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INHERITANCE OF GENETIC MARKERS FROM TWO POTATO DIHAPLOIDS AND THEIR RESPECTIVE PARENT CULTIVARS

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SUMMARY

In the first inbred generation (I_1) of cv. Black 4495 a dark green, slowly growing mutant (coded *ds*) was found, whereas the I_1 of its self-compatible dihaploid, B16, comprised this *ds* mutant and in addition the mutants virescens (*v*) and yellow margin (*ym*). The occurrence of *ds* and *ym* might trace back to diploid *S. phureja*, one of the ancestors of Black 4495. No lethal mutants were observed in I_1 of B16.
Analysis of I_1 of cv. Gineke revealed a simplex condition for virescence and either duplex or triplex heterozygosity for one lethal gene. On the other hand, the I_1 of its dihaploid, G254, segregated for virescence and for three different lethal genes. It is shown that both in B16 and in G254 homozygosity of an *S*-bearing translocation causes early death of embryo and endosperm, thus preventing seed development. From this study it appeared that the three lethal genes from G254 affect germination rate of the seeds. The genotypes at 11 loci of B16 and G254 are presented.

INTRODUCTION

Marker genes are of great value to genetic and breeding research. A clear-cut example are the complementary genes for embryo-spot, which enabled the selection of monoploid potato clones and hence the development of completely homozygous potato lines (HERMSEN & VERDENIUS, 1973; VAN BREUKELEN et al., 1977). Association of marker genes with particular chromosomes by means of trisomic analysis is possible now because the development of a trisomic series in diploid *S. tuberosum* is near to completion (RAMANNA & WAGENVOORT, 1976; WAGENVOORT & RAMANNA, 1978). There are several reports on marker genes in dipoid potatoes (DODDS & LONG, 1955, 1957; SIMMONDS, 1965; DE JONG & ROWE, 1972). In this article morphology and genetics of some previously described markers and some new ones are presented.

MATERIAL AND METHODS

The material used is included in Table 1. Both B16 and G254 are highly fertile, fully self-compatible dihaploids and originated from cvs Black 4495 and Gineke respectively.

The seeds were sown in petri-dishes. Upon germination they were carefully pricked out and put at 5 × 5 cm into boxes filled with sterilized seedling compost. Utmost care

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Table 1. Material used for genetic analysis.

Source	Seeds sown	Seeds germinated	Percentage germination
Black 4495 selfed	400	343	85.8
B16 dihaploid selfed	520	502	96.5
Gineke selfed	1270	1180	92.9
G254 dihaploid selfed	606	511	84.5

was taken not to lose any plantlet in the early stages of growth. Each plant was numbered and assessed at weekly intervals over a period of 1–2 months.

RESULTS

The I_1 of cv. Black 4495 and its dihaploid B16. The I_1 generation of cv. Black 4495 consisted of 343 plants. A clear-cut segregation into 268 normal and 75 dark green, slowly growing plants was observed ($\chi^2_{3:1} = 1.797$; $P = 0.20$). As the dark green mutant is probably a monogenic recessive character, the tetraploid parent must have the simplex constitution at the locus controlling this character. The symbol *ds* (abbreviation dark + slow) is used to indicate the most characteristic features of the mutant: its dark green colour and its overall slow growth. The *ds*-plants are not lethal, although their viability does vary. Most of them tuberize, and some even flower especially when grafted onto tomato rootstock.

The I_1 generation of the dihaploid B16 displays three mutants (fig. 1), but no lethals.

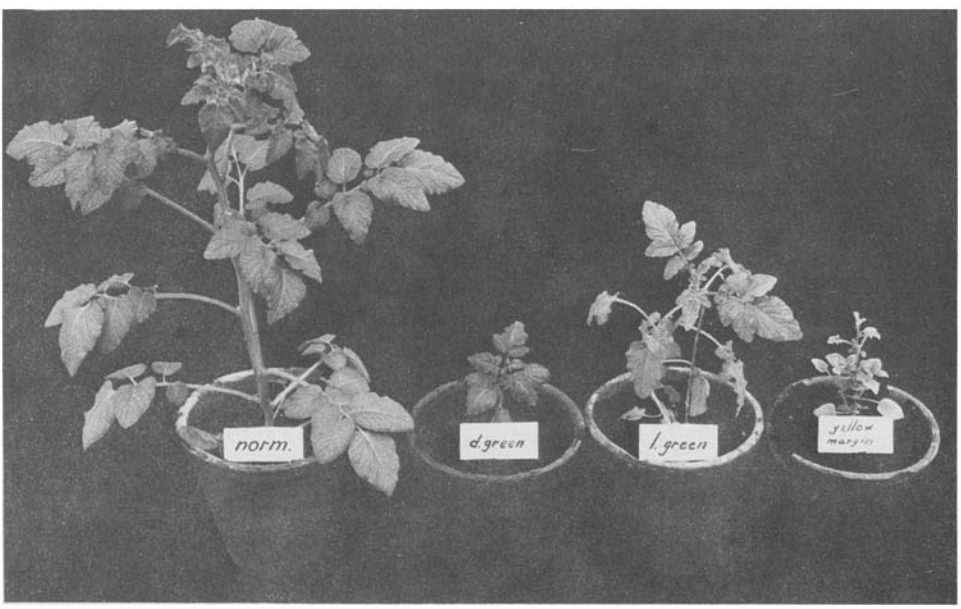

Fig. 1. Normal plant (height ca. 25 cm) and three mutants from the self progeny of B16.

The first mutant, *virescent*, is characterized by its light green coloration and a retardation in its early growth. The differences between normal and virescent plants are most noticeable when normal plants grown in the greenhouse are about 25 cm high (Fig. 1). The character causes relatively little harm, only a retardation in growth, tuberization and flowering. Virescent is monogenic and recessive (HERMSEN, 1978b). The symbol *v* seems acceptable, although allelism with the gene *v* localized by HERMSEN et al. (1973) has not yet been demonstrated.

The second mutant is *dark green + slowly growing*, described above and occurring in the I_1 of Black 4495, the tetraploid parent of B16.

The third mutant is *yellow margin*, described by SIMMONDS (1965) and symbolized *ym*. This mutant is also monogenic and recessive (unpublished data). It is characterized by small roundish leaflets with yellow or reddish margins; the plants may have a bushy appearance (Fif. 2). Yellow-margin plants may tuberize but rarely produce flowers even when grafted onto tomato stock. The occurrence of *ds* and *ym* in B16 might trace back to *S. phureja*, one of the ancestors of Black 4495.

With three recessive mutants in a heterozygous condition in B16 the segregation pattern in its I_1 is rather complicated (Table 2, 1st column). In calculating the expected frequencies the theoretical ratios for each mutant may be multiplied. For virescent a 6:1 ratio is expected and not a 3:1 ratio, owing to a linkage between *v* and *S*-bearing translocation as described previously (HERMSEN, 1978b). For darkgreen + slowly growing (*ds*) and also for yellow margin (*ym*) 3:1 ratios are expected. From these expectations the theoretical frequencies can be calculated for each of the 8 expected phenotypes (Table 2, column 2). Weekly observations of all plants over a period of 1—

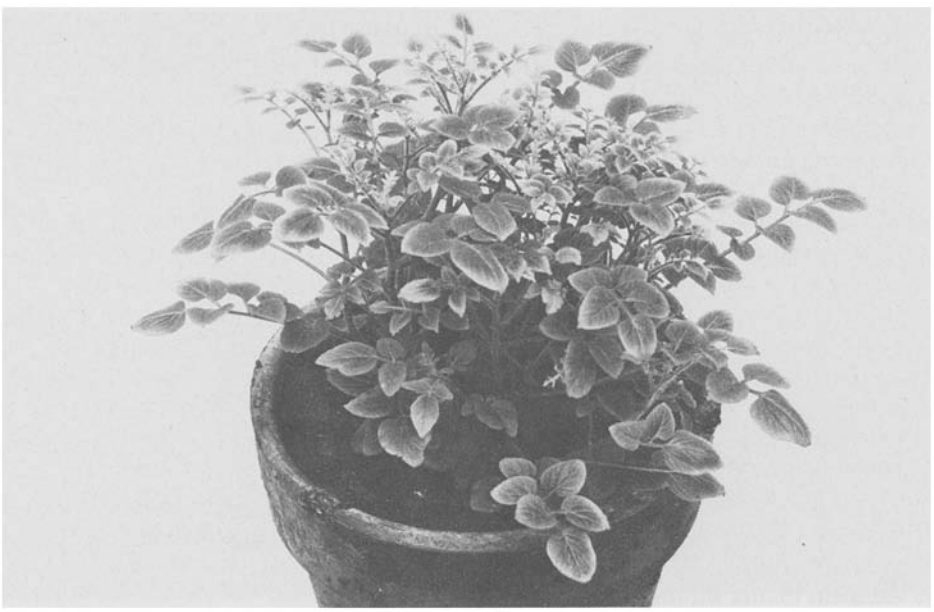

Fig. 2. A yellow margin plant with bushy appearance.

Table 2. Frequencies of 8 expected phenotypes and χ^2 test of four clearly distinguishable classes in I_1 (B16). ym = yellow margin mutant; ds = dark green and slowly growing mutant.

Expected phenotypes	Expected frequencies	Observed phenotypes	Expected frequencies	Numbers	
				expected	observed
normal	54	normal	54	242.0	222
virescens(= v)	9	virescens	9	40.3	38
ds	18		21	94.1	106
ds + v	3	ds			
ym	18				
ym + ds	6				
ym + v	3	ym	28	125.5	136
ym + ds + v	1				
χ^2 = 4.108; P = 0.30 – 0.20 (3df)				Total	502

2 months and the placing of them into groups in the greenhouse according to phenotype indicated that not all 9 phenotypes could be distinguished with certainty.Normal plants and virescent plants could be classified unambiguously. Darkgreen and slowly growing plants (*ds*) are also easily recognizable, but plants with the combination *ds* + *v* are not always clearly distinguishable from *ds* plants; therefore these phenotypes have been grouped together. Yellow-margin is also a clearly recognizable character, but all four *ym*-phenotypes had to be put into one group due to difficulties in classifying them separately. The expected and observed frequencies could therefore be determined for four classes: *n*, *v*, {*ds* and *v* + *ds*}, {*ym*, *v* + *ym*, *ds* + *ym* and *v* + *ds* + *ym*}: see table 2 last two columns. As appears from the χ^2 test the observed ratios fit the expectation (P > 0.20). The genotype of B16 may be symbolized by *VvDsds*Ym*ym*; that of tetraploid Black 4495 by *VVV·Dsdsdsds* YmYmYm·. Then the chance of obtaining a triple heterozygous dihaploid is at least $^1/_2 \times {}^1/_2 \times {}^1/_2 = 12.5\%$.

The I_1 of cv Gineke and its dihaploid G254. In the I_1 generation of cv. Gineke 1180 out of 1270 seeds germinated (93%). Of these, 53 did not emerge from the soil. The remainder gave rise to 826 normal and 282 virescent plants with light necrotic spots or flecks and with different vigour ($\chi^2_{3:1} = 0.120$; P > 0.70). Nineteen plants were not scorable owing to deformed cotyledons. It may be assumed that Gineke is simplex for the virescent character. Germinated seeds not emerging from the soil may be caused by fungi, but a lethal gene may also be involved. The ratio 1127 : 53 does not fit 35 : 1 (duplex selfed, chromosome assortment) but it does 27:1 (triplex selfed, chromatid assortment with $\alpha = {}^1/_7$).

The I_1 generation of G254 displays results which differ greatly from those of the I_1 of B16. From 511 germinated seeds the following results were obtained (Table 3 and Fig. 3):
173 normal plants,
33 virescent plants without necrotic spots,
118 germinated seeds, but seedlings not emerging from the soil,

Table 3. Frequencies of 16 expected phenotypes and χ^2 test of five clearly distinguishable phenotype classes in I_1 (G254).
l (ne) = lethal, non-emerging (gene symbol L_1); l (yc) = lethal, yellow cotyledons (gene symbol L_2); l (td) = lethal, tiny dwarf (gene symbol L_3).

Expected phenotypes	Expected frequencies	Observed phenotypes	Expected frequencies	Numbers	
				expected	observed
normal	162	normal	162	181.7	173
virescens (= v)	27	virescens	27	30.3	33
l (ne)	54				
l (ne + yc)	18				
l (ne + td)	18				
l (ne) + v	9	l (ne)	112	125.7	118
l (ne + yc + td)	6				
l (ne + yc) + v	3				
l (ne + td) + v	3				
l (ne + yc + td) + v	1				
l (yc)	54	l (yc)	63	70.7	64
l (yc) + v	9				
l (td)	54				
l (td + yc)	18	l (td)	84	94.7	115
l (td) + v	9				
l (td + yc) + v	3				
$\chi^2 = 6.135$; P = 0.20–0.10 (4 df)				Total	503

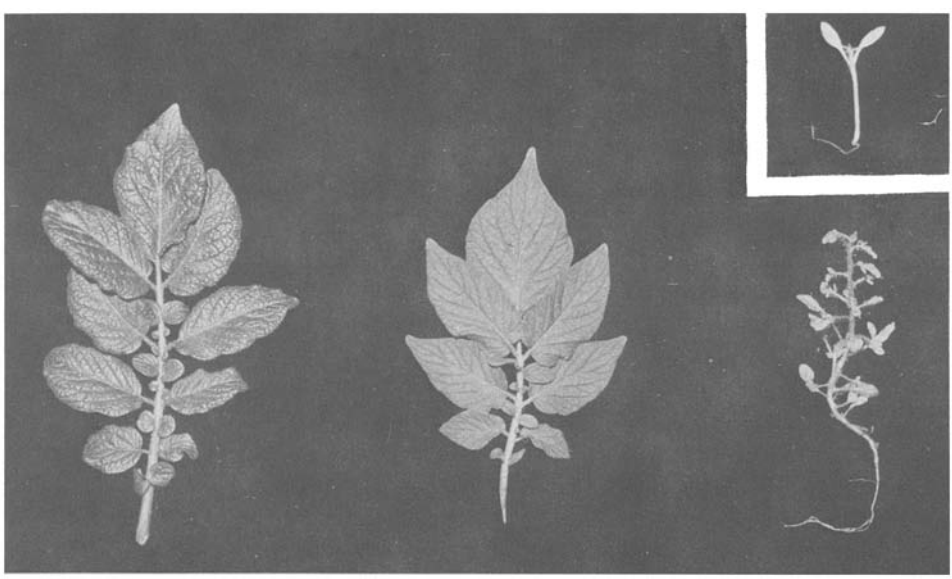

Fig. 3. Leaf of normal (left) and virescent (centre) plant; 'tiny-dwarf' lethal (right) and 'yellow-cotyledon' lethal (inset) from the self progeny of G254.

64 plants developing to the cotyledon stage, then turning yellow and dying (Fig. 3, inset).
115 tiny dwarf plantlets with single stems carrying many small, generally simple leaves on relatively long petioles,
8 plants not scorable owing to deformed cotyledons.

Apart from the last category of 8 plants three different types of lethals are apparent: non-emergent lethals (also in I_1 of Gineke, supporting the above genetic explanation), lethal in the cotyledon stage and tiny dwarf lethals. Like in I_1 of B16 the ratio normal: virescent is expected to be 6 : 1 (HERMSEN, 1978b). When taking the three types of lethals in I_1 of G254 together in one class and assuming that they are inherited independently and each is controlled by a single recessive gene then the ratios are as follows: Expected 181.9 normal: 30.3 virescent: 290.7 lethal
Observed 173 normal: 33 virescent: 297 lethal
($\chi^2 = 0.813$; $P > 0.50$).

When the three types of lethals are put into separate classes we have to consider the phenotypic effect of the presence of more than one type of lethal genes in a plant. If it is assumed: 1) that the gene for non-emergence lethality is epistatic to the genes for the other lethal types and 2) that the gene for tiny-dwarf lethality is epistatic to that of yellow-cotyledons lethality, then the results may be summarized and tested as explained in Table 3. The observed ratios are in agreement with the expected ratios ($P > 0.10$).

From the foregoing data it is concluded that Gineke is simplex for virescence and triplex for three lethal genes. G254 may be assigned the genotype $VvL_1l_1L_2l_2L_3l_3$ (for symbols see Table 3). The probability of finding such quadruple heterozygous dihaploid from Gineke is 8.3%.

A clear-cut difference in average germination time was found between the plant types in I_1 of G254. Average germination time of normal and virescent plants was 6.5 days, that of the lethals 'tiny dwarf' and 'yellow cotyledons' 7.2 days and that of the group 'non-emergent embryos' 11.2 days.

DISCUSSION

Among the I_1-plants from B16 only recessive mutants were found. No lethal plants due to homozygosity of the *S*-bearing translocation (HERMSEN 1978a) occurred. In view of the high germination rate of the seeds (96%) it may be concluded that ovules with embryo- and endosperm carrying the translocation in the homozygous condition, do not develop into seeds. If this is the case relatively low numbers of seeds per berry are expected upon selfing B16, and this was found indeed (22.7 seeds per berry).

Among the I_1 plants from G254 59% were found to be lethal. However, the lethals consist of three different types and none of the groups showed a frequency which approached 50%, which is the expected frequency of lethal translocation homozygotes. As in the case of B16, the number of seeds per berry upon selfing G254 is relatively low (83.4) and the germination rate of these seeds is high (85%). Therefore, it is highly probable that also the translocation homozygotes of I_1 of G254 do not develop into seeds and die in early stages of embryo development.

The data on germination rate of the I_1 plants from G254 allow the following

Table 4. Survey of known mutant genotypes in the self-compatible *S. tuberosum* dihaploids B16 and G254.

Character	Genotypes		Remarks and references
	B16	G254	
Virescens	*Vv*	*Vv*	Linked with S-translocation (HERMSEN, 1978b)
Yellow-margin	*Ymym*	*YmYm*	This article
Dark green and slow growth	*Dsds*	*DsDs*	This article
Incompatibility	S_3S_4/S_x	S_1S_3/S_1	Self-compatible (OLSDER & HERMSEN, 1976)
Flower colour	*XXYy*	*XxYy*	Complementary dominant (unpublished results)
Lethal, not emerging	L_1L_1	L_1l_1	This article
Lethal, yellow cotyledons	L_2L_2	L_2l_2	This article
Lethal, tiny dwarf	L_3L_3	L_3l_3	This article
Non-acceptance of pollen of *S. verrucosum*	$A_1a_1a_2a_2$	$A_1a_1A_2a_2$	Independant dominant (HERMSEN et al., 1974)

conclusions. The virescence gene has no effect on germination rate, whereas the lethal genes have, especially the genes for non-emergent lethals. An embryological study may reveal the effects of the lethal genes on embryo development and final embryo size. In addition, it would be interesting to trace the effects of translocation homozygosity on embryo development.

The genotypes at 11 loci of both B16 and G254 are presented in Table 4. It is confirmed that autotetraploidy associated with vegetative propagation enable potato cultivars to maintain a high level of heterozygosity and to store a large number of deleterious recessive mutant genes.

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