erreichen. Die Maiszüchtung gibt uns hier ein Beispiel. Die vorstehende Arbeit sollte einen Weg für die Rapszüchtung aufzeigen und als Anregung für ähnliche Überlegungen bei anderen Kulturpflanzen dienen.

Zusammenfassung

Aufbauend auf der Erkenntnis, daß durch eine ausschließliche Berücksichtigung des Ölgehaltes in der Rapszüchtung eine Minderung der Ertragsleistung eintreten kann, wurden einzelne Ertragsfaktoren, ihre Variabilität und Vererbung sowie die korrelativen Bindungen dieser Faktoren mit dem Ölgehalt untersucht. Auf Grund der Ergebnisse wurden Zuchtverfahren zur Erreichung des Zuchtzieles „höchster Fettertrag" entwickelt und diskutiert.

1. Die Samengröße ist ein wesentlicher Ertragsfaktor. Sie ist vererbbar, wird jedoch durch Umwelteinflüsse stark modifiziert. Die genetisch bedingte Samengröße ist nur bei einheitlichem Reifegrad des Pflanzenmaterials erkennbar. Die potentielle Samengröße wird erst in der Vollreife erreicht (Tab. 1 u. 4).

2. Die Variationsbreite der Samengröße ist in den einzelnen Linien einer Sorte geringer als in der Sortenpopulation. Die Linien unterscheiden sich signifikant von der Sortenpopulation (Tab. 2). Die Variationsbreite der Samengröße in einem Sortiment ist etwa gleich groß wie die Variationsbreite der Linien einer Sorte (Tab. 3).

3. Samenertrag und Schotenzahl weisen eine sehr enge korrelative Bindung auf und können gemeinsam beurteilt werden (Tab. 5). Bei normalem Aussaattermin differenzieren sich die Pflanzen stärker in bezug auf diese beiden Merkmale als bei verspäteter Aussaat (Tab. 6).

4. Genetisch bedingte Unterschiede im Samenertrag und in der Schotenzahl sind an Einzelpflanzen nicht erkennbar. Es konnten keine signifikanten Beziehungen zwischen dem Samenertrag der Einzelpflanze und dem Samenertrag der Nachkommenschaften nachgewiesen werden.

5. Die mittlere Schotenlänge am Haupttrieb einer Pflanze ist repräsentativ für die mittlere Schotenlänge aller Schoten einer Pflanze. Die endgültige Schotenlänge ist bereits in der Grünreife erreicht. Signifikante Unterschiede in der Schotenlänge bei verschiedenen Linien einer Sorte konnten nachgewiesen werden (Tab. 7).

6. In den einzelnen Linien einer Sorte waren keine signifikanten Beziehungen zwischen der Schotenlänge und dem Ölgehalt oder der TKM nachweisbar (Tab. 8). Die Schotenlänge ist daher kein Vorauslesemerkmal für die Merkmale Ölgehalt und TKM.

7. Eine enge negative Beziehung zwischen dem Ölgehalt und der Samengröße konnte innerhalb der Linien einer Population und auch für die Linienmittel nachgewiesen werden. Gleichzeitig wurden innerhalb der Linien vorwiegend negative Beziehungen zwischen dem Ölgehalt und den übrigen Ertragsfaktoren festgestellt. Die Linienmittel variierten in bezug auf diese Merkmale jedoch unabhängig voneinander (Tab. 9, Abb. 1 u. 2).

8. Durch die Zusammenstellung der Linienanalysen und durch Sortenanalysen ist eine Einschätzung des züchterischen Wertes der Linien und Sorten möglich (Tab. 10, Abb. 5).

9. Auf Grund der gewonnenen Erkenntnisse werden die sich daraus ergebenden zuchtmethodischen Möglichkeiten diskutiert und ein optimales Neuzuchtschema entwickelt (Abb. 3 u. 4). Auf die allgemeine Bedeutung der gewonnenen Erkenntnisse für die Zuchtarbeit besonders bei Frembestäubern wird hingewiesen.

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Potential value of a dwarf mutant in breeding coumarin deficient forms of white sweet clover (*Melilotus albus*)

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With 9 Photographs

Introduction

Breeding white sweet clover (*M. albus*) form with a low coumarin content is based on: a) selecting natural mutants with a low coumarin content (BRINK, 1934; DWORAK, 1937; GOPLEN et al., 1956; GOPLEN, 1961; GREENSHIELDS, 1958; STEVENSON et al., 1940; STUCZYŃSKI et al., 1964; UFER, 1934); b) carrying out interspecific crosses with *M. dentatus* (gene controlling low coumarin present) (SMITH, 1948; BAENZINGER et al., 1958; RUDORF et al., 1958).

Utilization of natural mutants to obtain low coumarin forms seems the easier way to approach. Transference of the low coumarin gene from *M. dentatus* to *M. albus* entails considerable disruption of the genetic equilibrium which is a characteristic of distant crosses (SMITH, 1943, 1954). A whole cycle of back-crosses and repeated selection becomes necessary if forms possessing the low coumarin gene and a genetical make-up which can be utilized in a breeding program, are to be produced.

Success in the selection and stabilization of coumarin deficient forms of *M. albus* is dependent on the development of suitable methods for control testing the plant material. Both quick, easy methods (qualitative) useful in selection and precise quantitative methods for the determination of coumarin content, are important. Knowledge gained about the genetical mechanism governing the inheritance of this character decides the choice of suitable breeding program.

The presence of the low coumarin character in natural and in artificially induced mutants (MICKE, 1958; SCHEIBE, et al. 1958) is frequently associated with dwarf habit. The variety 'Alpha', described by STEVENSON (1937) as a dwarf branching mutant is a classical example here. A number of mutants similar to the 'Alpha' type have been described (CLARKE, 1931; HARTWIG, 1942; KIRK, 1931; STUCZYŃSKI, et al., 1964).

The present paper concerns the possibility of using another type of dwarf mutant, containing the low coumarin gene, in a breeding program for coumarin deficient forms of *M. albus*.

Methods

While holding a fellowship at the University of Wisconsin, Dept. of Genetics, Madison, Wisc., USA, in 1959, the author carried out crosses in the greenhouse between a dwarf form of *M. albus*, a natural mutant from prof. W. K. SMITH's collection and the variety 'Evergreen' with a normal growth habit. The form with the natural growth habit was used as the female parent. Four seeds were obtained from this cross and planted out in pots in the spring of 1960 in the greenhouse of the Dept. of Genetics and Plant Breeding of the College of Agriculture in Poznań. The plants obtained from these seeds showed a normal growth habit, grew much more vigorously than the mother form (Evergreen), flowered abundantly in the first year and gave a very good seed set after self-pollination (insect-proof of greenhouse). Samples of 100 seeds from each hybrid were planted out in boxes in the autumn. Segregation ratios were established 3 to 4 weeks after emergence. The coumarin content of the F_2 plants with a normal growth habit was gauged using the qualitative test of RUDORF and SCHWARZE (1958). Plants showing a low coumarin content were transplanted to pots and samples were taken from them for quantitative coumarin tests (the chemical analyses were carried out by the Chemistry Laboratory, Dept. of Fodder Plants, Institute of Soil Science and Cultivation of Plants at Gorzów Wlkp.). In the succeeding year the low coumarin plants were again self-pollinated. In the F_3 samples of 50 seeds were planted out in boxes. It was established which of the lines had become stabilized as to growth habit and in which lines segregation was still taking place. F_3 plants homozygous for growth habit and a low coumarin content were transplanted to pots and further reproduced in isolation. Seeds obtained from these plants (F_4) were planted out in boxes. They will be planted out under field conditions in the spring 1964, under spatial isolation but with possibilities for cross pollination between them. Seeds obtained from them will be used for field yield tests.

Results

a) Description of the parent forms

The *M. albus* variety Evergreen, developed by Ohio Exp. Sta., is late maturing. It matures two weeks later than other varieties of this species. It makes more rapid growth early in the spring of the second year. The plants grow abundantly but only a few thick stems are formed. The variety is valuable for its application in soil improvement because of the large amount of plant growth obtained under favourable conditions. Evergreen is characterized by a high coumarin content. The coumarin content of young but fully developed leaves is approximately 3.5% of the dry weight value (GORZ, 1961).

The dwarf form of *M. albus* here described, is a natural mutant, quite different from the dwarf types described as "dwarf branching", "dwarf spreading" or "dwarf bunch" (CLARKE, 1931; HARTWIG, 1942; KIRK, 1931; STEVENSON, 1937). The different growth habit is noticeable from the first stages of vegetation. Emergence is a few days later than in the plants used as standards. The hypocotyl is considerably shorter and the cotyledons are not pushed out above soil level in a characteristic fashion at emergence. Under

Fig. 1. Dwarf mutant form of *M. albus* in the first year of growth.

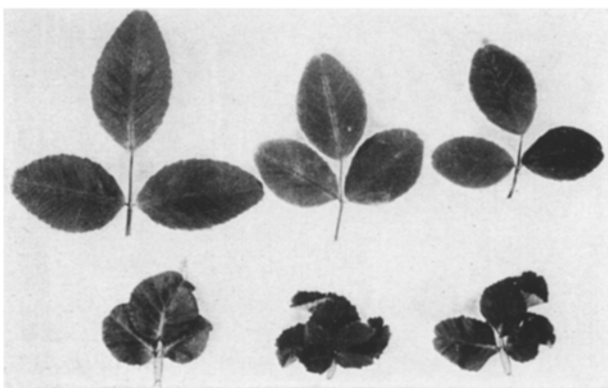

Fig. 2. *M. albus* leaves. Top row: leaves of plants with a normal growth habit; bottom row: leaves of dwarf mutant.

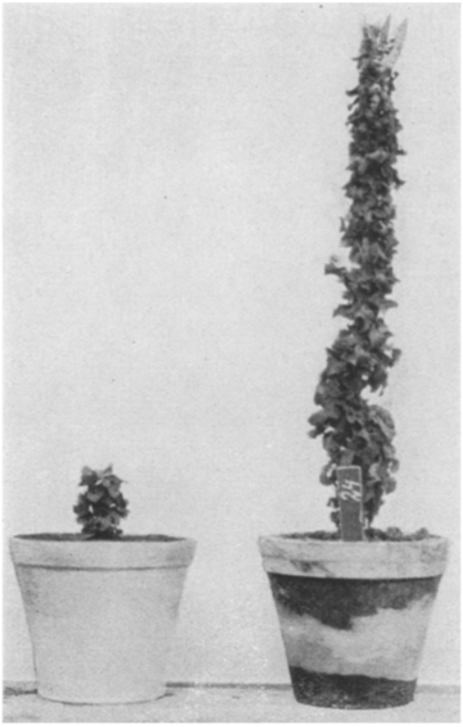

Fig. 3. Dwarf mutants of *M. albus*. First year of growth on the left; third year of growth on the rigth.

Fig. 4. Dwarf mutant inflorescences at full bloom and before flowering.

conditions of low light intensities (in late autumn and early spring) when the hypocotyl in plants with a normal growth habit is excessively elongated in the dwarf form a rosette of leaves is formed even a little below the soil surface. The initial slow and unusual development makes it possible to mistake the genus of these plants. In the first year of growth the dwarf type plants form a thick, much forshortened main stem without side-branches and with densely crowded leaves (Fig. 1). The leaves themselves are non-typical having shortened and thickened leaf and leaflet petioles and the leaflets are rounded in shape and frequently contain folds (Fig. 2). In the first year of growth the plants reach a height of 5—8 cm (Fig. 3). Under greenhouse conditions flowering took place in the third year of growth. Inflorescences develop at the tip of the stems only. They are short and crowded the flowers being very close together (Fig. 4). As a rule the dwarf plants do not branch. In isolated instances two equally developed, thick shoots may appear (Fig. 5). Flower parts are also shortened, particularly the sepals and petals. As a result developing buds open early (Fig. 6, 7). There are further differences in seed shape. Seeds are smaller, shorter and flattened (Fig. 8).

Cytological analyses showed the course of meiosis to be regular. There was no evidence of structural changes in the chromosomes (deletions, deficiencies). Hence it is of interest that even under self-pollination the fertility was good. The dwarf form contains traces of coumarin (Fig. 9).

Fig. 5. Dwarf mutant form with two branches. Third years' growth.

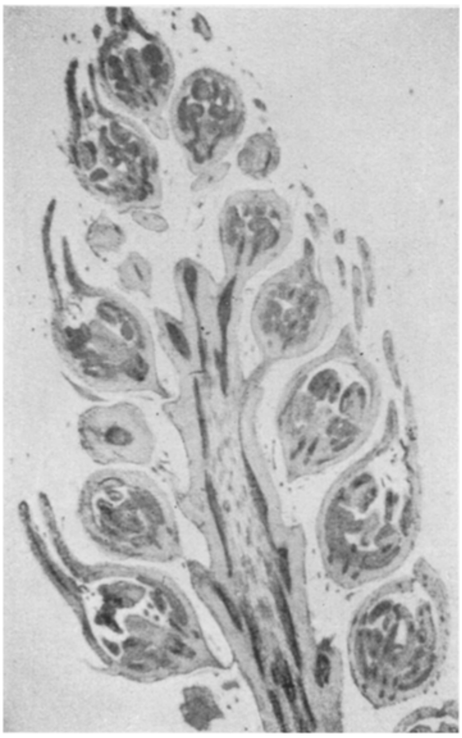

Fig. 6 and 7. Sections through inflorescences at the stage of bud development. — Fig. 6. Form with a normal growth habit. — Fig. 7. Dwarf mutant.

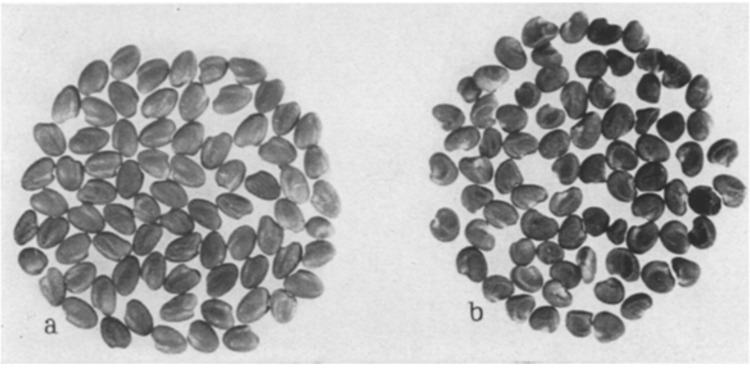

Fig. 8. Seeds. — a) form with normal growth habit var. 'Evergreen'; b) dwarf mutant.

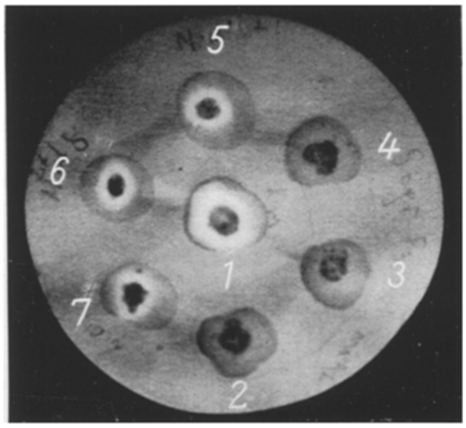

Fig. 9. Fresh leaf extracts with a drop of 10%NaOH under a fluorescent light. 1. *M. albus* var. 'Evergreen', 2. dwarf mutant; 3 & 4. low coumarin forms segregating out in the F_2; 5. 6 & 7. high coumarin forms segregating out in the F_2.

b) Description of hybrids of crosses: "normal growth habit" forms × dwarf types

Crosses between normal and dwarf forms are relatively successful. The form with the normal growth habit was used as the mother plant to assure easier and surer emasculation than it would be possible to achieve if the mutant had been used.

The four F_1 plants so obtained grew normally and were more vigorous than the mother form (the variety Evergreen employed in these crosses, had been selfed for 3 generations before use). In the F_2 generation obtained from self-pollination in the F_1 and originating from samples of 100 seeds from each hybrid plant, segregation ratios such as presented in Tab. 1 were obtained for the type of growth habit.

Table 1. *Type of growth habit segregation ratios in the F_2.*

Hybrid Plant No.	Number of plants: with normal growth habit	dwarfs
13/1	73	23
13/2	58	19
13/3	74	21
13/4	65	22
Total	270	85
Theoretical expected ratio 3:1	266	89 $\chi^2 = 0{,}24$

It may be seen that the observed ratio confirmed to the theoretical 3:1, one-factor ratio almost ideally, indicating that a single recessive gene governs dwarfness in the form described. The dwarf character is achieved in a definite way since the gene concerned causes the shortening of almost all the vegetative plant parts.

Some knowledge of the manner in which the ability to synthesize coumarin is inherited would be more important for future breeding work. A segregation into coumarin containing types and coumarin deficient types was established in the F_2 (Fig. 9). The method used for gauging the coumarin content was the RUDORF and SCHWARZE (1958) qualitative test as modified by the workers of the Institute of Soil Science and Cultivation of Plants at Gorzów Wlkp. (STUCZYŃSKA et al., 1963). Out of 270 plants with a normal growth habit there were 62 which could be classified as having a low coumarin content which is a segregation ratio very close to 3:1 ($\chi^2 = 0.658$). Quantitative tests showed that a plant coumarin content classed as "low" by the qualitative test varied between 0.1—0.001% on a dry weight basis. The last group may for all practical purposes be considered coumarin free. The frequency distribution of coumarin content in plants of the low coumarin content group is shown in Table 2.

Table 2. *Variation in the coumarin content (as a percent of dry weight) in plants segregating out in the low coumarin content group in the F_2*).*

Class mid points	0.10	0.08	0.06	0.04	0.02	0.009	0.008	0.006	0.004	0.002	0.001	n	$\bar{x}$
Frequency	11	7	3	4	4	5	6	3	5	9	5	62	0.0394

*) three replicate analyses were carried out for each plant

The above distribution provides little grounds for drawing conclusions. In general the variation in coumarin content seems to be considerable. It is impossible to decide whether this variation is caused by genes with a modifying influence or by sampling errors in the analyses. Nevertheless these variations in coumarin content are quantitatively within the limits characteristic for plants grouped together on the basis of a low coumarin content. The segregation is distinct and intermediate types were not observed.

Plants with a normal growth habit and a low coumarin content were further reproduced in isolation (in a greenhouse) as it was expected that some of them would be heterozygotes as to growth habit. The progeny of some of these plants were found to segregate into normally growing types and dwarfs while the progeny of others were found to be homozygotes as to growth habit. There was no further segregation as to coumarin content in the F_3 i. e. a low coumarin content had become genetically stabilized.

The results indicate that the dwarf mutant type of *M. albus* described, is a double recessive mutation. The mutation took place in genes at two non-linked loci hence the law of independent assortment holds good.

Discussion

In breeding for forms with a low coumarin (level) content in *M. albus* it is of fundamental importance: a) whether the lack or presence of coumarin is genetically controlled; b) what the mechanism of inheritance is once it is known to exist.

While seeking forms with a low coumarin content many plant breeders have found the coumarin content to vary with the year, phase of vegetation, stage of leaf development etc. Even forms which had been selected for their low coumarin content, have often been found to revert to the "bitter" type in the succeeding year (UFER, 1934). A more exact genetical analysis was made more difficult by the lack of a plant which would be used as a coumarin free standard. The analytical techniques available contributed additional difficulties. The fluorescence test for coumarin (UFER, 1939) was not specific enough since it concerned a whole complex of fluorescing organic substances. This was later brought out by studies which employed paper chromatography, electrophoresis and fluorometry.

It was not until the work on the hybrid of the *M. albus* × *M. dentatus* cross (SMITH, 1948) that it was clearly shown that there is a genetical mechanism in control of coumarin synthesis. The more detailed studies of SMITH (1948), GOPLEN et al. (1957), on the progeny of diallelic crosses of the following forms:

Arctic — containing free coumarin
Pioneer — containing bound coumarin
(*M. albus* × *M. dentatus*) — no coumarin,

show that at least two genes govern coumarin synthesis. *Cu* (coumarin present) and *cu* (no coumarin) control its presence. The second gene would be responsible for the kind of coumarin found in the plants e. g. a *B* genotype would result in the presence of free coumarin while the *bb* genotype would lead to the formation of bound coumarin.

RUDORF and SCHWARZE (1958) proposed a somewhat different hypothesis. They suggested that two recessive genes cu_1, cu_2 lower the coumarin content but differ in the extent of their action, the gene cu_1 making the content 1/10 lower than the initial value and the gene cu_2 making the content 1/100 lower than the initial value.

HASKINS and GORZ (1957) proposed a further hypothesis as a result of the work with the interspecific hybrid *M. albus* × *M. dentatus*. According to this hypothesis the gene *cu* in a homozygous condition transferred from *M. dentatus* to *M. albus* caused a marked lowering of the coumarin content. When in a heterozygous condition (*Cu cu*) the same gene causes an intermediate coumarin content. The gene *b* from the form Pioneer changes free coumarin to bound coumarin when in a homozygous condition (*bb*).

The above few examples show that opinions as to the manner of inheritance of the ability to synthesize small quantities of coumarin are not very well defined and change as the methods available improve. The terms free and bound coumarin used to be employed in the literature. More recent work has shown their use to be of doubtful value. According to SCHÖN (1961) and HASKINS (1961) who used paper chromatography "bitter" substances always occur as glucoside of the cis-o-cinnamic acid. Free coumarin is an artefact arising during the process of extraction. It is sufficient to inactivate β-glucosidase in the plant tissues before extraction not to find free coumarin.

Our own investigations indicate that a low coumarin content is more likely to be governed by a single recessive gene. Although there is a certain amount of variation in coumarin content among the plants which have the low coumarin gene it is not of the order which would give rise to difficulties when a plant form is to be classified as having a low coumarin content. In order to decide whether one or, as suggested by RUDORF and SCHWARZE (1958), two genes are active in this case, additional, perceptive genetical analyses would have to be carried out aided by highly sensitive methods of testing for coumarin content. These however, do not seem to be of primary importance for the planning of future breeding programs.

Natural dwarf mutant forms of *M. albus* occur rather frequently. The bushy form with delicate, leafy shoots is best known — variety 'Alpha'. STEVENSON (1937) established it to be a single recessive mutation of the normal growth type. He described it as a "dwarf branching" form. CLARKE (1931) studied a similar dwarf mutant and described it as "dwarf bunch". This too was a single recessive. When these two forms are crossed the F_1 progeny have the normal growth habit. The segregation taking place in the F_2 was interpreted as a 9:6:1 ratio with the double recessive being lethal. The two recessive genes active in this cross were described as *bd* (branched dwarf) and *sd* (spreading dwarf). In later years a number of other dwarf branching forms were isolated, which differed somewhat from the 'Alpha' type in their growth habit but they continued to be referred to as "dwarf branching" for convenience.

HARTWIG (1942) crossed a number of different dwarf forms and his results suggested that several non-allelomorphic genes are responsible for the dwarf branching character in common biennial white blossomed sweet clover. Four such genes are known and they have been named d_1, d_2, d_3, d_4.

The mutant used in our crosses differs from the forms so far described in its growth habit. It has been established that it is a single recessive of the normal growth type but it is still difficult to draw further conclusions about the mutated gene. It is planned to carry out a cross between this mutant and the Gorzów mutant, which is of the 'Alpha' type (STUCZYŃSKI et al. 1964), in the near future so as to gain further information concerning the genetical character of these two mutations.

Quite apart from any further studies of a more theoretical character, the finding that two different recessive genes, which segregate independently, are responsible for a low coumarin content and growth habit, is useful for further breeding work with the low coumarin *M. albus*. This gene can be transferred to vigorous forms with a luxuriant growth habit by simple crossing, so giving rise to valuable initial breeding material. The task here presented is easier and simpler than the one encountered when the low coumarin gene is transferred from wild species. There are no genetical abnormalities which cause lowered fertility or lowered vitality in the progeny.

It is at present difficult to evaluate the possible practical advantages of the low coumarin plants with a normal growth habit which were selected as they segregated out. The hybrids have been inbred for 3 generations (self pollinated). In plants which are natural cross pollinators this may result in a certain depression in vitality. A proper evaluation of the practical usefulness of this plant (yields under field conditions) will only be possible when seeds from cross-pollinations between coumarin deficient plants are obtained.

Summary

1. Crosses were carried out between a *Melilotus albus* variety with a normal growth habit and a natural dwarf mutant form.

2. The dwarf mutant is of no value for breeding since it has a completely changed developmental physiology and growth habit. It is of value in that coumarin is present in trace amounts only.

3. The segregation ratios found in the F_2 and in further generations showed that the dwarf form is a double recessive. Two genes at two non-linked loci are mutated.

4. Progeny of the above cross were self-pollinated in consecutive generations and tested for their coumarin content. A number of lines were established which are characterized by a low coumarin content and a normal growth habit. They will be subject to further breeding work.

Zusammenfassung

1. Zwischen einer Sorte von *Melilotus albus* mit normalem Wuchshabitus und einer spontanen Zwergmutante wurden Kreuzungen durchgeführt.

2. Für Züchtungszwecke ist diese Mutante infolge ihrer völlig veränderten Entwicklungsphysiologie und ihrer Wuchsform zwar wertlos, sie weist aber einen äußerst geringen Cumaringehalt auf.

3. Nach dem Spaltungsverhältnis in der F_2 und den folgenden Generationen ist die Zwergmutante doppelt rezessiv, 2 nicht gekoppelte Gene sind mutiert.

4. Die Nachkommenschaft der obigen Kreuzung wurde mehrere Generationen lang selbstbestäubt und auf ihren Cumaringehalt untersucht. Es wurde eine Anzahl Linien mit niedrigem Cumaringehalt und normalem Wuchshabitus gefunden, die das Ausgangsmaterial für weitere Züchtungsarbeiten bilden werden.

Acknowledgement

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KURZE MITTEILUNG

Studien über die durch Radioaktivität hervorgerufene Verunreinigung bei landwirtschaftlichen Erzeugnissen und Lebensmitteln bei Euratom

Die Europäische Atomgemeinschaft (Euratom), Brüssel, hat für die nächsten zehn Jahre gemeinsame wissenschaftliche, statistische und bibliographische Studien über die Bestimmung der durch Radionuklide hervorgerufenen Verunreinigung bei Lebensmitteln beschlossen. Sie hat eine Beratergruppe berufen, die unter Leitung von Dr. Jammet vom Atomzentrum in Paris steht.

Dieser Arbeitsstab besteht aus zwei französischen, zwei italienischen, zwei deutschen, einem belgischen, einem luxemburgischen und einem niederländischen Experten, die jährlich zu Sitzungen zusammentreten und die Funktion einer wissenschaftlichen Beratergruppe wahrnehmen.

In diesen Arbeitsstab wurden aus der Deutschen Bundesrepublik Professor D. Kuprianoff, Bundesforschungsanstalt für Lebensmittelfrischhaltung Karlsruhe, und Professor P. F. Pelshenke, Bundesforschungsanstalt für Getreideverarbeitung Detmold, berufen.

Die Aufgabe dieser Beratergruppe besteht darin, die Verseuchung unserer Lebensmittel durch Radioaktivität durch wissenschaftliche Studien zu verfolgen, einheitliche Sicherheitsnormen für den Gesundheitsschutz der Bevölkerung und der Arbeitskräfte aufzustellen und die Erfahrungen in den einzelnen Ländern der Europäischen Gemeinschaft auszutauschen.