

Sodium Azide-Induced Mutants of Peas That Accumulate Pisatin

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ABSTRACT

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Sodium azide which is both a powerful mutagen and a phytoalexin inducer, has been utilized to develop M-2

generation pea plants that accumulate pisatin in the absence of an externally applied inducer.

Sodium azide in the acid form has been shown to be a powerful mutagen in barley and peas (2, 3). Its effectiveness is enhanced because it acts as a base substitution mutagen without producing any apparent chromosome aberrations (3). Sodium azide is also a phytoalexin inducer (4). The dual mechanism of action of sodium azide suggested the possibility that mutagenic compounds which induce the production of phytoalexin in healthy pea tissue might be utilized in the development of mutant peas which continually produce the phytoalexin, pisatin.

A 10^{-3} M solution of sodium azide was prepared by dilution of a sodium azide stock with potassium phosphate (KH_2PO_4) buffer, and adjusting to pH 3 with concentrated phosphoric acid. The M-2 generation pea seeds (seeds from plants grown from mutagen-treated seeds) were developed as follows: Sound dry seeds of *Pisum sativum* 'Juneau', were placed in 1-liter Erlenmeyer flasks and covered with 750 ml of treatment solution. After 4 hours, the seeds were washed with distilled water and planted (without drying) in field plots or greenhouse pots. The M-2 seeds were harvested from these plants. For comparison, a like number of seeds were treated with either 10 or 20 Kr of γ -irradiation and also planted in field plots. In addition, seed lots of the cultivar Alaska which had been soaked for 4 hours in solutions (1 g/liter) of either ethidium bromide or acridine orange were planted in sand in a greenhouse. Dry M-2 seed from the above treatments were harvested at maturity and representative samples were surface-sterilized and planted. After two weeks, two leaflets were removed from each plant and assayed for pisatin in the following manner: The leaflets were soaked in 5 ml of hexane for one week, and the residue of this hexane extract was spotted on a silica gel G thin-layer chromatogram and developed in chloroform. The presence or absence of pisatin was evaluated, as reported previously (5), by exposing the developed chromatogram to HCl fumes and

subsequently checking for fluorescence under ultraviolet (UV) light (260 nm) at the R_f of pisatin. The seedlings which accumulated pisatin when assayed at 2 weeks were transplanted into sand and two newly formed leaflets from each plant were assayed after three weeks of growth. A total of 1,400 seedlings from the M-2 generation of azide-treated stock were assayed for pisatin. Only 1.14% of these seedlings continued to accumulate pisatin in new leaflets formed during both the second and third week. The quantity of pisatin extracted per gram of tissue was estimated (on the basis of UV fluorescence) to range from 10 to 50 μg (the remainder of the M-2 plants did not produce detectable pisatin). The viable seeds from these M-2 generation plants were bulked, grown, and assayed for pisatin in the M-3 generation, in which 19 M-3 seedlings were found to accumulate pisatin.

Pisatin-accumulating seedlings were also found in the M-2 generation of both γ -irradiated and acridine orange-treated stocks, but not in the M-2 generation of ethidium bromide-treated seed stocks.

Seven hundred seedlings grown from seed stocks which had received no mutagenic treatment were also assayed. Of these, only four seedlings accumulated detectable pisatin ($<10 \mu\text{g/g}$ tissue) in both the second and third week assays. M-1 generation plants from γ -irradiated (5-30 Kr) seed accumulated no pisatin in any tissue throughout the generation following the irradiation. However, freshly irradiated pods (200 to 5,000 r γ -rays) accumulated from 30 to 70 μg of pisatin per g of tissue. Thus the effects of γ -irradiation as a direct inducer appear to be transient unless a mutation is induced.

A wide range of morphological traits are seen in pea plants which continually produce pisatin (Fig. 1). The small, stunted M-2 plant to the left in Fig. 1 produced considerably more pisatin ($>50 \mu\text{g/g}$) than all other mutants. It is possible that the copious pisatin production in this plant limited its normal development. However, most of the stunted plants in the M-2 generation of the

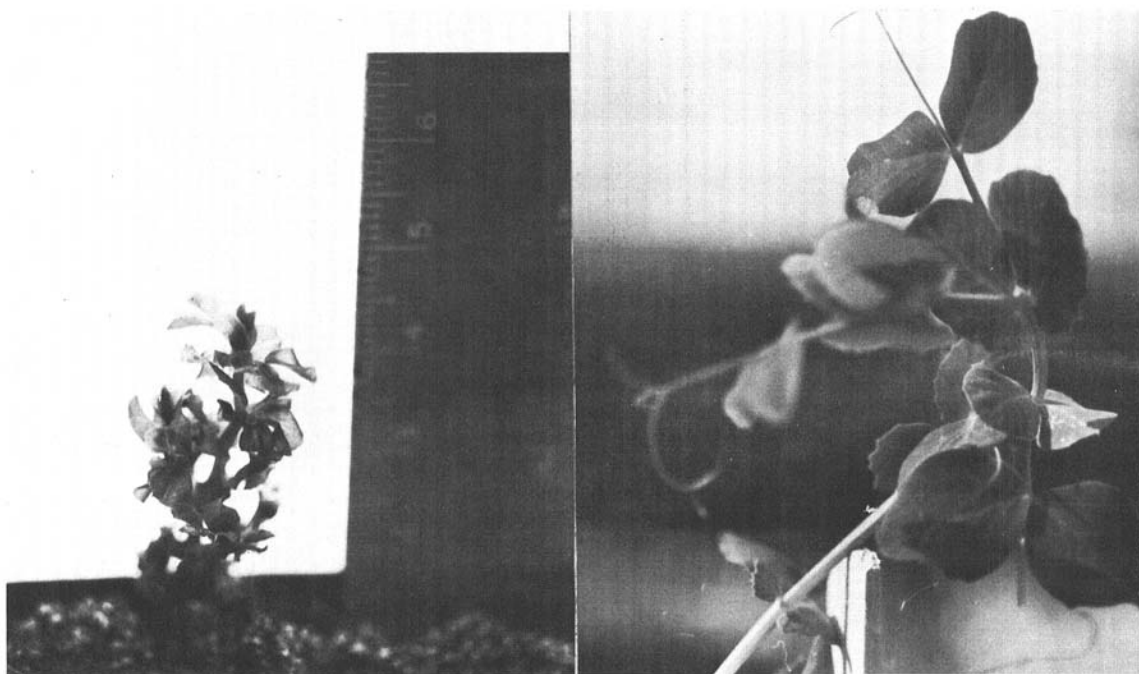


Fig. 1. Stunted (left) and normal-sized (right) mutant pea plants from the M-2 generation of azide-treated seed, which accumulate pisatin in the absence of an externally applied inducer.

azide-treated material did not continuously produce pisatin, and many of the pisatin producing mutants were not dwarfed (for example, the plant on the right in Fig. 1). Many of the pisatin producing seedlings did develop to maturity, but the production of viable seed was small. Because of a limited supply of seed, we have been unable to evaluate the disease resistance potential of mutant plants. However, the levels of pisatin which accumulate in the high producing mutants are less than half of those amounts (1) found in tissues challenged by nonpathogenic fungi.

Since pisatin accumulates in these uninjured mutant plants, it appears that pisatin production is not necessarily a wound response. These results also demonstrate that a phytoalexin inducer which is also a mutagen can be utilized to develop a mutant plant which produces phytoalexin in the absence of an externally applied inducer.

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