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BEAN MUTATIONS FROM AZIDE: AN IRON CHLOROTIC STRAIN, BUT NO INDUCED COLD HARDINESS

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Beans *(Phaseolus vulgaris L.)* will tolerate some ice in their tissue provided the temperature does not fall below -1^oC (1). The purpose of this study was to look for increased cold tolerance created by mutations resulting from azide seed treatments. Pinto UI-114, seeds were placed on wet paper towel for periods ranging from 2 to 17 hours and then submerged in an aerated azide solution for 2 hours before planting. The azide solution was a $10^{-3}M\ NaN_3$ in $0.1M\ NaH_2\ PO_4$ buffered to pH 3 (3).

The iron chlorotic mutation: During the course of preliminary experiments a chlorotic mutant appeared. Tests in the greenhouse and field showed that it grew well on acid soils, but classical iron chlorosis symptoms developed when it was grown on an alkaline silt loam with a 1% lime content. Yields were reduced as much as 40% unless the soil was treated before planting with adequate amounts of chelated iron. When enough iron chelate was added, the growth, appearance and yields were normal as compared to the standard pinto UI-114. A seed stock of this strain was developed and is available for photosynthesis and iron nutrition studies.

The seedling freeze study: Approximately 2500 treated seeds were planted directly in the field, grown to maturity and their seed harvested the first week in September. On September 14, the seed was planted in a one hectare plot along with control seed from untreated UI-114 parents. Many of the seedlings from the treated parents were undersized, some had misshaped leaves, occasionally with yellow mottling. Five of the seedlings were completely yellow and one was a true albino. Six of the seedlings were elongated such that the first two leaves were 15 to 20 cm above the soil surface rather than 5 cm as is normal.

In mid October, the temperature began to fall below freezing killing only a few seedlings because the dewpoint of the air was not reached. This let the water in most of the plants undercool rather than freeze (2). On October 25, the air temperature fell to -5^oC with a dewpoint of -6^oC and only two of the bean seedlings in the hectare survived. Seed from these two were germinated and as the first trifoliate developed, they were tested for increased freeze tolerance in a growth chamber with normal UI-114 seedlings as a control. In the first trial the leaves were misted with water droplets just as the temperature fell below freezing (Table 1). The droplets formed ice and nucleated the internal leaf water. In the second trial the leaves were not misted, so the dewpoint was never reached. A few of these seedlings survived by undercooling, but there were no obvious differences between strains. The final test was like the first, except the temperature was lowered to only -1.6^oC.

Table 1. Freezing tests showing percent survivors out of approximately 180 seedlings.

Conditions	Temperature °C ± 0.3	UI-114 Control	Selected Strains 1	Selected Strains 2
Nucleated	-3.1	0	0	0
Not Nucleated	-4.2	2.8	3.3	4.1
Nucleated	-1.6	0	0	0

Suggestions for selecting frost tolerant mutants: It is interesting that cold hardy weeds and vegetables do not undercool in dry freezing air in the field, but rather nucleate ice internally even though frost does not form on their leaves. This may be a prerequisite property for freeze tolerant seedlings as it avoids the rapid growth of ice crystals that occurs when undercooled water nucleates (2). Quite possibly at least two different mutations are required to give frost tolerance to bean seedlings; one that favors spontaneous nucleation in the tissue as the temperature falls below 0°C, and a second that allows the cells to tolerate a greater percentage of plant water as ice (1). If so, the chances of success in selecting for cold tolerance could be enhanced by screening for spontaneous nucleation and ice tolerance separately. Large numbers of seed can easily be treated and then grown in the field. The progeny from these plants can then be screened by planting them in the fall, but this should be done in a more humid area where the dew-point is normally above 0°C. Seedlings surviving temperatures below -1°C might then be expected to have an increased tolerance for ice. Seed from such plants could be increased, treated with azide and their progeny screened for spontaneous nucleation in a dry climate where the dewpoints in the fall are normally well below 0°C. This could lead to selection of the desired characteristics without requiring both mutations to occur simultaneously.

References

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