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# GENETIC VARIATION IN DIHAPLOID POTATO CLONES, WITH SPECIAL REFERENCE TO PHENOTYPIC SEGREGATIONS IN SOME CHARACTERS

(Preliminary Report)\*

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## Abstract

Haploidy provides a useful means for analyzing the genetic constitution of outbreeding crop plants, particularly vegetatively propagated ones. From such a view, 65 dihaploid clones ( $2n=24$ ) derived from the male sterile potato cultivar ( $2n=48$ ), Chippewa, were examined. These clones varied widely in their leaf, flower, stem and tuber characters, and the pattern of variation was continuous in most instances. An indication of discontinuity, however, was found in variations of some of the characters examined, showing segregation approaching either to disomic or to tetrasomic ratio; that is as follows: leaflet shape—5 narrow : 1 broad, stigma type—5 notched : 1 entire, tuber shape—1 long ovate : 4 oval : 1 round, stem pubescence—3 pubescent : 1 glabrous, stolon length—3 short : 1 long, meiotic behavior—3 normal : 1 desynaptic, corolla shape—1 stellate : 2 pentagonal : 1 sub-rotate, and photoperiodic response for tuberization—1 short day : 2 day-neutral : 1 long day. These segregation ratios may be interpreted as being equivalent to the gametic ratios expected from a duplex genotype depending on whether pairing of the chromosomes concerned is random or preferential. Thus, it is suggested that diploidization in the common potato genomes has been occurring in varying degrees in different chromosomes, so that segregations for some genes are still tetrasomic and others already disomic. Also, a consideration was made on the feasibility of dihaploids to offer promise of a new, efficient approach to potato breeding.

Haploidy seems to provide a useful means for making it possible to analyze the genetic constitution of crop plants that can not be inbred due to male sterility inherent in them. For this, a number of haploids have to be raised as an indispensable prerequisite. The common potato, *Solanum tuberosum* ssp. *tuberosum*, appears to afford a unique opportunity in this respect, because its dihaploids have become produced with comparative ease by employing PELOQUIN and HOUGAS' method<sup>10)</sup> and also because their clones raised once can be vegetatively maintained. With the common potato, however, despite of much

work having been so far performed to produce dihaploids in its numerous cultivars, no attempt has been yet made to demonstrate the feasibility of genetic analysis by means of haploidy, excepting the present author's earlier work<sup>5-8)</sup>, even though a few reports have been published by some workers<sup>9,11)</sup> in respect to a descriptive survey of the genetic variations among dihaploid clones.

This study has been thus undertaken to obtain any information as to whether such a basic, new approach to potato genetics is feasible or not as expected, and also as to whether haploidy offers or not promise of a new approach to potato breeding. In the present paper, an outline is preliminarily noted of the results obtained from this point of view with dihaploid clones derived from the male sterile potato cultivar Chippewa,

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and detailed descriptions of the results will be reported in further papers.

### Materials and Methods

Sixty five clones of dihaploids ( $2n=24$ ) derived from the male sterile cultivar, Chipewawa, of the common potato, *Solanum tuberosum* ssp. *tuberosum* ( $2n=48$ ), and 10 clones of their parental cultivar were used in this study. Of the former materials, 51 clones are ones which had been supplied from the Inter-regional Potato Introduction Station, Wisconsin, USA, being assigned the US-W numbers, and the rest are ones which had been raised in this laboratory. These plants all were planted in 25 cm pots under glass-house conditions, in a split plot design with 8-10 plants per clone, and grown for two years to replicate twice the trials. For assessing tuber-forming response to daylength, they were grown at both long and short day seasons, during the periods from March to June with the daylength of 13.5 hours on an average and from September to December with 10.5 hours, respectively.

All the characters studied were measured on individual plants at an earlier stage of flowering, excepting the stolon and tuber characters measured just after harvesting, and then clone averages were calculated in each of the characters measured. The methods employed for measurements have already been described elsewhere<sup>3)</sup> and hence will only be briefly mentioned in the following section 'Results'. Out of the data thus obtained, those on the characters whose variations were found to be discontinuous were subjected to  $\chi^2$ -test for examining segregation ratios. Cytological analysis was made using an acetocarmine technique modified by the present author<sup>4)</sup>.

### Results

The dihaploid clones used each had distinctive phenotypes in leaf, flower, stem and tuber characters. Most of these characters showed a continuous range of variation among the clones, but the following eight were indicative of discontinuity in their variations.

In Table 1 are summarized the data on phenotypic segregations of these characters.

#### 1) *Terminal leaflet shape*

This was expressed as a leaflet index, which is the ratio of width to length of terminal leaflets and shown in percentage. Mature leaves were measured for this character. The parental cultivar invariably had broad leaflets, their mean index value being  $64.7 \pm 0.5$ , whereas the dihaploid clones widely varied in their leaflet shape, showing the indices ranging from 39 to 63. The variation found here was not continuous but of a bimodal manner, and all the clones appeared to be grouped into two types; narrow and broad, giving the mean indices of  $49.4 \pm 0.45$  and  $61.4 \pm 0.5$ , respectively (Fig. 3). Of the 65 clones, 56 fell into the former type and 9 into the latter. This segregation was in a ratio not departing to any extent from 5:1, as seen from the chi-square value given in Table 1.

#### 2) *Stigma type*

Two types were clearly distinguished; one with the snicked and larger-sized apices, referred to as notched stigma, and the other with smooth-faced and smaller-sized ones, as entire or simple stigma. Of the 56 dihaploid clones which were successful to bloom, 47, like their parental cultivar, were of the former type, and 9 were of the latter, giving very good fit to a 5:1 ratio.

#### 3) *Tuber shape*

Measurements were made on comparatively well developed tubers, weighing more than 20 gr. Unlike the characters mentioned above, tuber shapes were fairly variable not only among different clones but also even within a clone. The range of variation was from 40 to 100 in tuber index value, which was expressed by the ratio (%) of transverse axis to long axis of tuber. In this case, however, there was recognizable an indication of trimodality in frequency distribution of the clones. Fig. 1 shows this situation. The mean index values of tubers involved in these three modes each were  $50.0 \pm 1.15$ ,  $66.9 \pm 0.95$  and  $92.5 \pm 1.46$ , and these tuber shapes were designated as long-ovate, oval and round, respectively (Fig. 4). The proportion of the clones with tubers taking such shapes was

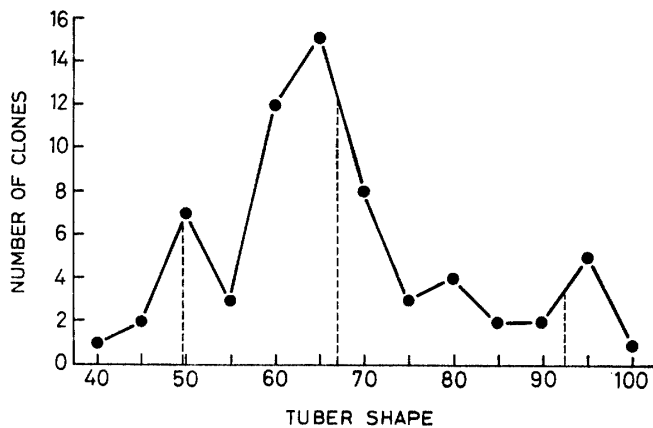


Fig. 1. Frequency distribution of the dihaploid clones with different tuber shapes, as indicated by tuber indices. The dashed vertical lines each indicate mean index values in the three tuber types.

10:42:13 in the order given above, and its ratio appeared to approach to 1:4:1. The parental cultivar almost always produced tubers with shapes ranging from oval to round, their mean index value being  $80.0 \pm 0.4$ .

#### 4) Stem pubescence

This character was examined in younger plants about 3 weeks after sprouting. The parental cultivar had well-marked hairs on

the stem. Dihaploids, however, showed pubescence varying from dense to almost absent among clones, and the frequency of the clones with pubescent and glabrous stems was 48 and 17, respectively. This segregation gave a very good fit to a 3:1 ratio.

#### 5) Stolon length

Arrested development of stolons is one of important characters distinguishing cultivated from wild species in tuberous *Solanum*, for stolons shortened by it give rise to tuberization being clustered tightly around the base of the shoot. In this study, stolon length of the parental cultivar with short stolons (as long as 15 cm or less) was employed as a criterion for assessing those of the dihaploid clones. The dihaploid clones were thus classified into two types; short and long, on the basis of stolon length. Of the 65 clones examined, 46 were of short type and the remaining 19 were of long type, this segregation being interpreted as a clear 3:1 ratio.

#### 6) Meiotic behavior

Eighteen out of the 56 dihaploid clones that bore flower buds showed various premeiotic disturbances, so that they could not be analyzed cytologically. The remaining 38 clones had meiosis in either normal or ab-

Table 1. Phenotypic segregations for the eight characters in dihaploid offsprings from the potato cultivar Chippewa

Character	Parental phenotype	Dihaploid offspring				
		phenotype	Observed frequency	Expected ratio	X <sup>2</sup>	P
Leaflet shape	Broad	Narrow	56	5	0.378	0.5-0.7
		Broad	9	1		
Stigma type	Notched	Notched	47	5	0.015	0.9
		Entire	9	1		
Tuber shape	Oval-round	Long ovate	13	1	1.459	0.3-0.5
		Oval	42	4		
		Round	10	1		
Stem pubescence	Pubescent	Pubescent	48	3	0.023	0.8-0.9
		Glabrous	17	1		
Stolon length	Short	Short	46	3	0.310	0.5-0.7
		Long	19	1		
Meiotic behavior	Normal	Normal	28	3	0.035	0.8-0.9
		Desynaptic	10	1		
Corolla shape	Sub-rotate	Stellate	16	1	0.571	0.7-0.8
		Pentagonal	28	2		
		Sub-rotate	12	1		
Photoperiodic response for tuberization	Day-neutral	Short day	18	1	0.507	0.7-0.8
		Day-neutral	33	2		
		Long day	14	1		

normal manner. Normal meiosis was found in 28 of the clones involved, giving a mean pairing frequency of  $10.5 \pm 0.39$  bivalents per cell, with a range of 7.86 to 11.88, and abnormal meiosis was encountered in the 10 clones, the pairing frequency per cell averaging  $1.44 \pm 0.23$  bivalents, with a range of 0.93 to 2.21. The latter case may be regarded as desynaptic due to the falling apart during from late diplotene to early diakinesis of chromosomes which paired normally at pachytene. Fig. 6 shows these two meiotic patterns observed in certain dihaploid clones. The segregation ratio, 38 normal: 10 desynaptic, found here was in a closer proximity to 3:1 rather than to 5:1. The parental cultivar was found to be normal in its pairing behavior and to have 2.40 quadrivalents, 0.95 trivalents, 16.50 bivalents and 2.55 univalents as a mean pairing frequency per cell.

#### 7) Corolla shape

This character was expressed as a corolla index, it being a ratio (%) of B to A in length, as illustrated in Fig. 2. Corollas of the parental cultivar were consistently of sub-rotate shape, with the mean index value of  $70.4 \pm 0.18$ , whereas those of the dihaploid clones could be classified into three types; stellate, pentagonal and sub-rotate, with the mean index values of  $52.8 \pm 0.36$ ,  $62.4 \pm 0.49$  and  $73.8 \pm 0.42$ , respectively (Fig. 5). The frequency of the clones assigned to each type was 16, 28 and 12 in the order given above, giving a good fit to a 1:2:1 ratio.

#### 8) Photoperiodic response for tuberization

Tuber-forming response to daylength was estimated by comparing spring- and fall-grown plants for their tuber yields. According to the degree of response, the dihaploid clones were likely to be grouped, though not critical, as follows: ones which give decreased or no tuber yields under long-day conditions of spring as compared with those under short-day conditions of fall, being designated as short-day type; ones in which long-day conditions are rather favorable for tuber formation, as long-day type; and ones which are capable of tuberizing under both conditions, as day-neutral type. Of the 65 clones, 18 and 14 were of short- and long-day types, respectively, and the remainder were

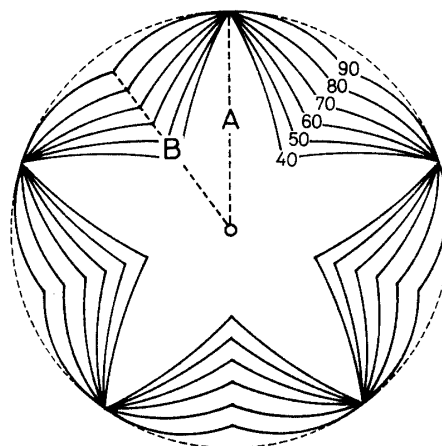


Fig. 2. Diagram illustrating corolla index in potato flowers. The index value is calculated as a ratio of B to A and represented in percentage. The figures given in the diagram each indicate the index values calculated in this way for various corolla shapes.

day-neutral, resembling the parental cultivar. The segregation for this character seems, therefore, likely to be interpreted as a 1:2:1 ratio.

## Discussion

The continuous variations found among the dihaploid clones for all of the characters examined, except for the eight ones, may be explainable as resulting either from the cumulative action of multiple sets of major genes with a polymeric effect or from the contribution of polygenes and their interaction with the above major genes. With respect to the eight characters, each of which has been described in the preceding section, however, an interaction of alleles other than polymeric ones should be presumed for interpreting the variations found in them, because there was recognizable an indication of discontinuity in the variations. The situation is presented in Table 1 with the data analyzed for the phenotypic segregations in the characters in question.

The dihaploid clones used here all have their origin in the egg cells from the parental cultivar. Consequently, phenotypic segregations in the former can be regarded as a reflection of gametic segregations in the latter. Since the common potato is a tetra-

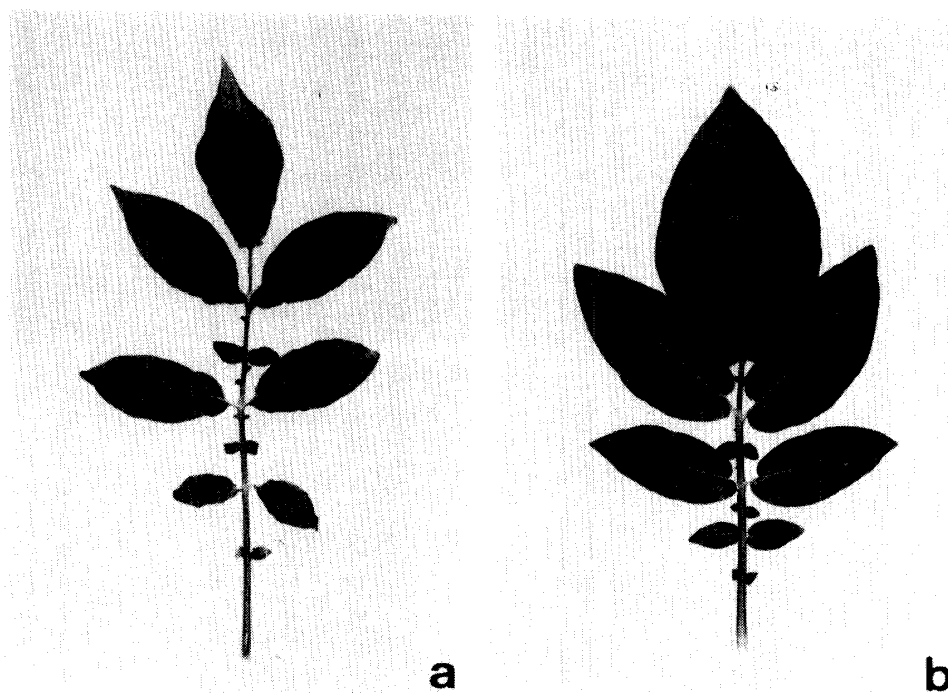


Fig. 3. Leaves of some dihaploid clones.  
a: Narrow leaflet (clone H-9).  
b: Broad leaflet (clone H-19).

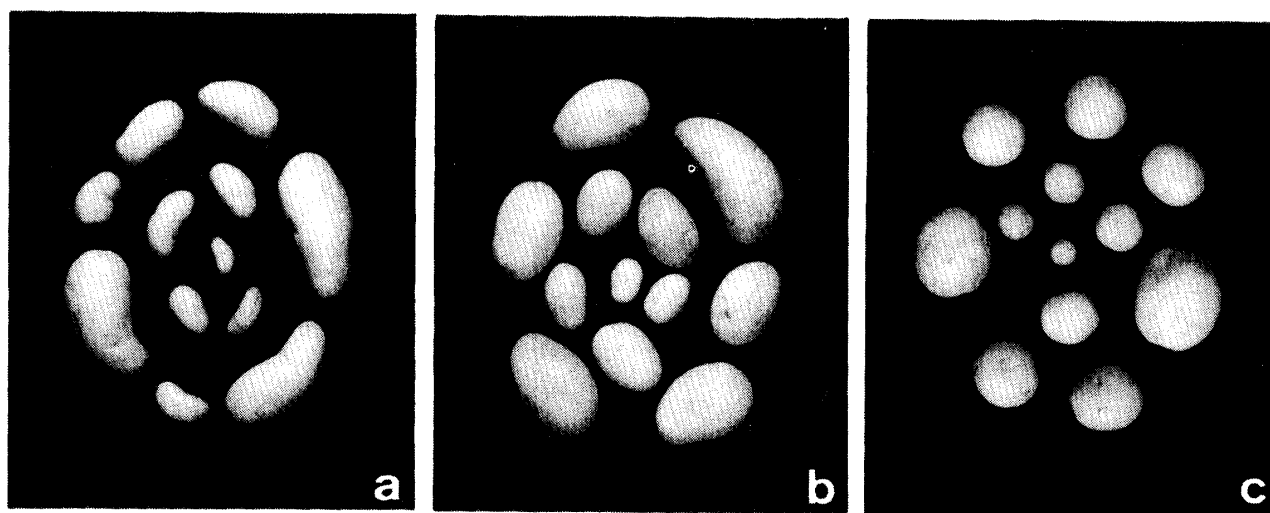


Fig. 4. Tubers of some dihaploid clones.  
a: Long-ovate (clone H-1).  
b: Oval (clone H-17).  
c: Round (clone H-34).

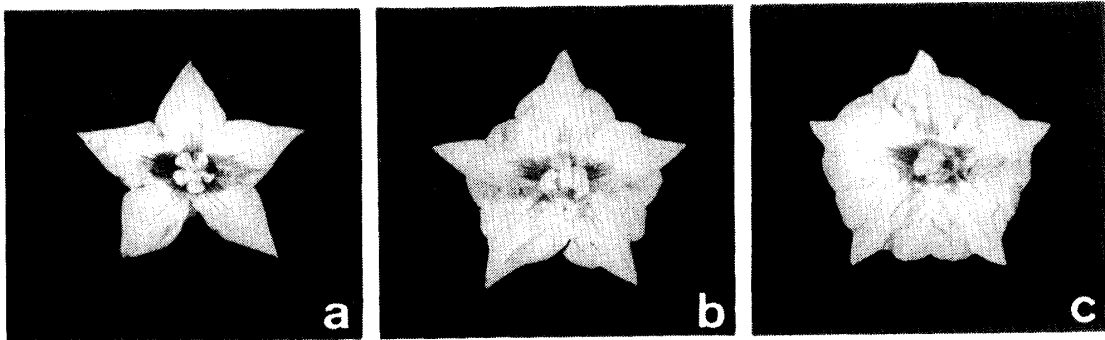


Fig. 5. Corollas of some dihaploid clones.

- a: Stellate (clone H-34).
- b: Pentagonal (clone H-14).
- c: Sub-rotate (clone H-28).

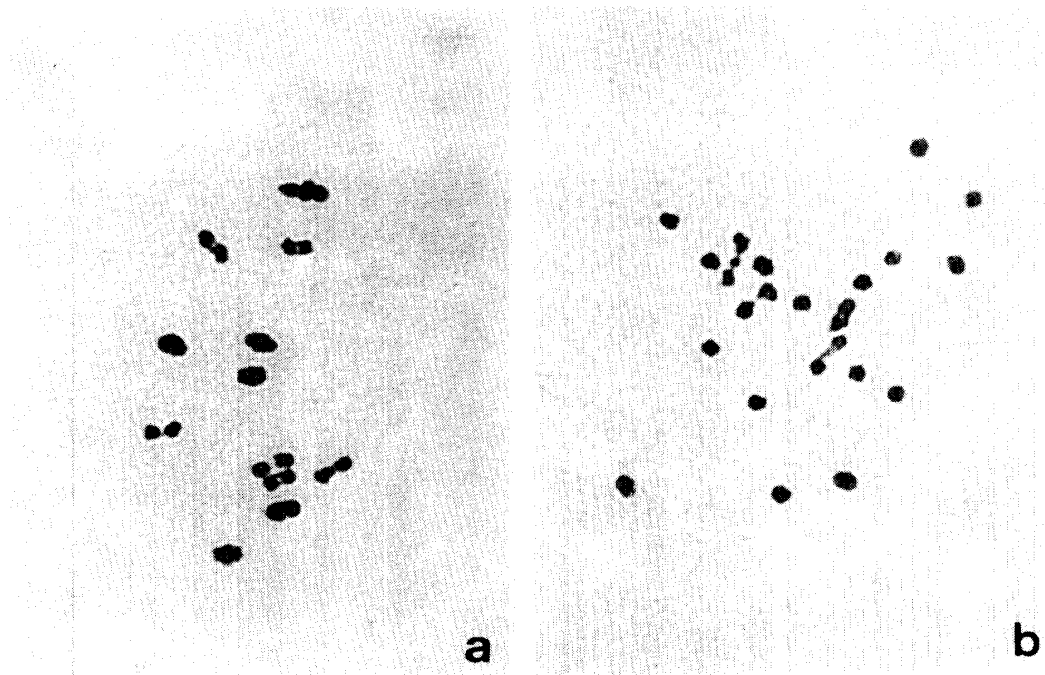


Fig. 6. First metaphase configurations in some dihaploid clones. (*ca.*  $\times 1700$ )

- a: Regularly paired 12 bivalents found in a meiotically normal clone (H-28).
- b: Loosely paired 4 bivalents and 16 univalents found in a desynaptic clone (H-14).

ploid with 12 chromosomes as the basic number, its constituent genome comprises the four sets of 12 chromosomes. If the four corresponding chromosomes from these four sets are highly homologous with one another, random pairing of chromosomes occurs, and, as a result, gametic segregation must become tetrasomic. If the chromosomes concerned are to a higher or lower degree differentiated structurally in two pairs from each other, then preferential pairing of chromosomes occurs in many cases due to their differential affinity, and hence it may be expected to lead the gametic outputs to segregate disomically. Now, assuming that any one of the parental genotypes is in the duplex condition, the gametic segregation expected from random pairing of chromosomes results in a ratio of either 5:1 or 1:4:1 according to whether dominance is complete or not, whereas that from preferential pairing results in either a 3:1 or 1:2:1 ratio. From the other tetrasomic genotypes, however, such gametic ratios can not be expected.

On the above assumption, the data presented in Table 1 may be interpreted as follows; the genes for leaflet shape, stigma type and tuber shape each are located in the chromosomes which still are in high homology with one another, and the genes for the other five characters each are carried by the four chromosomes which have already been so differentiated in two pairs from each other in their structure as to do not allow always random pairing. If this interpretation is valid, it may be considered that the genomes of the common potato have been undergoing diploidization genetically as well as cytologically in varying degrees among different chromosomes of the four sets involved in them, suggesting that the common potato, at least so far as the present cultivar concerns, is of segmental allotetraploid nature.

Such a view seems also to be evidenced by the fact that the four sets of chromosomes in the present cultivar apparently have a lesser tendency to pair as multivalents as compared with those in autotetraploid forms induced from some diploid *Solanum* species. In the former, as described already, there

were present 2.40 quadrivalents + 0.95 trivalents in mean frequency per cell, while in the latter 5.56 and 6.14 multivalents have been found in *S. polyadenium*<sup>12)</sup> and *S. verrucosum* (MATSUBAYASHI, unpublished), respectively.

On the other hand, the phenotypic segregations found in the dihaploid clones are of special interest also from breeding point of view. It is a reason for this that such segregations make it possible to disclose some deleterious recessives lurking in the parental genotypes and to expel them by selection. Breeding by this way has a important significance particularly in male sterile crop plant cultivars, such as the present material, which are not capable of yielding inbred progeny. In fact, the present author has found out, from among the present dihaploid clones, one clone which became superior, by doubling its chromosome complement, to the parental cultivar not only in tuber yield but in some tuber characters. Therefore, it will never be infeasible to improve to a higher extent the existing potato cultivars by utilizing their dihaploids, provided a number of desirable dihaploid segregants are exclusively selected including male fertile ones, intercrossed in favorable combinations and then the resultant hybrids are doubled for their chromosomes. Breeding schemes suggested by HOUGAS and PELOQUIN<sup>2)</sup> and also by CHASE<sup>1)</sup> seem thus to have a great promise for the future in potato improvement.

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バレイショの2倍性半数体における遺伝的変異, 特に  
 若干形質にみられる対立変異について  
 (予報)

松 林 元 一

要 約

半数体は自殖のきかない栽培植物の遺伝的組成を分析する上で極めて有力な手段を提供する。本研究では、この観点から、栽培バレイショ (*Solanum tuberosum* ssp. *tuberosum*,  $2n=48$ ) の雄性不稔品種 Chippewa から育成された2倍性半数体 ( $2n=24$ ) 65系統を用い、それらの特性を調べて次の結果を得た。

供試系統は地上部及び地下部の形態的ならびに生理的諸形質において、それぞれ固有の表現型をもち、しかも系統間で幅広い変異を示すことが明らかとなった。特に興味あることは、染色体数の半減に伴って下記8形質の変異に非連続的な対立性が認められたことである。すなわち、頂小葉形では5広葉:1細葉、柱頭型では5刻み型:1単頭型、塊茎の形状では1長卵形:4卵形:1球形、茎毛では3有毛:1無毛、ストロン長では3短:1長、染色体対合では3正常対合:1解対合、花冠形では1星形:2五角形:1車形及び塊茎形成の日長反応では1短日:2中性:1長日の分離がみられた。5:1もしくは1:4:1の分離は、母本品種における関係遺伝子がduplex組成になっており、それらを担う染色体が全く機会的に対合と分離を行なった結果であると考えれば説明がつく。しかし3:1もしくは1:2:1の分離については2つの解釈が成り立つ。1つは母本における関係遺伝子座が2染色体的であったとする見方であり、他はそれが4染色体的ではあっても染色体の対合に強い選択性が働いたためとする見方である。いずれにせよ、このような分離が、前記4染色体的分離とともにみられたという事実は、バレイショのゲノムを構成する染色体がそれぞれ遺伝的2倍体化の程度を異にしていることを示唆するものであり、さらに染色体数の半減によって母本に潜在する劣悪遺伝子を排除し、その倍加によって有用遺伝子の集積を図るという新しいバレイショ育種の技術的可能性を示唆するものと考えられる。