dicentric with 2 closely located prominent C-positive heterochromatic zones (figures 2, 4–6). The existence of this meta-dicentric marker chromosome has been documented unmistakably in all the metaphases examined from different individuals after several successive in vivo passage of the tumour. The position and size of the centromeres and the NF value of the cell are indicative of the fact that this meta-dicentric chromosome has arizen by breakage and fusion within each of the short arms of 2 acrocentric chromosomes and constitutes a regular member in the karyotype of this ascites tumour.

Previously it was generally accepted that the dicentric chromosomes are usually unstable[3]. But evidence is now accumulating on the spontaneous(?) occurrence of stable and transmissible dicentric chromosomes in natural populations as well as in different continuous cell lines. Recently an extensive study on the dicentric nature of the chromosomes with multiple heterochromatic bands has been made in some continuous mouse cell lines by Chen and Ruddle[11], and in continuous human lymphoid cell lines by O'Neill and Miles[8]. Long before the discovery of differential centromeric staining, the dicentric nature of metacentric chromosomes was also suspected by Manna[12] when he was working with LP 59 cell line. More recently the existence of a stable dicentric resulting from Y-Y translocation has been reported in man by Ghosal et al.[13]. In addition to the previous reports, our present finding on the occurrence of a stable and transmissible dicentric in MS 180 cells will add further cytological data to the problem of the stability of dicentric chromosomes.

Considerable controversy still exists regarding the exact nature of rods in mouse. After an extensive study on the chromosomes of a large variety of animals, White and others have adopted the view that all naturally occurring rods are acrocentric[3]. But recently, from the whole mount EM study, Comings and Okada[14] have concluded that the rods of mouse, sheep and goat are all telocentric with no evidence for a short arm. In our previous report[6] on the fusion metacentric of the house mouse of Asian variety, we have concluded that the rods of the mouse are all telocentric in nature. But the nature and placement of centromeres in the meta-dicentric marker of MS 180 cell line lead us to suggest that the chromosomes of this particular tumour are acrocentric in nature.

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Solanum verrucosum as a base for mutation breeding in potato

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Summary. Solanum verrucosum Schlechtd. is proposed as a base for mutation breeding in potato. Its mutability was demonstrated with N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea as mutagens.

The induction of mutations in a number of cultivated plants has become a valuable part of breeding programs, but not for the cultivated potato, Solanum tuberosum. This species has more than 100 wild relatives in Central and South America, which offer a large reservoir of potentially useful genes. Some of these have been used in breeding for resistance to Phytophthora infestans, virus diseases, frost, nematodes and insects[2,3]. However, the transfer of genes from some wild species to S. tuberosum is often difficult or impossible due to cross-incompatibility, sterility and different levels of ploidy. Additionally, some traits which would be most useful for potato improvement (e.g. leaf roll immunity) are not known to be present in any of the wild or cultivated Solanum species. Thus mutation breeding represents the only way to obtain new characteristics, as well as to reproduce existent characteristics which are not transferable from other species. However, mutation breeding in S. tuberosum presents some serious difficulties. The species is tetraploid ($2n = 48$), and therefore the probability of having recessive gene mutations expressed is low. Moreover, identification and isolation of a mutation from the highly heterozygous segregating progeny is very difficult. Therefore mutation experiments undertaken with S. tuberosum have involved only vegetative progenies of the M_1-generation, while seed progenies have not been previously fully analyzed[4–8]. To avoid these difficulties, a diploid Solanum species or derivative could be used as a base for mutation induction in potato. Theoretically then, such induced mutations could be introduced into the S. tuberosum genome. This would open new possibilities in potato breeding, which has been limited until now to crossing, selection, and the appearance of somatic mutations. The use of 'dihaploids' ($2n = 24$), derived from S. tuberosum, or allied diploid

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N-nitroso-N-methylurea (NMU) and N-nitroso-N-ethylurea (NEU) induced mutations in Solanum verrucosum Schlechtd.

	0.4 mM NMU	1.2 mM NEU	Control
Total No. of M_1 examined plants	252	323	120
Total No. of M_2 examined plants	2269	2127	1017
M_1 chlorophyll mutants	21.59%	14.88%	3.45%
M_2 chlorophyll mutants	42.45%	66.01%	1.59%
Dwarfs	10.94%	20.75%	0.20%
Leaf and flower macromutations	1.67%	2.45%	0.00%

species of Tuberosa cannot be seriously considered for this purpose. The problems inherent in the use of such material are several. Of these, their normal self-incompatibility and the associated severe inbreeding depression of 'self-compatibles' isolated from them[9] are probably the most serious. As a consequence of these traits, the isolation of recessive mutations is extremely complicated and difficult in all the diploid Tuberosa. Of the known diploid Solanum species, only the wild Mexican Demissa, Solanum verrucosum, appears to be theoretically suitable for large scale mutation induction in potato. S. verrucosum is known to have a unique combination of characteristics which would make it appear to be an ideal base for mutation breeding in potato. The species is diploid (2n = 24), self-compatible (only exceptional lines are self-incompatible), and is little subject to inbreeding depression even after several generations of selfing. It is also readily crossable with other wild diploid species as well as with S. tuberosum and the S. tuberosum haploids[3,10,11]. Additionally, this species has valuable genes both for hypersensitivity and field resistance to P. infestans[12,13] and its linking position between the series Tuberosa and the other tuber-bearing Solanum series has been suggested[14,15]. To test for this theoretical use of S. verrucosum as a dependable base for mutation breeding in potato, the range of mutability and the characters of the induced mutants in this species were investigated.

Material and methods. 0.4 mM N-methyl-N-nitrosourea (NMU) and 1.2 mM N-ethyl-N-nitrosourea (NEU) were applied to S. verrucosum seeds (24 h, 25 °C). 2 seed generations (M_1 and M_2) were observed for induced changes. In the M_1-generation, seed germination, seed coat shedding, seedling injury, growth apex damage, somatic chlorophyll mutations, leaf and flower abnormalities, anthocyanin pigmentation of stem, pollen stainability, plant survival and seed production were evaluated. The M_2-generation was tested for non-chlorophyll abnormalities of cotyledons, dwarfs, chlorophyll mutations, and leaf and flower macromutations.

Results and discussion. Of the characters evaluated, somatic chlorophyll mutations in the M_1-generation, as well as dwarfs, chlorophyll mutations, and leaf and flower macromutations in the M_2-generation were considered to be the most important in assessing S. verrucosum for mutability. The obtained frequencies of these (table) provide a useful indication of the value of S. verrucosum for mutation breeding in potato. They also establish a high mutagenic efficiency of the 2 alkylnitrosoureas in this species. The physiological damage caused by the mutagens under the conditions of these experiments was not serious. In the M_1-generation, both NMU and NEU slightly stimulated seed germination. They had a similar effect in decreasing the length of the hypocotyls, which were 17% shorter than the controls. Both mutagens also decreased plant survival by an average of 26%. The mutagens had no significant effect on seed coat shedding, leaf deformations, flower abnormalities and pollen stainability. Seed production was slightly decreased in the case of NEU only. NEU also significantly damaged the growth apex, however, the plants later recovered from this damage. In appearance, the M_1-plants varied greatly after the mutagenic treatments. The variability included height of plants, size and hairiness of leaves, branching, and a large spectrum of weak to stout and vigorous plants. In the M_2-generation, the spectra of chlorophyll mutations were broad, and differed significantly for the 2 mutagens. The macromutations induced with NMU and NEU involved pronounced changes in leaf morphology (e.g. cordate leaf, elliptic leaf, rugose leaf) as well as flower mutations, such as white flower, persistent flower, orchid flower and dark stigma. Many of them had pleiotropic effects.

In addition to these mutations, many other obviously mutated plants appeared. These changes were somewhat less dramatic in appearance and involved such characters as early tuberization, larger tubers, changes in anthocyanin pigmentation of flowers, size of flowers, etc. Most of these mutations were not followed in detail in the experiments. They do, however, suggest another potential role for S. verrucosum. Should serious consideration ben given to the possibility of producing a true-seed propagated strain of potato, the characteristics of this species would provide a sound base for such a development. With induced mutagenesis to provide the required variability, the natural fertility and relative freedom from inbreeding depression could be exploited without danger of loss or diminution by outcrossing to other species.

Comparing the macromutations to similar characteristics of other wild potato species, it was found that many of the S. verrucosum mutants resemble certain wild diploid species of both South and Central America. This indicates that S. verrucosum or a direct ancestor could have contributed significantly to speciation of some tuber-bearing Solanum species. The data therefore supports the Hawkes theory to this effect[15,16].

Conclusion. The results of this study indicate that S. verrucosum provides a most suitable base for mutation breeding in potato. The number and spectrum of the mutations was broad. This, and the known relative ease with which genetic information can be transferred from S. verrucosum to the Tuberosa, suggests a valuable new role for the species in potato breeding programs. The detailled results of induced mutagenesis in S. verrucosum will be published in subsequent papers.

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