Estación Experimental de Aula Dei, Zaragoza

# Cytogenetics of autotetraploid sugar beet (*Beta vulgaris* L.)

## Part I: Tetraploid varieties

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### Introduction

Artificially produced polyploids desintegrated into the presently cultivated crops on a rather large scale, especially in sugar beet, where tetraploid lines are used as one of the seed parents for the production of triploids. Cultivation, propagation and selection of tetraploids offer many problems as has been amply demonstrated in tetraploids of corn (RANDOLPH 1935, ALEXANDER 1957, SHAVER 1962 a. o.), rye (Literature review: ELLERSTRÖM and SJÖDIN 1963) and barley (Literature review: NILAN 1964). According to the forementioned investigations the cytogenetic structure and behaviour of these tetraploids differ in many ways from that of the diploids they were derived from. Studies were designed to find out if this also applies to diploid and tetraploid sugar beet.

When the first tetraploid varieties of sugar beet were investigated it was soon realized that these varieties were not stabilized on the tetraploid chromosome level (RASMUSSON 1953, KLOEN and SPECKMANN 1954), but it was thought that within a few generations aneuploidy would disappear. As euploid plants continue to produce aneuploids within their offspring (ROMMEL, unpublished results), tetraploid varieties will maintain a certain level of aneuploidy even in advanced generations (ROMMEL 1963). Therefore, euploid and aneuploid plants, which are the components of the "tetraploid variety", were studied for morphology, plant development, root yield and seed production. It was not attempted to classify the aneuploids according to their karyotype but to outline their general features and to explore their impact on yield.

### Material and Methods

The investigations were carried out from 1962 to 1964, using diploid varieties of sugar beet and their tetraploid counterparts. The tests, started in 1962 included two varieties, while the same tests repeated in 1963 included three varieties. The tetraploids were in the $C_4$ generation.

Seed samples were taken at random from the diploid and tetraploid varieties. In March seeds were sown in flats filled with soil and after germination all seedlings were potted individually. The plants were kept in the greenhouse until small leaves were collected for cytological investigations. Twice a spray of an insecticide was applied to keep the plants free from aphis and virus yellow. In May all plants were removed from the greenhouse and divided into two groups. One group from each variety was transplanted to the field to represent beets for factory use, while the other group was planted into seed beds to be used as seed beets the following year.

The factory beets were placed on a distance of 0.25 m within a row and 0.40 m between rows. In 1962 50 diploid and 200 tetraploid plants and in 1963 50 diploid and 100 tetraploid plants were taken from each variety. Tetraploid plants were planted at random without knowing their particular chromosome number. During the summer months notes were taken on plant development, morphology and vigour. In December the roots were harvested, topped, cleaned from soil and weighed individually.

The group with the steckling plants was placed into seed beds on a distance of 0.10 m within and 0.25 m between rows. Chromosome counts were carried out during winter time using stored material. The following spring the plants were recollected and transplanted to field plots in hemp isolation. Four plots represented one variety, consisting of one diploid and three tetraploid plots. The scheme of the test is explained in Table 1. Plant development was recorded from April to June and the seeds harvested in July.

Table 1. *Scheme of seed beet plots in hemp isolation.*

| Plot | No. of plants | Classification | No. of chromosomes |
|---|---|---|---|
| A | 24 | euploid tetraploid | 36 |
| B | 24 | tetraploid variety | 34, 35, 36, 37, 38 |
| C | 24 | aneuploid tetraploid | 34, 35, 37, 38 |
| D | 24 | diploid | 18 |

Cytological investigations were carried out using young leaves and the orcein technique described by TJIO and LEVAN (1950), modified by ROMMEL (1963).

### Results

Germination of the diploid and tetraploid varieties is recorded in Table 2. Because of the diploid and tetraploid seed samples deriving from different places and years figures should not be considered to be representative for a particular variety.

Table 2. *Germination of diploid and tetraploid varieties.*

| Year | Variety | No. of genoms | No. of seeds | No. of germs | Germination in % |
|---|---|---|---|---|---|
| 1962 | MLR | 2x | 600 | 853 | |
| | B 742-MM | 4x | 600 | 590 | |
| | Ebro 229 | 2x | 600 | 365 | |
| | Ebro 646 | 4x | 600 | 725 | |
| 1963 | MLR | 2x | 300 | 473 | 77 |
| | B 742-Im | 4x | 300 | 460 | 91 |
| | Ebro 194 | 2x | 300 | 248 | 45 |
| | Ebro 645 | 4x | 300 | 429 | 87 |
| | AJ-1 | 2x | 300 | 705 | 92 |
| | J 8413 | 4x | 300 | 240 | 62 |

Chromosome counts showed that the number of chromosomes ranged from $2n = 32$ to $2n = 39$ and that the percentage of aneuploids was 41.0 and 39.9 in 1962 and 33.7, 31.7 and 32.8 in 1963. Field notes, which were taken on plant size are combined with the results on chromosome counts and represented in

Table. 3. *Plant size and chromosome number in diploid and tetraploid sugar beet.*

| Year and no. of varieties | 2 n = | Plant size small no. | small in % | medium no. | medium in % | large no. | large in % | Total no. of plants |
|---|---|---|---|---|---|---|---|---|
| 1962 | | | | | | | | |
| 2 varieties | 18 | | | | | 100 | 100 | 100 |
| 2 varieties | 36 | 39 | 16.7 | 34 | 14.6 | 160 | 68.7 | 233 |
| | 32 | 1 | | | | | | 1 |
| | 33 | | | 1 | | | | 1 |
| | 34 | 7 | | 1 | | | | 8 |
| | 35 | 48 | 66.7 | 18 | 25.0 | 6 | 8.3 | 72 |
| | 37 | 53 | 62.4 | 15 | 17.6 | 2 | 13.3 | 85 |
| | 38 | 10 | | 2 | | 3 | | 15 |
| 1963 | | | | | | | | |
| 3 varieties | 18 | | | | | 144 | 100 | 144 |
| 3 varieties | 36 | 17 | 8.9 | 19 | 10.0 | 154 | 61.1 | 190 |
| | 34 | 3 | | | | | | 3 |
| | 35 | 17 | 53.1 | 8 | 25.0 | 7 | 21.9 | 32 |
| | 37 | 15 | 27.8 | 18 | 33.3 | 21 | 38.9 | 54 |
| | 38 | 3 | | 2 | | 3 | | 8 |
| | 39 | 1 | | | | | | 1 |

Table 4. *Plant size and percentage of aneuploids in tetraploid sugar beet.*

| Year | Plant size | Total no. of plants | Euploids no. | Euploids in % | Aneuploids no. | Aneuploids in % |
|---|---|---|---|---|---|---|
| 1962 | small | 158 | 39 | 24.5 | 119 | 75.5 |
| | medium | 71 | 34 | 48.9 | 37 | 52.1 |
| | large | 186 | 160 | 86.0 | 26 | 14.0 |
| 1963 | small | 56 | 17 | 30.4 | 39 | 69.6 |
| | medium | 47 | 19 | 40.4 | 28 | 59.6 |
| | large | 185 | 154 | 83.2 | 31 | 16.8 |

Table 3. Plants were classified into large, medium and small according to their size at the time the notes were taken. The large group represents the best developed plants within the variety under consideration. The results among the varieties were considered sufficiently consistent to be combined in 1962 and 1963 respectively.

Table 4 shows the percentage of aneuploid plants within the three groups. In both years and in five varieties the number of aneuploids was highest in the small sized and lowest in the large sized group. Aneuploid plants with $2n + 1$ chromosome seem to be slightly better conditioned for plant development than plants with $2n - 1$ chromosome.

Root weight of diploid and tetraploid plants is presented in Table 5. In both years the weight of the euploid plants is superior to the aneuploid plants. Moreover, the euploid tetraploids compete well with the diploid varieties they were derived from, while the average root weight of the tetraploids lies below or equals the average root weight of the diploids.

In 1962 out of 158 plants classified under "small" only two were found to reach the average root weight of the variety. The corresponding figures for 1963 are two plants out of 56. In contrast to this the opposite is not true, which means that a well developed crown of leaves is not bound to have high root weight.

Table 5. *Average root weight of diploid and tetraploid plants.*

| Year and variety | Diploid 2 n = 18 no. | in g | Tetraploid 2 n = 36 no. | in g | 2 n = 37 no. | in g | 2 n = 35 no. | in g | 2 n = 34, 38, 39 no. | in g | total no. | in g |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1962 | | | | | | | | | | | | |
| MLR | 50 | 673 | | | | | | | | | | |
| B 742-MM | | | 117 | 524 | 38 | 234 | 26 | 293 | 18 | 226 | 199 | 412 |
| Ebro 229 | 50 | 418 | | | | | | | | | | |
| Ebro 646 | | | 110 | 378 | 41 | 191 | 44 | 140 | 5 | 182 | 200 | 283 |
| 1963 | | | | | | | | | | | | |
| MLR | 50 | 578 | | | | | | | | | | |
| B 742-IM | | | 59 | 675 | 20 | 416 | 10 | 420 | 4 | 386 | 93 | 579 |
| Ebro 194 | 50 | 818 | | | | | | | | | | |
| Ebro 645 | | | 64 | 857 | 12 | 555 | 10 | 628 | 5 | 284 | 91 | 760 |
| AJ-1* | 44 | 178 | | | | | | | | | | |
| J 8413* | | | 64 | 474 | 21 | 368 | 11 | 217 | 3 | 167 | 99 | 414 |

* Attacked by deseases

The seed yield of diploid and tetraploid varieties is presented in Table 6, expressed in average seed yield per plant. In two years and in five varieties the seed yield of euploid tetraploid plants proved to be superior to the yield of the diploid variety. The tetraploid variety, without being selected for euploidy yielded more than the diploid one in three cases, and aneuploids when grown in one plot, in no case reached the diploid yield.

Table 6. *Average seed yield of diploid and tetraploid plants.*

| Year and variety | Diploid no. | in g | Tetraploid euploid no. | in g | variety no. | in g | aneuploid no. | in g |
|---|---|---|---|---|---|---|---|---|
| 1963 | | | | | | | | |
| MLR | 18 | 62.8 | | | | | | |
| B 742-MM | | | 22 | 97.0 | 24 | 75.8 | 24 | 48.3 |
| Ebro 229 | 16 | 33.4 | | | | | | |
| Ebro 646 | | | 20 | 41.8 | 19 | 40.3 | 12 | 23.8 |
| 1964 | | | | | | | | |
| MLR | 23 | 36.5 | | | | | | |
| B 742-IM | | | 24 | 52.3 | 24 | 41.9 | 23 | 26.7 |
| Ebro 194 | 23 | 49.3 | | | | | | |
| Ebro 645 | | | 24 | 57.1 | 22 | 47.3 | 24 | 22.7 |
| AJ-1 | 18 | 37.2 | | | | | | |
| J 8413 | | | 24 | 38.3 | 22 | 26.6 | 20 | 7.5 |

## Discussion

Autotetraploid varieties of sugar beet are characterized by the presence of a considerable number of aneuploid plants. Aneuploidy as described by LEVAN (1942) affects the plants in many ways and makes them distinguishable from euploid plants by morphological and physiological features. In tetraploid varieties of sugar beet consisting of euploid and aneuploid plants it is obvious that euploidy favours plant development. Plants with deviating chromosome numbers tend to be smaller and often exhibit few or narrow leaves and root and seed yield is lowered in comparison with euploid plants. In the experiments reported here, aneuploidy has caused considerable losses in root and seed yield, depending from the number of aneuploids which were encountered in the varieties investigated (Table 7).

Table 7. *Root and seed yield of euploid plants versus variety (euploids and aneuploids)*

| Year and variety | Roots | | | | Seeds | | | |
|---|---|---|---|---|---|---|---|---|
| | euploids | | variety | | euploids | | variety | |
| | ∅ weight | in % | ∅ weight | in % | ∅ yield | in % | ∅ yield | in % |
| 1962/63 | | | | | | | | |
| B 742-MM | 524 | 100 | 412 | 78.6 | 97.0 | 100 | 75.8 | 78.1 |
| Ebro 646 | 378 | 100 | 283 | 74.9 | 41.8 | 100 | 40.3 | 96.4 |
| 1963/64 | | | | | | | | |
| B 742-IM | 675 | 100 | 579 | 85.8 | 52.3 | 100 | 41.9 | 80.1 |
| Ebro 645 | 857 | 100 | 760 | 88.7 | 57.1 | 100 | 47.3 | 82.3 |
| J 8413 | 474 | 100 | 414 | 87.3 | 38.3 | 100 | 26.6 | 69.5 |

In competition with diploid varieties aneuploidy also seems to be a disadvantage for the tetraploid varieties. In field tests, diploid plants grow steadily with well developed crown of leaves which soon will cover the ground, whereas only one part of the tetraploids developes well, while the remaining plants are less vigorous. In plots of seed beets similar observations can be made. Root weight of tetraploids varies much more than that of diploids.

To raise the yielding capacity of the tetraploid varieties the elimination or at least reduction of the number of aneuploids is necessary. Chromosomal stability of the euploid plants seems to be the first need but has not been found yet. Mechanical selection can be applied but has to be repeated in each generation. The most secure but time consuming mechanical selection is based on chromosome counts herewith not considering the number of euploids which in fact are aneuploids. Once a certain relationship between morphological traits and aneuploidy has been established these features can serve for mechanical selection on a much broader scale. Selection for euploidy has to be carried out before planting because otherwise the number of plants in the field would be reduced too much. Seed selection on account of seed size proved to be very useful in cereals (MÜNTZING 1943, HELGASON and ROMMEL 1963), but was not effective in sugar beet as indicated by preliminary tests. Selection of mother beets on account of weight will be easy to apply, the same as selecting steckling plants in spring for size of roots. Probably in many stocks of tetraploid sugar beet some selection for euploidy was done in choosing the best sized roots for seed production.

The presence of aneuploids in tetraploid varieties makes the evaluation of the tetraploids in comparison with the diploids difficult. Results of comparative studies in diploid and tetraploid sugar beet carried out over a number of years (JÄHNL 1963) consider the average values of existing tetraploid varieties but not the potential values expressed in output of euploid plants. To apply genetical selection tetraploid stocks should be cleaned as much as possible of aneuploids to make the selection more appropriate. To reduce the number of aneuploids in tetraploid sugar beet may not seem to be so important in a crop where emphasis is placed on triploid seed production. But reducing the number of aneuploids in the tetraploid seed parent will also reduce the number of aneuploids among the triploid seeds (ROMMEL 1963) and raise the seed yield of the parent plants.

## Summary

Autotetraploid and diploid varieties of sugar beet were investigated for morphology, plant development, root and seed yield. The results obtained from the tetraploid varieties were evaluated according to the number of euploid and aneuploid plants found in each variety. Aneuploid plants often are characterized by delayed growth and poor root or seed yield, which reflects in the average yield of the tetraploid variety. Eutetraploid plants will compete successfully with their diploid counterparts. Until chromosomal stability of euploid plants will be found, aneuploid plants can be eliminated by mechanical selection only, which has to be repeated in each generation. A mechanical selection for euploidy will not only lower the amount of aneuploids in the tetraploid varieties, but also among the triploid hybrid seeds.

## Zusammenfassung

Autotetraploide und diploide Zuckerrübensorten wurden auf Morphologie, Wachstum sowie Rüben- und Samenertrag untersucht. Die Ergebnisse der tetraploiden Sorten wurden gemäß den in ihnen gefundenen Anteilen von euploiden und aneuploiden Pflanzen ausgewertet. Aneuploide Pflanzen sind oft durch verlangsamtes Wachstum und schlechten Rüben- oder Samenertrag gekennzeichnet, was sich im Durchschnittsertrag der tetraploiden Sorten widerspiegelt. Eutetraploide Pflanzen können erfolgreich mit den entsprechenden diploiden konkurrieren. Ehe nicht chromosomal stabile eutetraploide Pflanzen gefunden werden, können Aneuploide nur durch mechanische Selektion ausgelesen werden. Diese muß in jeder Generation wiederholt werden. Eine mechanische Selektion auf Aneuploide wird die Anzahl der Aneuploiden nicht nur in den tetraploiden Sorten herabsetzen, sondern auch unter den triploiden Hybridsamen.

## Resumen

Se investigó variedades autotetraploides y diploides de remolacha azucarera en relación con la morfología, el desarrollo y el rendimiento en raíz y semilla. Los resultados obtenidos de la variedad tetraploide se evaluaron de acuerdo con el número de plantas euploides y aneuploides halladas. Las plantas aneuploides se caracterizan frecuentemente por un crecimiento retardado y una producción pobre en raíz o semilla, propiedades que se reflejan en el rendimiento medio de las variedades tetraploides. Las plantas eutetraploides pueden competir con éxito con sus correspondientes diploides. Mientras no se alcance la estabilidad cromosómica de las plantas euploides, las aneuploides pueden únicamente eliminarse por medio de una selección mecánica, selección que debe repetirse en cada generación. Una selección mecánica para euploidía no sólo rebajará la proporción de aneuploides en las variedades tetraploides sino tambien dentro de las semillas híbridas triploides.

## Literature

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# Der Gendosiseffekt beim Nikotinabbau des Tabaks

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Mit 1 Abbildung

Die Auswertung von Kreuzungen zwischen nikotinhaltigen und nikotinfreien Tabaksorten hatte gezeigt, daß die Umsetzung des Nikotins zu Nornikotin, wie sie in den Blättern nikotinfreier Tabaksorten vor sich geht, ein Vorgang ist, der als direkte und einfache Genwirkung anzusehen ist.

$$\text{Gen} \bigcirc \xrightarrow{\text{Abbauferment}} \text{Nikotin} \downarrow \text{Nornikotin} \downarrow$$

Die Umsetzung des Nikotins, d. h. der Schritt vom Nikotin zum Nornikotin, kennzeichnet das dominante Merkmal, das auch den Wildtypen eigen ist. Unterbleibt dieser Schritt bei Verlust der Abbaufähigkeit, so reichert sich das Nikotin an, was für das rezessive Merkmal des Nikotinreichtums unserer meisten Gebrauchstabake charakteristisch ist.

Meine Vorstellung über die Natur des Erbmerkmals Nikotingehalt basiert nur auf Nikotinuntersuchungen. Nach Feststellungen der Tabakchemiker ist der Verlust an Nikotin von einer Anreicherung des Nornikotins begleitet, nikotinfrei also gleichbedeutend mit nornikotinhaltig, weshalb ich auch in dem oben aufgezeigten Schema den Begriff Nornikotin einführe. „It is now well known that nicotine is converted into nornicotine in the shoot, especially in the leaves" (SCHROETER, 1958).

Solche Einzelschritte einer Genwirk-Kette sind in letzter Zeit mehrfach aufgedeckt worden. Ich erinnere nur an die Bildungsprozesse von Ommochrompigmenten oder von Blütenfarben. Beim Tabak konnten entsprechend seiner amphidiploiden Natur zwei voneinander unabhängige Genorte nachgewiesen werden, die gleichsinnig den Nikotinabbau steuern (KOELLE, 1961, dort weitere Literatur), so daß ich bei Auswahl geeigneter Sorten und ihrer Bastarde eine Reihe von quantitativ abgestuften Abbaufaktoren aufstellen konnte, wie es die nachfolgende Übersicht zeigt.

Der Abbau-(A)Faktor könnte auch durch $a^+$ gekennzeichnet werden, da sich in ihm das Wildallel präsentiert. Die alte Schreibweise aber läßt hier die sich summierenden A-Faktoren deutlicher werden.

Übersicht

| genetisches Symbol | phänotypisches Merkmal | Sortenname bzw. Kreuzung |
|---|---|---|
| 1. $a_1a_1a_2a_2$ | nikotinhaltig nicht abbauend | Havanna II c |
| 2. $A_1a_1a_2a_2$ | abbauend | (Hav. II × Hav. III) $F_1$ |
| 3. $A_1A_1a_2a_2$ | abbauend Nornikotintyp | Havanna III 3 |
| 4. $A_1a_1A_2a_2$ | abbauend | (FO × Hav. II) $F_1$ |
| 5. $A_1A_1A_2a_2$ | abbauend | (FO × Hav. III) $F_1$ |
| 6. $A_1A_1A_2A_2$ | abbauend Nornikotintyp | FO |

Der Nikotinabbau ist eine fermentative Demethylierung (SCHROETER, 1961). Als direkte Wirkung dieser A-Faktoren dürfen wir also ein Abbau-Ferment annehmen. Da ein solches Ferment meines Wissens im Tabak noch nicht identifiziert ist, konnte ich die direkte Wirkung der A-Faktoren nur über den Unterschied im Nikotingehalt erfassen. Ich schließe aus einem höheren Nikotingehalt auf einen geringeren Abbau und damit auch eine geringere Anzahl von A-Faktoren, und umgekehrt aus einem niedrigeren Nikotingehalt auf einen stärkeren Abbau und damit eine höhere Anzahl von A-Faktoren. Dieser Schluß ist naheliegend, jedoch nicht zwingend. Da er im Einklang steht mit meinen bisherigen Versuchsergebnissen, scheint er mir berechtigt, solange nicht kompliziertere Erklärungsmöglichkeiten zwingend werden.

Neben dem Gendosisproblem ergibt sich anhand dieser 6 Genotypen die Frage nach einem Unterschied in der Wirkung gleichsinniger Gene, wenn diese im einen Fall, wie bei Versuchsglied 3, einander allel, im anderen Fall, wie bei Versuchsglied 4, auf zwei verschiedenen Genorten lokalisiert sind. Und weiter geben meine Versuchsergebnisse Anlaß, die Erscheinung eines hier auftretenden Dominanzwechsels und die damit eng zusammenhängenden widersprüchlichen Auffassungen von der Vererbung des Nikotingehaltes zu diskutieren.

## Material und Methode

Die 3 Versuchssorten Havanna II c (Hav II), Havanna III 3 (Hav III) und Forchheimer Ogrodowy