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Mutation breeding for resistance to blackspot bruise and low temperature sweetening in the potato cultivar Lemhi Russet

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Abstract

A mutation breeding method was developed to select clones of Lemhi Russet that have resistance to blackspot bruise and low temperature sweetening. Following irradiation with gamma rays from a Cobalt^{60} source, over 2000 tuber eye pieces were planted directly to the field and tubers from the resulting crop were individually evaluated for blackspot bruise potential. Selection for blackspot bruise resistance continued for five clonal cycles. Selection for low temperature sweetening began in the M_3 and continued for three cycles. Ten clones were selected, eight with significantly ($p = 0.05$) better blackspot bruise resistance, and two with increased resistance to low temperature sweetening. The results confirm the possibility for the development of a system to improve single selectable quality characteristics in potatoes.

Introduction

Selection and propagation of somatic mutations has long been an accepted practice for potato variety improvement (Miller, 1954). These somatic mutations are largely naturally occurring sports and involve changes in physical appearance such as tuber color, vine architecture and other visible traits (Easton & Nagle, 1981; Holm, 1988; Webster & Rieman, 1949). Some mutations have resulted in useful cultivars (Webb & Miller, 1954; Whitson et al., 1914).

Beginning with Stadler (1928), induced mutations were demonstrated to have potential as a tool in crop improvement efforts world-wide. Numerous cultivars of many crops have been released for commercial production as a result of mutation breeding efforts (Micke et al., 1987). These cultivars are mostly self-pollinated crops including soybeans, barley, rice, wheat, and common bean. Sporadic efforts to induce mutations in potato, beginning with Asseyeva & Blagovidora (1935), have been theoretical rather than practical (Hovenkamp-Hermelink et al., 1987; Kolesnikova & Maksimova, 1977; Roer, 1967; Upadhya & Purohit, 1973; Van Harten & Bouter, 1973), and have resulted in only two new potato cultivars (Micke et al., 1987). Potatoes are a difficult subject for mutation breeding due to clonal propagation, a high level of heterozygygosity, and tetraploidy. The bulkiness of the propagules limits the manageable population, while clonal propagation by itself results in the complex problem of chimeras (Seinkard, 1970). Tetraploidy reduces the expression of mutations in the M_1 and crossing with mutation-bearing parents produces little in the way of acceptable progeny, reducing the impact of any beneficial mutations. The best use of induced mutations in potatoes appears to be the improvement of existing cultivars (Jauhar & Swaminathan, 1967).

The cultivar Lemhi Russet was released in the

United States in 1980 (Pavek et al., 1981). Within a few years of entering the commercial arena, Lemhi Russet was identified as having an unacceptable tendency for blackspot bruise (Stark et al., 1985). Blackspot bruise is caused by the condensation of phenyl substrates into melanin following damage to the cell membranes (Mulder, 1955; Raper, 1927). Low incidence of blackspot bruise is necessary if potatoes are to be used for processed products.

Another desirable characteristic in a potato cultivar is the ability to maintain low sugar levels following storage at temperatures between 4 and 8 °C. Currently, no russet-skinned cultivars with long tuber shape used in North America or Europe have this trait. A number of breeding selections and wild species have been reported with this ability (Ehlenfeldt et al., 1990).

This research was conducted to determine the potential of mutation breeding to improve an existing cultivar for single, selectable tuber quality traits. The study employed a system for handling a large population while selecting independently for one of two desirable traits. The practical goal was to select Lemhi Russet mutants with better resistance to blackspot bruise or cold temperature sweetening.

Materials and methods

In 1986, virus-free breeder seed of Lemhi Russet was obtained from the Tetonia, Idaho Research and Extension Center. Individual eyes were removed from whole tubers along with a two cm diameter portion of the underlying flesh. The eye pieces were stored at 4 °C for 1 week.

Mutation induction was done with radiation at the Idaho National Engineering Laboratory using a $Cobalt^{60}$ gamma-ray source. The eye pieces were arranged in a 5 cm layer perpendicular to the source. Approximately 2000 eye pieces were irradiated, 500 at each of four exposure levels, including 2500, 3000, 3500 and 4000 rad, at a rate of 2400 rad/h. Following exposure the eye pieces were allowed to heal for a 3 week period, then planted directly to the field. Control eye pieces were subjected to the entire process except radiation exposure.

All field trials were conducted at the Aberdeen, Idaho Research and Extension Center. The M_1 plants were physically spaced at 1 m^2 to allow for maintenance of plant identity. Plants were harvested individually and each tuber was treated as a separate unit. Blackspot bruise potential was measured for each tuber using a variation of the abrasive peel method of Pavek et al. (1985). A three cm diameter area on the stem end of the tuber was abraded on a large basaltic stone, on the surface of which was maintained a constant flow of clean, running water. Following a 24 hour incubation period, the tubers were rated for the severity of the blackening present on the abraded area. A rating scale of 1 to 5, with 5 indicating severe blackening, was used for all trials. After rating, the tubers were allowed to heal, then stored at 4 °C so the unabraided portion could be used for seed. Only blackspot bruise potential was evaluated in the M_1. Response to low temperature sweetening was considered the secondary trait of interest and screening began in the M_3.

The M_2 and subsequent clonal generations were handled in a similar manner to a typical breeding population. Plot designs were as follows; the M_2 (1987) consisted of unreplicated plots, each with four plants; the M_3 (1988) consisted of three replicates of 15 plants per plot; the M_4 and M_5 1989, 1990) consisted of four replicates of 24 plants per plot. All plots were planted within the first two weeks of May and harvested in the last week of September or first week of October. Within-row seed piece spacing was 0.25 m and row width 0.9 m, and all plots were single row. The trials were sprinkler irrigated and managed typically for the surrounding production area. All selections were vigorously rogued and periodically tested for leaf-roll virus, PVX and PVY.

In the M_2, and subsequent generations, each plot was subsampled and evaluated for blackspot bruise potential. In the M_3 and subsequent generations, they were also evaluated for low temperature sweetening. For blackspot bruise, approximately two months after harvest a ten tuber sample was evaluated using the method of Pavek et al. (1985). The amount of low temperature sweetening was measured based on product color after frying. After 3 months of storage at 4.5 °C a three tuber sample

Table 1. The number of potato clones remaining after each cycle of selection for resistance to blackspot bruise and low temperature sweetening.

Clonal Generation	Clones Evaluated for Blackspot Bruise	Clones Evaluated for Fry Color
M_1	5692	not evaluated
M_2	226	not evaluated
M_3	59	145
M_4	18	8
M_5	8	2

was sliced into 1.25 cm strips, fried for 3.5 min at 190 °C, then rated using the USDA Fry Color Chart (0–4 rating with lower number = lighter color).

In addition to the selection variables, each clone was evaluated for total yield, marketable yield, tuber size, and tuber yield. Two data sets were compiled, one included all blackspot bruise resistant selections remaining at the end of the M_5 and a Lemhi Russet control, and another included similar data for the low temperature sweetening resistant selections. For each data set, with-in year mean squares were calculated and Bartlett's test for homogenicity of variances was completed across years. With one exception, all data variables in both data sets showed homogeneity of variances allowing a single ANOVA across years. The exception was tuber size in the blackspot bruise data set. A log transformation was calculated, resulting in homogenous variances across years, and the ANOVA was completed on the transformed data. Means separation of the clones was done using Duncan's Multiple Range Test.

Results and discussion

A summary of the selections made through the M_5 is found in Table 1. In the M_1, a total of 5692 clones were grown and evaluated. The M_1 population was made up of single tubers harvested in 1986 from approximately 2000 plants. By the M_5, only ten clones remained, eight with blackspot bruise resistance and two with reduced low temperature sweetening.

The radiation dosage had a large impact on the number of mutants selected for both blackspot bruise and low temperature sweetening resistance (Table 2). No resistant selections were made from the group irradiated with 2500 rad. Of the selections remaining in the M_5, eight of ten were irradiated with 3500 or 4000 rad. The 4000 rad treatment was physiologically injurious to the eye pieces as evidenced by reduced stand and greatly reduced vigor in the M_1 plants. The 3500 rad treatment appeared to give the overall best results and this dosage will be used in future studies.

Blackspot bruise resistance

Selection was based entirely on blackspot bruise potential through the M_3, with no clones eliminated for yield or agronomic inferiority. Selection in the M_1 was arbitrarily based on a blackspot bruise score of two or less in order to quickly reduce the population to a manageable size. In the M_2, selection was based on a blackspot bruise score of 3.7 or less. In subsequent generations selection was based on a statistically lower score ($p = 0.05$) in comparison with the Lemhi Russet checks.

The nonirradiated, control eye pieces were planted, harvested and evaluated in the same manner as the irradiated eye pieces. In the M_1, fifteen control plants were evaluated. All tubers from these plants, with the exception of one, scored a blackspot rating of 4 or 5, typical of Lemhi Russet tubers. The exception was a tuber with a score of 2, which placed it among the acceptable selections. In the M_2, fifteen control plots originating from five M_1 plants (3 tubers each) were planted and evaluated. The fifteen included one plot derived from the low scoring control tuber from 1986. The blackspot rating from all fifteen plots ranged from 4.1 to 4.9, typical of Lemhi Russet. In 1988, three control clones, plus a Lemhi Russet control from the original seed source were included with the trial. Among these was the control clone derived from the original low scoring tuber. The non-irradiated control clones scored 4.3 to 4.8, while the Lemhi Russet control scored 4.3. It was concluded that the nonirradiated control clones were identical to the original Lemhi Russet for blackspot bruise response and the radiation treatments were resonsible for the changes observ-

Table 2. Number of selections in the M_3 and M_5 for resistance to blackspot bruise and low temperature sweetening resulting from each of four mutation inducing irradiation dosages.

Dosage (Krad)	Clonal Generation					
	M_3			M_5		
	Blackspot Selections	Fry Color Selections	Total	Blackspot Selections	Fry Color Selections	Total
2500	0	0	0	0	0	0
3000	7	4	11	1	1	2
3500	9	4	13	3	1	4
4000	7	0	7	4	0	4

ed in the treated clones. Consequently, beginning with the M_3, the original Lemhi Russet seed stock source was used as a control clone.

In the M_5, only eight selections remained that showed consistently lower blackspot bruise scores. The clones were compared for blackspot bruise potential, total yield, marketable yield, tuber size, and tuber number (Table 3). All eight clones had significantly lower blackspot bruise scores than Lemhi Russet. However, all eight clones also had significantly lower total yields and all but one had lower marketable yields than Lemhi Russet. Two of the clones, LM-116 and LM-232, had yields similar enough to Lemhi Russet to have agronomic potential.

Inspection of tuber size and number provided some explanation for the reduction in yield potential for each of the eight clones. All of the clones had a reduction in either size or number of tubers, and in some cases, both. Reduction of tuber size was consistently associated with reduced vine vigor, while reduced tuber number was not.

The reduction in blackspot bruise potential of the eight clones, although significant, is probably not sufficient to make them commercially valuable. The severity of the blackspot bruise problem in Lemhi Russet means a substantially larger increase in resistance is needed to make them commercially acceptable. If the increase in blackspot bruise resistance is heritable some or all of the clones may replace Lemhi Russet as a valuable breeding parent.

Although no clones were recovered with commercially acceptable blackspot bruise characteristics, sufficient progress was made to substantiate the value of the breeding system used. Similar progress in blackspot bruise resistance in a cultivar with

Table 3. Blackspot bruise rating, total and marketable yield, average tuber size and tuber number of Lemhi Russet and eight potato clones selected via mutation breeding for resistance bo blackspot bruise[1].

Clone	Blackspot bruise rating	Total yield	Marketable yield	Tuber size	Tubers per hectare
		mt/ha		- g -	--- x 1000 ---
LM-332	3.27 a	36.4 cd	27.3 b	180 abc	200.0 bc
LM-363	3.55 ab	36.3 cd	28.6 b	160 cd	221.2 bc
LM-330	3.60 ab	38.5 bc	28.8 b	178 abc	216.0 bc
LM-257	3.61 ab	32.8 d	24.5 b	175 bc	194.3 c
LM-232	3.69 b	42.1 b	28.5 b	161 cd	265.1 a
LM-116	3.81 b	42.5 b	35.0 a	199 a	215.1 bc
LM-295	3.85 b	34.7 cd	26.2 b	148 de	230.6 b
LM-382	3.95 b	38.4 bc	24.8 b	137 e	277.0 a
Lemhi Russet	4.63 c	48.1 a	37.9 a	182 ab	266.6 a

[1] Mean separations were done using Duncan's Multiple Range Test. Means with the same letter are not significantly ($p = 0.05$) different.

only a marginal problem could result in one or more useful mutants.

Low temperature sweetening

The population evaluated for resistance to low temperature sweetening in the M_3 included 59 clones selected for blackspot bruise resistance plus 86 clones saved because of other obvious phenotypic changes. As with the blackspot bruise evaluations, the nonirradiated control clones were compared in the M_3 with a Lemhi Russet control from the original seed stocks and the irradiated clones. The nonirradiated controls had fry scores ranging from 3.3 to 3.7 and the Lemhi Russet a 3.3. These were typical for Lemhi Russet and significantly worse than the selected mutants. From the M_3 on, the original Lemhi Russet seed stocks were used as a control.

Selection in all generations was based on a french fry score statistically ($p = 0.05$) lower than the check clones. After four cycles of selection, only two clones remained. These two clones and a Lemhi Russet control were compared for french fry color, total yield, marketable yield, tuber size, and tuber number (Table 4). Both of the resistant clones had significantly lower fry scores across years, following cold storage, than did Lemhi Russet. Total and marketable yields of the two clones, although numerically smaller, were not significantly different from Lemhi Russet. LM3-479 had significantly smaller tuber size than Lemhi Russet and both clones produced similar tuber numbers. It is of interest to note that LM3-479 carried a second mutation that eliminated the russetting on the skin.

Both clones with reduced low temperature sweetening retained Lemhi Russet's problem with blackspot bruise. This will eliminate both clones from commercial production but they will be used for hybridization. The overall mutation breeding method again showed considerable potential for improving an existing variety for a single selectable trait.

General discussion

The ability to isolate improved mutants for both resistance to blackspot bruise and cold-temperature sweetening confirms the effectiveness of this mutation breeding scheme. This has important implications for variety improvement and presents another alternative to traditional breeding. It will allow the selection of varieties with improvement in a single trait. Such varieties can then be clonally propagated and used without further genetic manipulations.

Regardless of improvement, all of the Lemhi Russet mutant selections still express unacceptable levels of blackspot bruise susceptibility. This will eliminate them from consideration for release as new varieties, and will limit their useful potential to that of parental stock in the traditional hybridization program. The parental potential of these selections is yet to be determined and depends on several factors. The mutations may be present as periclinal chimeras. This is relatively unimportant if the mutant is propagated and used directly as an improved variety. If used as a parent, the mutation will only be heritable if the selected mutation is present in the L2 cell layer. Also, in most of the remaining selections, the beneficial trait is accompanied by a reduction in vigor. This may be the result of pleiotropic effects of the mutated gene(s); or the beneficial traits may be by-products of abnormal physiolog-

Table 4. Fry color, total and marketable yield, average tuber size and tuber number of Lemhi Russet and two potato clones selected via mutation breeding for resistance to low temperature sweetening[1].

Clone	Fry Color	Total Yield	Marketable Yield	Average Tuber Size	Tubers per Hectare
		mt/ha		-g-	x 1000
LM2-441	1.67 a	34.2 a	26.7 a	173 ab	199.8 a
LM3-479	2.11 a	36.4 a	28.4 a	162 b	224.3 a
Lemhi Russet	2.95 b	39.8 a	31.1 a	185 a	215.8 a

[1] Mean separations were done using Duncan's Multiple Range Test. Means with the same letter are not significantly ($p = 0.05$) different.

ical processes resulting from the disruption of the action of an important developmental gene. In both cases, the trait will be heritable but carry the detrimental effects of the mutation into subsequent generations. In some mutants, the beneficial trait and the loss of vigor may be due to separate mutation events and these mutants may be useful parents and allow selection away from the reduced vigor in the progeny. Genetic analysis is needed to characterize each of the reported mutants and determine their value as parents.

Lemhi Russet is currently an important parent in the breeding program at Aberdeen. An improved mutant with parental value will replace or supplement Lemhi Russet's role and will become a valuable parent in furture hybridization efforts.

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