dium, however, as cited in Penningsfeld and Forchthammer (5), differ considerably from the one described in the present paper. Moreover, shoot production per capitulum was much lower.

Murashige et al. (4) have shown that *Gerbera jamesonii* can be multiplied vegetatively by shoot tip culture. This method is more rapid than the capitulum explant system but requires a great number of mother plants. The use of capitulum explants has the advantage of leaving the mother plant intact.

The culture of portions of young capitulum at the stage of inflorescence buds has proved more productive than that using fully developed inflorescences. For optimal plantlet production, Pierik et al. (8) use quartered inflorescences which, at best, yield 8 to 12 shoots after 8 weeks. With the technique described in this paper, each capitulum provides 20 to 25 explants, each of which can produce up to 12 shoots in 12 weeks, for a total production of 240 to 300 plantlets per capitulum per 12 weeks.

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In Vitro Storage of Potato Tuber Explants and Subsequent Plant Regeneration

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Abstract. **Isolated tuber explants of potato (*Solanum tuberosum* L. 'Russet Burbank') were stored in a modified White's medium at 24°C under a photon flux density of 25 μmol $s^{-1}m^{-2}$ in a 14-hr photoperiod for 18 months. The explants turned green, but no further visible changes occurred during the period of storage. The stored explants showed no decline in totipotency compared to the fresh explants when transferred to a modified Murashige and Skoog medium. Preliminary indications suggest that this relatively simple storage technique has the potential to be an alternative to cryopreservation of potato germplasm.**

Germplasm storage by conventional means in a vegetatively propagated crop such as potato, taxes heavily on manpower and resources due to the large volume of the tubers and the controlled atmospheric conditions required. Moreover, potato tubers have a limited storage life and are subject to continued attack by diseases and insects (2). Recently, tissue culture storage methods for genetic conservation of crops have been investigated (3).

Grout and Henshaw (1) reported the results of cryopreservation of shoot tips of *Solanum gonicalyx* in liquid N (−196°C). After 3 weeks of storage in liquid N, however, only 20% of the shoot tips survived, and of these, only about 50% produced shoots directly whereas the rest formed green callus. The success of this method also depends on the cooling and thawing rates of the explants, and on the presence or absence of cryoprotectants such as dimethyl sulfoxide. Westcott et al. (10) established a technique to regenerate whole plants from shoot tips of potato which enabled the recovery of the numerous plants from each explant. This technique seems applicable to Andean potato clones. However, only 20% of the shoot tips excised from a single *Solanum tuberosum* subsp. *tuberosum* clone initiated multiple shoots after callus formation. Therefore, the prospects of using shoot tips as explants for successful germplasm storage and clonal multiplication of *Solanum tuberosum* cultivars remain unknown at present.

In vitro methods for successful regeneration of plantlets from cultured tuber disks have been reported (4, 5, 6, 9) as a means of rapid clonal multiplication of potato plants.

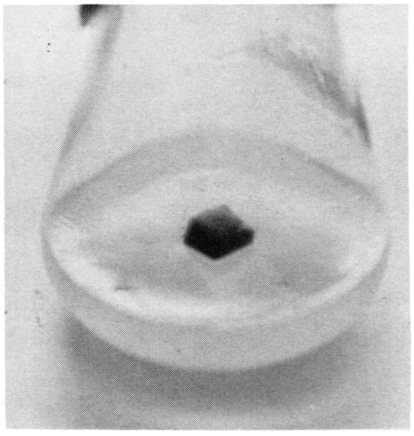

Fig. 1. A tuber explant after 18 months of storage in a modified White's medium.

To extend the use of this method for genetic improvement of potato, it would be advantageous to store the tuber disks in another culture medium for a prolonged period of time prior to plantlet regeneration. This action would facilitate germplasm preservation for later use or for exchanges among breeders. The culture medium required for storage of tuber disks should be such that these explants would remain dormant, show no signs of active cell division and differentiation, and retain their totipotency for an extended period of time. This research was initiated to determine if potato tuber tissue could be stored in vitro, and if plants could be regenerated after transfer to a shoot initiation medium.

Field-grown tubers of potato ('Russet Burbank') harvested and stored for 6 weeks at 4°C were used in this study. Tubers weighing about 200 g, free of any visible defects, were washed thoroughly with water and then surface sterilized by immersing in 0.5% sodium hypochlorite solution for 20 min. Under aseptic conditions, the skin was removed, and from each tuber mid-section, explants 8 × 8 × 5 mm in size were cut from the cortex region. Twenty-five explants were taken and rinsed in sterile water and transferred to 125-ml flasks (one explant per

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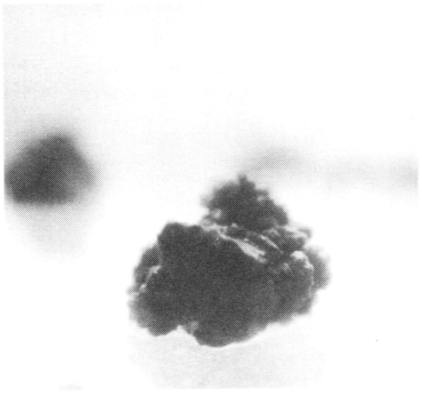

Fig. 2. Globular bodies developed from the stored explant, and photographed 4 weeks after transfer to a modified MS medium.

flask) containing 40 ml of modified White's medium. This medium consisted of inorganic salts and a vitamin supplement (11), sucrose (0.029 M), and solidified with agar (10 g/liter). No growth regulators were added. The flasks were capped tightly with cotton wool and double-layered aluminum foil. Cultures were maintained at 24° under a photon flux density of 25 μmol $s^{-1}m^{-2}$ in a 14-hr photoperiod provided by cool-white, fluorescent lights. The explants were observed every week during the next 18 months. Within 10 days after transfer, all explants changed from creamy white to green in color. Thereafter, no further development was evident except for an occasional development of a few small protuberences in some explants at the edges directly in contact with the medium. None of the explants turned brown or split open, and all of them retained their green color and fleshy appearance during the 18-month period (Fig. 1).

After 18 months of storage, 22 explants were transferred to a plantlet regeneration medium similar to that devised by Lam (6) but with concentrations of some growth regulators slightly modified. This medium consisted of the Murashige and Skoog major and minor salts (7), Nitsch and Nitsch organic addenda (8), and the following: 6-benzylamino purine (4.4×10^{-6}M), kinetin (2.3×10^{-6}M), zeatin (1.8×10^{-6}M), gibberellic acid (1.4×10^{-6}M), napthaleneacetic acid (1.0×10^{-7}M), indoleacetic acid (5.7×10^{-6}M), casein hydrolysate (1.0 g/liter), and sucrose (7.3×10^{-2}M). The medium was solidified with agar (9.0 g/liter). The pH was adjusted to 5.6, and 40 ml of the medium were dispensed to 125-ml flasks. To study the effect of storage on the regenerative capacity of the isolated explants, 24 fresh explants obtained from potato tubers, that had been kept at 4°C for 8 weeks after harvest, were used as a control treatment. These explants were transferred directly to separate flasks containing the same modified Murashige and Skoog (MS) medium. The cultures were maintained at 22° ± 1° under a photon flux density of 60 μmols^{-1}m^{-2} on a 16-hr photoperiod.

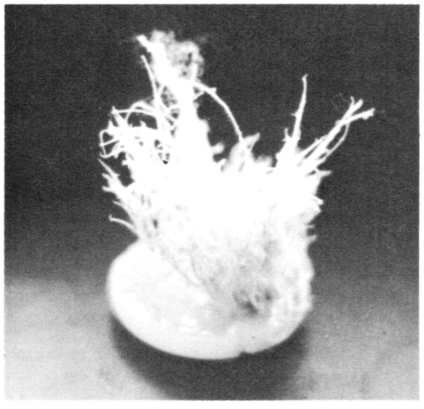

Fig. 3. Plantlet formation in the modified MS medium. Plantlets and the agar based medium taken out of the culture bottle and photographed after 16 weeks.

Seven to 10 days following transfer to the MS medium, the green tuber explants that were stored in the White's medium began to proliferate from the edges, soon followed by the formation of green globular structures (Fig. 2). After 8 weeks, 14 explants developed shoot primodia from these globular structures and grew into normal plantlets (Fig. 3). Roots were borne mostly aerially on the shoots. In 7 explants, the globular bodies turned brown and failed to produce shoots. Meanwhile, the fresh explants that were transferred directly to the MS medium turned green within 14 days. In a majority of explants, the same sequence of events described for the stored explants followed. The 1st shoot primodia developed after a period of 10 weeks, however, 2 weeks longer than for the stored explants. The number of explants with shoots, number of shoots per explant, and number of explants senesced were counted after 16 weeks in the MS medium.

The results reported in Table 1 suggested that 18 months of storage in White's medium had no undesirable effect on the regenerative capacity of the isolated tuber explants, compared to fresh explants following transfer to the MS medium. When grown in the greenhouse, the regenerated plants seemed uniform and produced tubers that conformed to the cultivar characteristics. The possibilities exist, therefore, to use this relatively simple technique for germplasm storage in potato. There are several advantages inherent in this method. The culture environment under which the explants were stored in the White's medium is inexpensive to maintain, and large numbers of tuber explants could be stored in a small space. Following storage, normal plantlets with shoots and roots can be produced in a single step eliminating problems associated with undifferentiated callus. In contrast to shoot tips stored in liquid N, higher levels of survival were obtained with stored tuber explants. This characteristic is desirable to eliminate the possibility of selection in the stored germplasm.

The results of this study suggest the possibility of using tuber tissue as explants for germplasm storage in potato. These explants showed no decline in regenerative capacity after 18 months of storage, and it is conceivable that they are amenable for prolonged storage. Further experimentation on the longevity of explants in relation to the storage temperature and the composition of the storage medium involving different cultivars is warranted. It has also been reported (4) that tuber explants of some potato cultivars do not regenerate as readily as others in the same MS culture medium. Widespread use of this technique as an alternative method for germplasm preservation in potato requires it to be applicable to other cultivars of the subspecies *tuberosum* as well as other tuber bearing *Solanum* species of importance to the genetic improvement of potato.

Table 1. Plantlet regeneration from stored and fresh potato tuber explants in a modified Murashige and Skoog medium.

Tuber explants	Explants with shoots (%)	Avg no. of shoots per explant[z]
Stored	64	17.8
Fresh	67	20.1

[z]Difference not significant, as determined by *t* test.

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