating cryoprotectants. The properties exhibited by each of these cryoprotectants have a specific role in the vitrification process. The vitrification media used were combinations of DMSO, ethylene glycol, propylene glycol, acetamide and polyvinyl pyrrolidone in varying concentrations. DMSO and ethylene glycol are well-known permeating cryoprotectants that protect intracellular components against freezing damage. Propylene glycol is an excellent glass former[5] aiding the formation of a non-crystalline amorphous solid that is characteristic of vitrification. Acetamide has been reported to have specific toxicity neutralization properties for DMSO[6]. Polyvinyl pyrrolidone, a high molecular weight (40,000) non-permeating cryoprotectant, protects against membrane damage that occurs when very high cooling rates are used, as is the case in vitrification. The possibility of cryopreserving nauplii by vitrification was investigated by employing four different vitrification solutions with total cryoprotectant concentration of 28% v/v, 28% v/v, 32% v/v and 38% v/v. The high concentration of cryoprotectant in the vitrification solution made stepwise addition mandatory. Addition was done in three serial steps at 15°C, 4°C, −10°C in order to minimize toxicity to the least possible level[7]. The nauplii exhibited tolerance to the toxicity of the vitrification solutions but did not revive after freezing to −196°C though morphological damage was unperceivable. Probably, the cryoprotectant concentration of the vitrification solution employed, though substantial, was not high enough to achieve total vitrification (prevention of both extracellular and intracellular ice formation). Our efforts to further raise the concentration of the vitrification solution resulted in a sharp rise in mortality.

Alternatively, we have investigated the efficacy of the conventional slow cooling approach in developing a protocol for cryopreservation of prawn nauplii. This methodology uses comparatively lower concentration of cryoprotectant, thus reducing greatly the magnitude of cryoprotectant toxicity. In the slow cooling approach to cryopreservation the critical factor is the cooling rate, which has to be very precisely controlled. This was surmounted by using a rate-controlled programmable freezer, Kryo 10, from Planer Biomed, UK. This microprocessor-controlled unit uses liquid nitrogen to cool samples, and is capable of cooling at rates as fine as 0.01°C/min. Stage VI nauplii were treated separately with 15% v/v concentrations of DMSO, ethylene glycol and methanol. The addition of diluted cryoprotectant was done in two steps at 15°C. After an equilibration period of 15 min, the samples were loaded into 2 ml ampoules and cooled to subzero temperatures in the Kryo 10. The freezing protocol employed was a 3-ramp cooling program with a start temperature of 15°C, a cooling rate of −1.5°C/min and manual seeding at −6°C using a pair of tongs dipped in liquid nitrogen. On reaching final temperatures of −30°C and −40°C the frozen samples were removed from the freezing chamber of the Kryo 10 and thawed rapidly at a thawing rate >300°C/min. The slow dilution procedure was adopted to preclude the possibility of an osmotic imbalance affecting the survival of the nauplii. High percentage survival was seen in all the cases, but the nauplii protected with ethylene glycol exhibited more active twitching movements than those protected with either DMSO or methanol. The thawed nauplii were extremely sensitive to the rate of dilution as rapid dilution caused the nauplii to evert their contents. This effect was more pronounced in the vitrified samples (Figure 1 *a*) than those that were cooled slowly. Eversion in the samples that were cooled slowly is not entirely unexpected, even while considering the low concentration of cryoprotectant used. During slow cooling, ice crystal formation and growth take place in the extracellular medium in the direction of least solute concentration. This raises the tonicity of the extracellu-

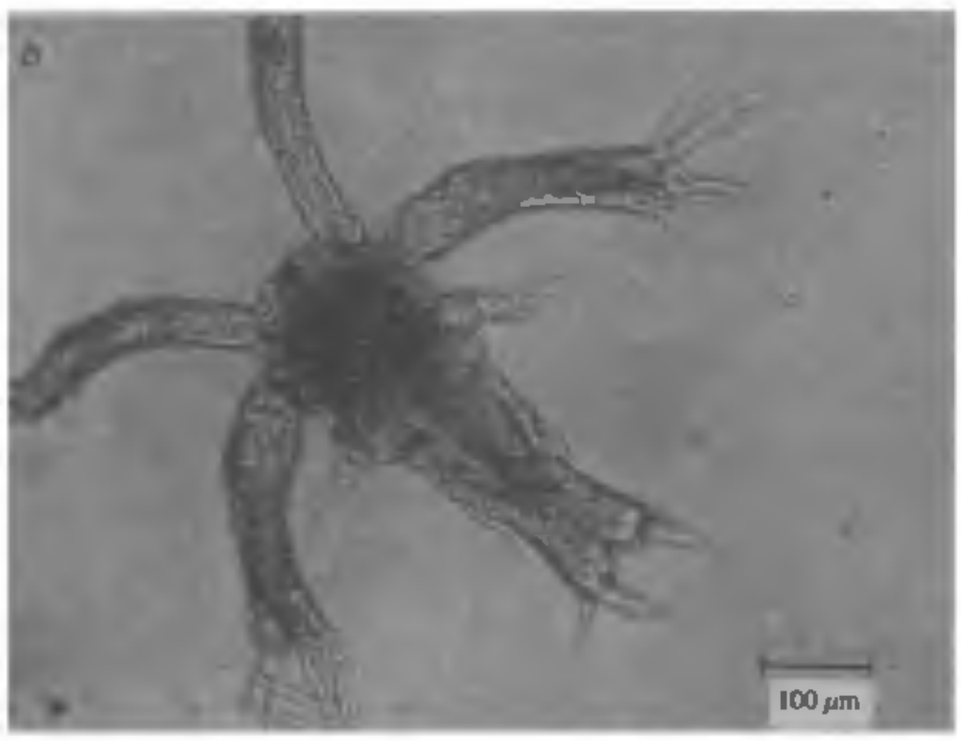

Figure 1. *a*, Eversion (arrow) in vitrified stage II nauplius of *P. indicus* when the vitrification medium was diluted step-wise. *b*, Stage VI nauplius of *P. indicus* frozen to −40°C using the slow cooling procedure and thawed at >300°C/min.

lar medium, thereby forcing the cell to first dehydrate by efflux of water and then equilibrate by influx of cryoprotectant. This exchange causes an intracellular build up of cryoprotectant, that continues until the freezing point of the cryoprotectant is reached, which is always, much below that of the freezable water. Rapid thawing, though helpful in preventing intracellular recrystallization, does not allow sufficient time for the cell to equilibrate with the surrounding medium, making slow dilution necessary. The high percentage of survival, 82% in nauplii frozen to −30°C and 63% in nauplii frozen to −40°C, (Figure 1, *b*) using 15% v/v ethylene glycol suggests that rapid thawing and slow dilution can be incorporated in the protocol for cryopreservation of nauplii of penaeid prawns (Table 1). However, it remains to be seen, and will be the focus of our immediate investigations, if slow thawing and rapid dilution will yield better results.

Table 1. Freezing of prawn nauplii—experimental protocol

Cryoprotectant	Ethylene glycol, 15% v/v
Addition	Two steps at 15°C
Equilibration time	15 min
Cooling rate	−1.5°C/min
Seeding	−6°C
Final temperature	−40°C
Thawing rate	Rapid >300°C/min
Dilution	Slow

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ACKNOWLEDGEMENT. We thank Department of Biotechnology, New Delhi for financial support.

Received 14 December 1992; accepted 3 March 1993

Effect of mixed inoculation with isogenic strains of *Bradyrhizobium* on total nodules of pigeonpea

Madhu Bala* and Y. D. Gaur

Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110 012, India

*Present address: Department of Biocybernetics, Institute of Nuclear Medicine and Allied Sciences, Lucknow Marg, New Delhi 110 054, India

Interstrain competition is known to influence the proportion of nodules formed by rhizobia from a mixed population. Here we show that inoculation with mixed strains of *Bradyrhizobium* not only influences the proportion of nodules but also the total number of nodules formed on pigeonpea.

COMPETITION among strains of rhizobia for occupying infection sites on root system is a common phenomenon operating in soil[1]. This leads to formation of varying proportions of nodules by competing strains. Some non-nodulating strains of rhizobia may block nodulation by nodulating strains[2–4]. Instances of bacteria such as *Erwinia herbicola* affecting nodulation by *Rhizobium meliloti* are also known[5]. However, there is very little information about the influence of mixed strains of rhizobia on the total number of nodules. Such influence we report here.

A wild-type strain of *Bradyrhizobium* sp. (*Cajanus*)