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# Characteristics and Inheritance of Seed-Ageing Induced Mutations in Lettuce (*Lactuca sativa* L.)

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*With 3 tables*

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## Abstract

Mutations affecting qualitative traits were induced by seed ageing in lettuce. The mutant plants were isolated in the $A_2$ generation and included chlorophyll-deficient types (chlorotica, lutescens, chlorina-virescens, luteo and viridalbo maculata), and morphological variants (dwarf and narrow, thick and curly leaf types). The leaf mutants were found to be either partially or completely sterile. Segregation pattern of the mutants in $A_3$ generation showed that, except for the maculata types, all chlorophyll deficiencies and the dwarf mutant are controlled by single recessive nuclear genes. The genetic status of the leaf mutants was not clear, due to possible pleiotropic effect of the mutant genes in inducing gametophytic sterility. The maculata mutants exhibited sorting out of the normal and chlorophyll deficient regions during vegetative development and segregated for different degrees of chlorophyll deficiency in selfed progenies. The maculata mutants probably originated by plastome mutations induced by nuclear mutator genes.

**Key words:** *Lactuca sativa* — aged seeds — induced mutations — characteristics — inheritance.

There are several reports indicating that heritable point mutations are induced in seeds as they age during storage (Stubbe 1935, Abdalla and Roberts 1969, Floris and Meletti 1972 and Dourado and Roberts 1984). This phenomenon has been reviewed recently by D'Amato (1986) and Roberts (1988). Even when seeds have been subjected only to mild ageing treatments such that viability has not fallen below 92 %, it has been shown that significant increases in mutation occur in both pea and barley (Dourado and Roberts 1984).

Most mutations resulting from seed ageing are recessive and, providing that they are not lethal to the gametophytes, they are transmitted to the progeny of the aged seeds and become visible as they segregate in the second generation ($A_2$). Recently we (Rao et al. 1987) studied the induction of phenotypic mutations and their quantitative relationship with loss of seed viability under a wide range of storage conditions in lettuce, which is an obligate self pollinating, widely grown salad vegetable crop. During the course of study a number of mutations affecting the normal chlorophyll development and other morphological characters were detected in the progenies of aged seeds. Some of these mutations have not hitherto been reported in lettuce. The purpose of this paper is to describe characteristics and the probable genetical status of these mutants.

## Materials and Methods

Achenes of lettuce (*Lactuca sativa* L. cv. 'Trocodero Improved') which had an initial moisture content of 5.5 % and viability of 98 % were used in this study. The seeds were artificially aged by storing at 40 °C with 9.8 % moisture content in sealed laminated aluminium foil packet for 12 and 19 days which led to a reduction in their germination rate from 98 % to 79 and 23 % respectively, and at 50 °C with 9.9 % moisture content for 54 hours or 5.5 % moisture content for 30 days which reduced germination from

frequence 98 % to 67 or 68 % respectively. Altogether the progeny from 655 control and 1447 aged seeds were genetically analysed. There were no significant differences in frequencies of mutations induced between different treatments nor was there any evidence that any type of mutation was associated with one ageing treatment or another (RAO et al. 1987). The plants from the control (not aged) and the aged seeds (designated as $A_1$) were grown to produce seeds in field plots under a glass shelter or in pots in a glasshouse during summer. At maturity, the seeds from each plant (designated as $A_2$) were harvested separately, dried and following temporary storage at 3 °C they were sown in seed compost in Plant Pak trays in a glasshouse maintained at 15 °C. After 3—4 weeks growth of the seedlings, each line was screened for putative mutant phenotypes. Lines exhibiting only normal phenotypes were discarded, while from those segregating for the mutant phenotypes a random selection of normal plants was grown to maturity to produce the $A_3$ seeds. After a brief storage at 3 °C, the $A_3$ seeds collected from each of the $A_2$ plants were sown in Plant Pak trays in a glass house at 15 °C and the 3—4 weeks old seedlings were scored to determine the segregation ratios and thereby to infer the genetical status of the mutants. Where the putative $A_2$ mutant phenotypes survived and produced seeds (e.g. chlorotica, chlorina-virescens, maculata and thick-leaf mutants), selfed progenies of them were grown to test that the mutants bred true to their phenotype.

The terminology used to describe the chlorophyll-deficient mutants observed in this study follows that devised by BLIXT (1961) for peas.

## Results

### Characteristics

The mutant phenotypes first appeared in the $A_2$ generation. These included several chlorophyll-deficient types and others which had changes in the gross morphology, especially of the leaves. The characteristics of the mutants are described below.

The following four basic patterns of chlorophyll deficiency were seen segregating in the progenies of the phenotypically normal $A_1$ plants.

**Chlorotica:** The mutants were uniformly light green in colour and were slower growing than the normal plants. They survived until maturity and produced viable seeds.

**Lutescens:** The cotyledons were normal green at the time of emergence, but turned pale yellow subsequently. The first true leaves were also pale yellow and the seedlings survived for only 3—4 weeks.

**Chlorina-virescens:** The first leaves were initially yellow green but they became green during further development. The plants were less vigorous than their normal sibs, but they survived and produced seeds. This mutant was similar to the virescent chlorophyll deficiency described earlier by RYDER (1971).

**Maculata:** The maculata phenotypes were variegated for chlorophyll-deficient and normal green areas on the shoots. Two basic patterns of variegation were evident. In viridalbo-maculata the chlorophyll-deficient areas of the variegated leaves were white, while in luteo-maculata they were yellow. In both the mutants, the variegated pattern was seen from the time of emergence, and the first true leaves had different proportions of normal and chlorophyll deficient sectors. During the course of further growth, the mutants exhibited somatic segregation and produced either all normal green or some normal and some variegated and, in a few cases, some completely chlorophyll-deficient leaves, the number being proportionate to the amount of variegation present in the seedlings. The mutant plants survived until maturity and produced seeds. A cytoplasmically inherited variegated phenotypic mutation similar to the viridalbo-maculata was reported previously in lettuce by WHITAKER (1944).

### Morphological variants

**Narrow-leaf:** The mutants had very narrow and thick leaves. Although the plants bolted and flowered, they were sterile and did not produce any seeds.

**Dwarf:** The mutant plants were stunted and had small, dark green and shiny leaves; they survived only for a few weeks even when grown under favourable conditions.

**Thick-leaf:** The mutants had thick, leathery and dark green leaves with blistered surfaces. The plants exhibited considerable sterility as they produced a high proportion (about 74 %) of sterile achenes.

**Curly-leaf:** The mutants had thin, frilled and twisted leaves with deeply indented margins. The plants were spindly in appearance and

Table 1. Segregation of mutant phenotypes in the $A_2$ generation of aged lettuce seeds

| Mutant type | No. of segregating families | Segregation within the family | | Total |
|---|---|---|---|---|
| | | Normal phenotypes | Mutant phenotypes | |
| Chlorotica | 1 | 69 | 19 | 88 |
| Lutescens | 1 | 109 | 26 | 135 |
| Chlorina-virescens | 1 | 122 | 19 | 141 |
| Viridalbo-maculata | 1 | 265 | 1 | 266 |
| Luteo-maculata | 9 | 916 | 9 | 925 |
| Dwarf | 1 | 170 | 25 | 195 |
| Narrow-leaf | 1 | 186 | 21 | 207 |
| Thick-leaf | 1 | 158 | 26 | 184 |
| Curly-leaf | 1 | 87 | 15 | 102 |

produced abortive floral stocks after prolonged vegetative growth.

## Inheritance

The frequency of mutant phenotypes segregating in the $A_2$ families, relative to the normal phenotypes was less than what would be expected on the basis of the normal Mendelian monohybrid ratio, assuming that most of the induced mutations are simple recessives (Table 1). It is therefore evident that the mutated $A_1$ plants were chimeric and had only a small sector affected by the mutation, presumably derived from a mutation in a single embryonic cell. Nevertheless, segregation of the mutant phenotypes should conform to typical Mendelian ratios in $A_3$, although there would be a general deficit in the number of segregating families (see BLIXT 1961).

The progenies of plants heterozygous in $A_2$ for the mutant alleles, chlorina, chlorina-virescens, lutescens and dwarf showed a 3 : 1 segregation ratio, suggesting the monogenic recessive inheritance of these traits. Test for

Table 2. Segregation ratios in the $A_3$ generation of aged lettuce seeds

| Mutant type | No. of families studied | No. of families segregating | Segregation in families | | | | |
|---|---|---|---|---|---|---|---|
| | | | Normal phenotype | Mutant phenotype | Assumed segregation | $\chi^2$ (Probability) | Heterogeneity $\chi^2$ (Probability) |
| Lutescens | 19 | 11 | 713 | 204 | 3 : 1 | 3.71 (0.1—0.05) | 13.57 (0.2—0.1) |
| Chlorotica | 16 | 7 | 604 | 206 | 3 : 1 | 0.08 (0.8—0.7) | 4.16 (0.7—0.5) |
| Chlorina-virescens | 18 | 8 | 650 | 232 | 3 : 1 | 0.80 (0.5—0.3) | 9.88 (0.2—0.1) |
| Luteo-maculata | 33 | 1 | 123 | 25 | 3 : 1 | 5.19 (0.05—0.02) | — |
| Viridalbo-maculata | 5 | 0 | — | — | — | — | — |
| Dwarf | 17 | 8 | 413 | 133 | 3 : 1 | 0.12 (0.8—0.7) | 4.84 (0.7—0.5) |
| Narrow-leaf | 17 | 2 | 152 | 10 | | | |
| Thick-leaf | 8 | 5 | 382 | 56 | Anomalous ratios* | | |
| Curly-leaf | 9 | 1 | 97 | 1 | | | |

* See text

heterogeneity showed that the segregation ratios across families are homogeneous. As can be seen from the segregation data, the proportion of heterozygous dominants in $A_2$ is less than the expected ratio (2/3), obviously due to the chimeral nature of the mutant plants (see Table 2).

The segregation ratios of the leaf mutations are not clearly understood. There were too few mutant phenotypes segregating in the $A_3$ to account for a monohybrid segregation ratio (Table 2). Although the data are suggestive of digenic inheritance, there were too many mutant phenotypes in the segregating $A_2$ families to allow this possibility.

The $A_3$ segregation pattern of the two maculata mutants suggested their origin to a cytoplasmic gene mutation, probably the plastome. While none of the normal siblings of the viridalbo-maculata and of two of the luteo-maculata mutants studied segregated in $A_3$, segregants were found in the progeny of one of the ten normal $A_2$ siblings of the third luteo-maculata mutant (Table 2). On the other hand, the mutants themselves did not breed true; instead, they segregated for different degrees of chlorophyll deficiency in their progenies — producing a few to many variegated, some totally green and some totally chlorophyll deficient off-springs (Table 3). The proportion of green, variegated and chlorophyll deficient offsprings in the progeny seemed to be dependent upon the degree of variegation present in the mother plant.

## Discussion

As can be seen, the chlorophyll deficient chlorotica, lutescens and chlorina-virescens and the dwarf phenotypes followed the pattern of segregation typical of Mendelian inheritance in $A_3$ which suggested that the mutant phenotypes are controlled by single recessive nuclear genes. In case of the chlorophyll-deficient maculata mutants, the somatic sorting out of the normal and chlorophyll-deficient areas during vegetative growth and the segregation for different degrees of chlorophyll deficiency in the selfed progenies are indicative of plastome mutations. Since all maculata mutants were first isolated in the $A_2$ generation, it is probable that these putative plastid mutations were induced by nuclear genes in a homozygous recessive state. The fact that none of the normal siblings of the mutants, except one, segregated in $A_3$ argues against this; but, since maculata mutants occurred with very low frequencies in $A_2$ (see Table 1), the small number of progenies examined could have easily been the reason for not observing any segregants. However, more detailed studies are called for before arriving at any definite conclusion.

It is interesting to note that mutations affecting leaf morphology also induced either partial or complete sterility in this study. Assuming a monohybrid segregation ratio, there was a general deficiency of the mutant phenotypes in the segregating progenies. Probably mutations affecting the leaf morphology had a pleiotropic effect in also causing gametophyte sterility, and therefore resulted in the aberrant segregation ratios observed in the $A_3$ generation. Genes linked to factors causing pollen abortion and showing abnormal segregation ratios have already been reported in lettuce (LINDQVIST 1960). For example, disturbed segregation ratios at loci u and v (for leaf lobing and anthocyanin pigmentation, respectively) have been explained on the basis of linkage with a gametophytic factor affecting fertility. Aberrant ratios were also found for genes of the linkage group lg-g-h-i involving loci for leaf colour (lg and g), hearting (h) and anthocyanin pigmentation (i). It is also possible that segregation ratios may have been distorted by haplontic and diplontic selection (GAUL 1961).

Table 3. Segregation in selfed progenies of the maculata mutants

| Mutant type | No. of progenies examined | Seedling phenotype Normal | Variegated | Chlorophyll deficient |
|---|---|---|---|---|
| Luteo-maculata | 3 | 196 | 22 | — |
| Viridalbo-maculata | 1 | 51 | 78 | 37 |

## Zusammenfassung

### Charakterisierung und Vererbung der durch künstliche Alterung erzeugten Mutationen beim Salat (*Lactuca sativa* L.)

Durch künstliche Alterung von Salatsamen wurden Mutationen in qualitativen Eigenschaften ausgelöst. Die in der $A_2$-Generation isolierten Mutanten umfaßten Typen mit Chlorophyllschädigungen (chlorotica, lutescens, chlorina-virescens, luteo und viridalbo maculata) und morphologisch veränderte Formen (Zwergwuchs; schmale, verdickte und gekräuselte Blattformen). Die Blattmutanten waren entweder teilweise oder ganz steril. Bei der aufspaltenden $A_3$-Generation wurde deutlich, daß mit Ausnahme der maculata-Typen alle Chlorophyll- und Zwergwuchsmutanten von einzelnen rezessiven Kerngenen bedingt waren. Die genetischen Verhältnisse der Blattmutanten sind wegen möglicher pleiotroper Wirkungen mutierter Gene, die die gametophytische Sterilität hervorgerufen haben, noch ungeklärt. Während der vegetativen Entwicklung entmischten sich die normalen und chlorophyllgeschädigten Partien der maculata-Typen. In den Selbstungsnachkommenschaften dieser Pflanzen spalteten Formen mit unterschiedlich starken Chlorophylldefekten heraus. Die maculata-Typen sind wahrscheinlich durch Plastom-Mutationen entstanden, die ihrerseits durch mutationsfördernde Kerngene ausgelöst wurden.

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