Mutation of florets and inflorescences in *Melilotus* sp.

J. K. JARANOWSKI

Institute of Genetics and Plant Breeding. College of Agriculture, Poznań, ul. Wojska Polskiego 71c

(Received: March 3, 1972.)

Abstract

A mutant of melilot (*Melilotus* sp.) consisting in the formation of altered florets and inflorescences is analysed and described. The genetic background and inheritance of the changed characters are discussed.

INTRODUCTION

In genetic and breeding studies of the group of leguminous plants in the Institute of Genetics and Plant Breeding of the Poznań, College of Agriculture, an interesting case has been noted of deviations in the floret and inflorescence structure in melilot (*Melilotus* sp.). These changes are mutational in character, therefore they seem rather interesting from the botanical point of view.

PLANT MATERIAL AND METHODS

In 1966 crosses were performed with a complex hybrid [(*Melilotus albus* × *Melilotus officinalis*) × *Melilotus albus* f. *annua*] as mother plant and *Trigonella grandiflora* as paternal plant. The cross was carried out with previously bred tetraploids of these forms. Three seeds were obtained from which only one germinated. The seedling, and later the plant grew rapidly and flowered early. Its seeds sown in October in the glasshouse flowered as early as the beginning of February, whereas the controls (maternal type) under the same vegetation conditions did not flower until the beginning of April. The plant obtained from the cross had a distinctly changed structure of florets and inflorescences. By self-pollination the material was bred up to the fifth generation. Back-crosses were also performed.

The results of observation and investigations of a population of 50 plants from the 4th generation are reported here.

During the entire vegetation period the plants grew in the glasshouse. They were seeded in autumn. In the vegetation period the morphological traits of the plants, the beginning of bud setting and flowering were noted. During full bloom the inflorescences were examined and described in detail. At the same time analysis of pollen grains was performed with particular reference to their viability (ability of staining) and morphology (size and shape).

For detailed analysis of the flower structure, entire inflorescences from particular plants with flowers at various development stages were fixed. Morphological observations of the flower structure were performed with a stereoscopic microscope.

From some florets permanent preparations were made by the routine paraffin technique.

RESULTS

The further generations of the plants investigated continued to grow rapidly and flower early. As regards the latter character, however, they varied widely. The beginning of bud setting varied from Jan. 30 to May 10. Mostly dates earlier than in the controls prevailed. The time between bud setting and beginning of flowering varied in the particular plants from 19 to 58 days. Mostly it lasted around 30 days, it was longer only in 7 plants. The correlation between the length of this period and the complexity of the inflorescences was rather regular. Plants with a very complex inflorescence structure showed a longer time interval between bud setting and flowering.

The plants grew very vigorously, reaching a height of 2 m, they branched and flowered profusely. Flowering lasted over the entire spring-summer season until late autumn. The flowers were pale cream-coloured, owing to the use in the crosses of *M. officinalis* with yellow flowers.

The plants, beside vigorous growth and early flowering, had morphological characters quite similar to those of the "mother" melilot form.

The main traits analysed in the plants were the inflorescences and florets. Since the plants resembled melilot, the florets of this maternal plant were assumed as reference.

The inflorescence of melilot is a raceme. Normally it is monopodial with a distinct main axis. The lateral axes of first order are the flower pedicels with single florets at their tips. The florets are typical in shape for *Papilionaceae*. The flower formula is as follows

$$K(5)C5A(9)+1G1$$

The hybrid plant had a changed inflorescence. It was a raceme with branchings of 2nd, 3rd and higher orders. The florets were also complex. Seeds obtained by selfpollination gave in the subsequent generation plants segregating distinctly as regards the structure of florets and

Photo 1. Inflorescences of type I. On the left normal (control) inflorescence

inflorescences. This segregation was also noted in the population subjected to analysis.

Three basic types of inflorescences were distinguished. Within each type a certain variability occurred so that subtypes could be established. Their classification is as follows:

Type I. Inflorescence as a single raceme (Photo 1)

Subtype 1 — single florets, normal,

2 — several florets in lower part of inflorescence complex, the remaining ones normal,

3 — complex flowers up to 2/3 of inflorescence, apical ones normal,

4 — all florets on the entire length of axis complex.

Type II. Inflorescences — complex racemes (Photo 2).

- Subtype 1 — lateral axes of 2nd order in lower part of inflorescence, in middle part complex florets, in apical part single ones,
- 2 — lateral axes of 2nd order in lower part of inflorescence, remaining florets complex,
- 3 — lateral axes of 2nd order up to $2/3$ of inflorescence, in apical part complex florets.
- 4 — lateral axes of 2nd order on the entire length of inflorescence,
- 5 — in lower and middle part of inflorescence lateral axes of 3rd and higher orders.

In this type of inflorescence additional irregularities appeared, the flower buds withered in various parts of the inflorescence (Photo 3).

Type III. Inflorescence — racemes not developing flowers (Photos 4, 5).

In this type the inflorescences developed, but were deformed, the flower buds were poorly developed, they remained green and did not open. The inflorescences after reaching this stage of development did not change and remained in this form on the plant frequently for more than six weeks. They remained fresh and green as long as did cut shoots in vases (in this form they were rather decorative). Plants with this type of inflorescences resembled in appearance *Chenopodiaceae* (e.g. *Beta* sp.).

The plants with changed inflorescence showed a very wide scale of variability in the structure of the particular florets (Tab. 1, 2). Beside

Table 1

Structure of 30 analysed florets from type I inflorescences

Complexity of florets	Number of florets	Percent	Number of		
			petals	anthers	pistils
Single (normal)	7	23,3	5	10	1
Single (changed)	7	23,3	6	8—10	1
Double	9	30,0	9—18	9—18	1—2
Triple	4	13,5	12—17	15—27	3
Quadruple	2	6,6	25—30	35—40	4
Quintuple	1	3,3	22	35	3

Photo 2. Inflorescences of type II. On the left normal (control) inflorescence

Photo 3. Inflorescences of type II with partially aborted florets. On the left normal (control) inflorescence

Photo 4. Inflorescences of type III. First on the left normal (control) inflorescence

Photo 5. Comparison of inflorescences of II and III type at time of flowering. From left: normal (control), two of II type and the remaining three of III type

Table 2

Structure of 30 analysed florets from type II inflorescences

Complexity of florets	Number of florets	Percent	Number of		
			petals	anthers	pistils
Single	2	6,7	6—7	11	1
Double	8	26,7	7—16	7—16	1—2
Triple	4	13,3	7—13	8—16	1—2
Quadruple	4	13,3	21—28	12—26	2—4
Quintuple	4	13,3	28—44	19—36	2—5
Sixtuple	2	6,7	34—39	32—41	5
Septuple	3	10,0	18—41	16—33	2—5
Octuple	3	10,0	35—48	31—36	4—5

single ones of almost normal shape, modified single ones were seen, with a changed number of petals in the corolla and sepals, up to very composite florets where there were several flowers on one pedicel (Photo 6).

Analysis of the cross sections of fixed florets demonstrated a high viability of the pollen grains and visualised the structure of the ovules. In normal pistils there were on the average three ovules attached to the ovary wall, whereas in the changed pistils 1—4 ovules were present, frequently changed in shape.

On the crosssections of normal florets the differentiation of the tissues of the gynoecium and androecium, corolla, petals and sepals was distinctly visible. In the modified florets, the histological structure of these organs was strikingly similar. This seems to indicate their homogenous origin and confirms the supposition that the quantitative changes in the constituents of the flower are the result of transformation of certain organs into others.

The flowers from type III inflorescences had a completely different structure. The anatomical sections showed that the particular florets grew on shortened pedicels, surrounded by reduced sepals. From the receptacle, primordia of 2nd order pedicels grew, ending in undifferentiated protuberances of homogenous cells (Photos 7, 8). Further development and differentiation which would have probably led to the formation of highly complex florets was blocked. These florets or rather flower buds did not develop further, they remained in this stage for a very long time (6—8 weeks) and then died off gradually.

Beside the changes in the structure of the florets, a high variability was noted in the length of the entire inflorescences. The mean lengths of their main axes varied within the limits of 10.0 to 32.5 cm, and of the inflorescences proper from 8.0 to 24.0 cm.

The fertility of the plants with modified inflorescences and florets was markedly lower than that of controls. The causes of this lowered fertility may lie in the different structure of the florets. Cytological analysis, particularly of the course of meiosis in the process of microsporogenesis, did not show any definite disturbances. The type of chromosome conjugation was characteristic for artificial polyploids, that means that the bi- and tetravalent sets as well as the anaphasal separation were normal in most cases. This resulted in an exceptionally high pollen grain viability for tetraploids, reaching values above 90 per cent. It was lower only in four plants. The shape and size of the pollen grains were typical for the tetraploid maternal outset form. The pollen grain viability and other morphological characters were not correlated with the complexity of the floret and inflorescence structure. It was only observed that highly composite florets produced less pollen grains.

The modifications in the floret structure produced the phenomenon of herkogamy making fertilization difficult in the absence of insects. The failure of the two petals to unite forming the typical keel which encloses the gynoecium and androecium resulted in an open-type flower so that the stamina did not contact the stigmata or else the absence of the "explosion" phenomenon did not let the pollen grains reach the stigmata. Artificial placing of pollen grains on the stigmata or tripping of the inflorescences gave good results and stimulated the

Photo 6. Complexity of floret structure and changed side axes of the inflorescences.
Upper left — five normal (control) florets

Photos 7. 8. Median longitudinal section of florets (florete buds?) of the type III inflorescence

setting of pods and seeds. It is probable that under conditions of panmixia when fertilizing insects have access to the plants, pod setting would be more profuse. In the present experiments, however, inbreeding was indispensable.

DISCUSSION

The normal structure of flowers characteristic for definite taxons is conditioned by heredity. The development of flowers and flowering result, on the other hand, from physiological processes, frequently modified by habitat conditions.

The cases of disturbances or deviations in the flower structure described in the literature concern mostly hybrids from wide crosses

Fig. 1. Structure alternation of calyx (a), standard (b), wings (c), keel (d), pistil (e).
First from the left normal (control) organs

(Gajewski 1953; Kazimierski 1960, 1961) or induction of mutation (Monti et al. 1969). These authors suggest that changes in the structure of flowers of wide hybrids may be the result of disturbances in the production of some specific substances which arise in various growth zones. This argumentation is logical. The cooperation of two different genomes in hybrids may actually lead to disturbances in physiological processes, and, consequently, to definite phenotype alternations in the flower structure or in the processes of flowering. At any rate, the disturbances observed in hybrids were attributed to one common cause that is abnormal production, transport, activation and desactivation of growth substances. The conclusion, on the other hand, that these disturbances may result from some chromosome deficiency in the hybrid organism is subject to discussion. The studies of Kazimierski, for instance, (1960, 1961) give, namely, no information on the behaviour of plants grown from seeds formed in the changed flowers. It is, therefore, difficult to say whether these changes were hereditary. Teratologic changes of part of the flowers may frequently be also due to viruses.

The disturbances in the structure of flowers and inflorescences which are the subject of the present study are most probably not the result of wide crosses. The outset (maternal) plant originated it is true, from an intergeneric cross, but it was not a hybrid. The forms used for crossing (melilot and trigonella) differ markedly in morphology and development, and it is hard to imagine that, in the case of a hybrid, segregation, in a narrow scope at least, or certain intermediate states would not occur in further generations. All the plants of the subsequent generation had morphological traits typical for the "maternal" form, that is melilot. The karyological similarity of the components used for the crosses consisted in the same basic chromosome number ($n=8$). The forms used for the crosses were tetraploid, thus, they had a somatic chromosome number $4n=32$. It is further improbable that the chromosome homology of these species belonging to different genera would be so high that it would result in normal meioses and pollen grain viability as high as that noted in the descendants. These criteria eliminate the hybrid character of the plants examined. Moreover, attempts of back-crosses to trigonella did not give any results, whereas with the maternal melilot form seeds could easily be produced.

The melilot form used for the crosses was not an autotetraploid but an allopolyploid. It was obtained by crossing at the tetraploid level according to the scheme: (*M. albus* $\times$ *M. officinalis*) $\times$ *M. albus* f. *annua*. The progeny of these crosses did not exhibit in several successive generations any deviations in the structure of flowers and inflorescences.

It may, therefore, be assumed that pollination with *Trigonella grandiflora* pollen was the factor inducing some mutation. Most probably,

the seeds obtained were the result of diploid parthenogenesis. Possibly, the disturbances in meiosis during macrospore formation, which resulted in a diplospore, caused mutation in one of the chromosomes. The absence of definite meiotic configurations rules out changes in larger chromosome segments. Point mutation is also but little probable, since alternation involves, beside changes in the flower and inflorescence structure, also certain developmental processes as for instance earliness.

This hypothesis agrees well with the phenomena observed. In four successive generations derived from the "hybrid" plant, definite segregation to plants with changed inflorescences and florets took place. This segregation was repetitive, since there always appeared inflorescences which could be classified to the three basic types.

If this was case of mutation, it should be explained why this mutation is of dominant character, and where lies the source of variability in the segregates of the successive generations, that is, beginning with plants with normal inflorescences and flowers, over various degrees of deviation up to extreme cases of suppressed flowering.

This mutation, as is mostly the case, is a defect as compared with the normal evolutionally conditioned situation. Presumably, a part of the genetic information responsible for biochemical-physiological processes leading to normal processes of flowering and inflorescence and flower development, was changed. There must exist a determined quantitative balance of alleles conditioning this process. Hence, alternation of even one allele is not compensated by the presence of normal homologues. Consequently, the phenomenon of alternation of the given trait, appears predominantly. The variability of the segregant types in the successive generations may be conditioned by the tetrasomic type of segregation. The outset plant which had a changed inflorescence was probably a heterozygote in the *triplex* system. Owing to inbreeding, genetic systems of the type of heterozygotes *duplex* and *simplex* and of homozygotes *quadruplex* and *nulliplex* appeared probably in combined chromosome-chromatid segregation. In the light of this, the observed segregation types were conditioned: plants with normal flowers and inflorescences — a return to the normal *quadruplex* system; plants with single but changed flowers — a heterozygote in the *triplex* system; plants of type II with inflorescences in the form of complex racemes (panicles) flowering normally — a heterozygote in the *duplex* system. Plants with complex inflorescences with dying off flower buds in various parts of the inflorescence — a heterozygote in the *simplex* system, and finally the group of plants with changed inflorescences, but with blocked differentiation of parts of the perianth, in which flowering was suppressed — a homozygote in the *nulliplex* system. The quantitative relations of the segregants in the successive generations seem to support these hypothetic considerations. The lack of full agreement is due to the

fact that the generations were recorded according to the population and not the pedigree principle, because the material and the elimination in each generation of definite types (to quote selection as the result of lack of flowering) did not allow the former treatment.

Deviations in the flower structure of not modifying but mutational type have been earlier reported by some authors. Monti and Devreux (1969), after treating pea seeds with ethyl disulphate, obtained mutation of flowers which they described as "*stamina pistillodia*". It consisted in a partial change of the androecium into gynoecium. Genetic analysis demonstrated that it was a monogenic and recessive mutation.

Another mutation has been reported in detail in *Lupinus angustifolius* described as "*polypistillia*" (Jaranowski, 1972). It was isolated in the line of a remote generation of the cross *Lupinus angustifolius* × *Lupinus linifolius* (acc. to botanical systematics separate species, genetically, hybrids give the Mendel segregation). The changes in floret structure consisted in the formation of additional carpels which caused the development of 3—7 pistillate flowers, the number of the other perianth parts remaining unchanged. Genetic tests demonstrated a typically monomeric recessive conditioning of this change.

The deviations in the flower and inflorescence structure here described are certainly conditioned by a definite physiological process in ontogenesis (Monti et al. 1969; Heslop-Harrison, quoted after Monti, 1969; Listowski, 1970; Wardlaw, quoted after Listowski, 1970; Lubimowa, 1958; Gajewski, 1953). This is, however, a different problem worth studying in further investigations.

The mutation which was the subject of the present study has no major significance in practical breeding. Traits such as earliness, vigour, profuse flowering would seem favourable. A basic shortcoming, however, is the difficulty or even impossibility of genetic stabilisation. The favourable phenotypes appear only in heterozygotic condition, and even here with a high dose of variability as regards the tetrasomic type of heredity. Homozygotic systems lose these characters, and the type *nulliplex* homozygote has no breeding value since it does not flower. This mutation, however, is interesting from the botanical and evolutionary point of view, and if it is subjected to investigations, it may bring the elucidation of a number of interesting processes as regards plant development physiology.

REFERENCES

- Gajewski W., 1953. Trochę obserwacji nad zaburzeniami w rozwoju kwiatów u *Geum* L., Acta Soc. Bot. Pol. 22: 587—604.
- Jaranowski J., 1972, Polypistilly in *Lupinus angustifolius* L., Phytomorphology (in press).
- Kazimierski T., 1960, An interspecific hybrid in the genus *Lupinus* (*L. albus* × *L. jugoslavicus*). Abnormalities in flower structure, Gen. Pol. 1(1): 16—24.
- Kazimierski T., 1961, Certain variations in flower structure of interspecific hybrids of *Lupinus*, Gen. Pol. 2(1): 119—127.
- Lubimova W. F., 1958, O nasledovanii teratologicheskikh izmienienii u pshe-nichnopirejnikh gibridov, Biul. Gław. Bot. Sada 32: 53—59.
- Listowski A., 1970, O rozwoju roślin, PWRiL, Warszawa.
- Monti L. M., Devreux M., 1969, Stamina pistillodia. A new mutation induced in pea, TAG. 39—1.
- Strasburger E., 1967, Botanika, PWRiL, Warszawa.

Mutacja kwiatków i kwiatostanów u Melilotus sp.

Streszczenie

Przedmiotem badań była populacja 50 roślin, stanowiąca czwarte pokolenie generatywne pochodzące od jednej rośliny uzyskanej w wyniku zapylania [(*Melilotus albus* 4x × *Melilotus officinalis* 4x) × *Melilotus albus* f. *annua* 4x] × *Trigonella grandiflora* 4x. Rośliny charakteryzowały się zdecydowaną zmianą budowy kwiatów i kwiatostanów. Wyróżniono trzy typy kwiatostanów: typ I — kwiatostany w postaci pojedynczego grona; typ II — kwiatostany w postaci grona złożonego; typ III — kwiatostany w postaci grona złożonego, nie zakwitające. Poza zmianą struktury kwiatostanów wystąpiły poważne zmiany w budowie kwiatków, powodując ich skomplikowaną złożoność. Testy cytologiczne (przebieg mejozy i żywotność ziarn pyłku) oraz genetyczne (brak segregacji) wykluczają mieszańcowy charakter badanych roślin. Przyjmuje się, że zapylanie pyłkiem *Trigonella grandiflora* stymulowało partenogenezę diploidalną. Zaburzenia w makrosporogenezie, doprowadzające do powstania diplospory spowodowały mutację, której efektem były alternacje w budowie kwiatów i kwiatostanów. Może to być mutacja zarówno strukturalna, jak i punktowa. Postuluje się raczej mutację „obszerną”, ale nie powodującą charakterystycznych konfiguracji meiotycznych. Segregacja w kolejnych generacjach trzech typów kwiatostanowych jest wynikiem tetrasomicznych założeń dziedzicznych. Układy heterozygotyczne warunkują zmienne typy kwiatostanów i kwiatów, natomiast homozygotyczne w układzie *quadruplex* kwiaty i kwiatostany normalne, natomiast w układzie *nulliplex* kwiatostany bardzo złożone z blokadą różnicowania się części kwiatków (nie kwitnące).