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Papaver somniferum — achievements, incertitudes, expectations

ION I. BĂRĂ*, ECKARD WELIMANN**

* Statiunea de Cercetari „Stejarul", Str. Alexandru cel Bun 6, 5600 Piatra Neamt, România
** Institut Biologie II, Schanzlestrasse 1, 7800 Freiburg, Federal Republic of Germany

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ABSTRACT

The poppy (*Papaver somniferum* L.) has had in the past, has now and will have in the future a special position among medicinal plants. It is of increasing scientific interest due to its utility, aesthetic, alimentary and pharmaceutical values. At present we possess much information on this plant from the fields of biochemistry, morphology, genetics and pharmacy.

Key words: Papaver somniferum

INTRODUCTION

The existence of the human species, from its beginning up to the present, represents a continuum of experiences, discoveries and projects. Life in the midst of often hostile nature, forced the human being to learn its enigmas, adversities and resources, its useful parts which could serve as his food, for the treatment of diseases, the adornment of his house and even of himself.

In this confrontation and cooperation between man and nature going on for thousands of years, medicinal plants have taken on one of the most important roles. Among them, the poppy (*Papaver somniferum* L.) has had, has now and certainly will have in the future, a special position bringing together the most different interests and preoccupations. Man's interests regarding the poppy have a decorative, aesthetic, alimentary and pharmaceutical nature (Duke 1973); evidence of its uses date from the old neolithic days, from Niederwil (Switzerland). Since the poppy has not only a historical meaning, but also can be looked upon as a plant of the future, we consider it useful to present a review of the research done on it world-wide. In this way, the main results obtained up until now can

serve both for future investigations and to prevent unnecessary financial and intellectual effort being spent on resolving solved problems.

VARIABILITY

A summary on *P. bracteatum* (Nyman and Bruhn 1979) has already been published and our present work is another contribution to the biology of the *Papaver* genus. Although this plant is derived from a small area in the west of Mediteranea (Schultze-Motel 1979), the poppy has expanded over a large area, now being cultivated in Asia, Asia Minor and Europe (Goina et al. 1967).

The many thousand-year long „agricultural history" of this species displays itself in the multitude of varieties found in culture in different parts of the world. This reflects the effect of prolonged artificial selection, unconscious at the beginning, conscious and scientific later. The opinion is held that the individual variability within the species has a high amplitude — this includes morphological (Kaicker at al. 1975), physiological and biochemical (Nyman and Hall 1976, Nyman 1980a, b) as well as cytogenetical characteristics (Bără and Floria 1979, Bără et al. 1981).

There are differences both between variaties and among individuals of the same variety in regards to their length, dimensions of the capsules and number of branches, quantity of seeds, oil and alkaloid contents. In some cases there are correlations between opium production and capsule weight, opium content and precocity or leaf number, plant length, capsule number, number of stigmatic rays, quantity of dry substance etc. (Khanna and Singh 1975, Deneva 1975).

Natural mutants have been discovered, which are characterized by a small opium production but produce thebaine. These mutants, determined by a simple gene, have a red latex (Nyman 1980a).

The early introduction of the poppy into culture brought about the necessity of knowing such aspects of the species biology as the process of seed germination and the most favorable conditions for seed germination and sprouting. Research done on this question has pointed out that the range of optimum temperatures for germination is quite large, between 13-33 C (Bare et al. 1978).

Of course, the main use of the species has remained in the phytopharmaceutic field. This is why most of the observations and experimental data have been gathered regarding the conditions that favor high alkaloid production and regarding the structures and mechanisms that play an important role in opium production and accumulation.

No correlations have been found between alkaloid synthesis and meristematic activity but some have been found between morphine quantity and

the plant part. The stamens of closed flowers contain a high quantity of alkaloids (Sárkány et al. 1974).

The environmental conditions at harvesting and plant age also have a great influence on the opium quantity (Tookey et al. 1975). If plants are harvested 29-33 days after anthesis, approximately 6.7 kg opium per ha can be obtained, whereas if they are harvested 4-6 days after anthesis the opium quantity is much smaller, about 5.36 kg per ha.

Sometimes, no correlations have been found between opium production (quantity) and average temperatures or rainfall (Ohno and Kinoshita 1977). On the other hand, the opium content is smaller if the average temprature is high during the anthesis. Regarding this aspect, there are data showing that early varieties are more sensitive to the environment than late ones. Some poppy varieties obtained from different parts of the world are adapted to those environmental conditions and to no others. For example, Khanna (1975) cultivated under similar conditions in Lucknow (India), 190 poppy varieties from Europe together with Indian varieties and noticed that the European varieties needed a longer photoperiod. Similar observations made on the Ikkansu (Japan) poppy variety, pointed out that the quantity of opium is high if the water quantity is low and high temperatures prevail during anthesis and capsule formation.

The poppy photosensitivity was noticed on other occasions (Gentner et al. 1975). They established that the poppy needs 14-16 hours daily for the flowering phase which is induced by 2 or more long photoperiods or by a longer illumination period of 24 hours. There is also a close correlation between the opium production and the mineral nutrition of *P. somniferum* (Costes et al. 1976).

The NO_3^- anion proved to be the most efficient in production of fresh or dry substance and in the increase of morphine content, while NH_4^+ with urea have a depressive effect. Phosphates stimulate flower proliferation and fruit growth, but not the opium quantity.

Mg^{2+} and Ca^{2+} are important factors. Lack of deficiency of Mg^{2+} effects the stem length and flowering but not the morphine quantity. Ca^{2+} deficiency causes a drop in the alkaloid content, while its excess increases the plant length and capsule number. Na^+ favors plant growth and morphine output.

Addition of a micro-nutrient mixture increases opium yield per hectare (Ramanathan 1979). Studies which intended to examine the interdependence between phenotype, genotype and environment (Khanna and Singh 1975) pointed out that the differences in the opium content between species are genetically determined (Kaicker et al. 1978).

A high phenotypic variability of *P. somniferum* has been noticed, the steadiest character being the capsule number per plant. A high inheritability of leaf form has also been found. In the inheritance of opium content, the genes have a non-additive effect, while in oil-content inheritance, they

show an additive action (Hlavacková 1973). The opinion prevails that there are ten genes (polygenes) responsible for the determination of oil content. In this context we can affirm that the inheritance of the oil content is high while that of the morphine content is low. The oil components were also established. The following acids have been found: palmitic (12%), stearic (3%), oleic (20%) and linoleic (65%) Sengupta and Mazumder 1976).

MUTAGENESIS

Papaver somniferum L. has been investigated in regards to the effects of physical and chemical mutagenes. Radiation treatments (doses ranging from 5 to 40 Krad, with 16 rad per min) induced a different behavior of plants in respect to the morphine content (Popov et al. 1974). Due to ionizing rays the alkaloid content was higher in some *P. somniferum* individuals (Ileva et al. 1975). For example, by applying a 4 Krad dose, the morphine content was increased by 29.17% (Malik et al. 1977). Chemical mutagene treatments had similar effects on alkaloid content (Ivanova 1975).

CYTOGENETICS

We know the chromosome number of *P. somniferum* — 2n=22 (Bără and Floria 1979, Bără et al. 1981, Koul et al. 1972, Hammer and Fritsch 1977), the karyotype differences between varieties (Bără et al. 1981) etc. There are three types of centromere position: medial, submedial and subterminal. The chromosome length varies from 3.24 to 2.12 μm. No satellites or secondary constructions have been found. The differences between varieties consist especially in the centromere position and chromosome length (Bără et al., unpublished data).

Attempts to obtain interspecific hybrids have not lead to any clear results so far. However, there are reports of the existence of hybrids between *P. somniferum* (2n = 22) and *P. setigerum* (2n = 44) (Malik et al. 1979) and even the existence of some gene interchange between the two partners of the hybridization (Grover 1979). The problem is debatable because it has not been proven conclusively that these are two distinct species and that *P. setigerum* is not a subspecies of *P. somniferum*.

BIOCHEMICAL ASPECTS

The physiological and biochemical data from many laboratories are quite numerous. Multiple aminopeptidase forms from plants at different ontogenetic stages have been described (Benesová et al. 1980). The morphine

content of different varieties has been determined (Nyman and Hansson 1978, Bără et al. — in preparation) and a negative correlation between the morphine content and some chemical compounds has been established. For example it is suggested that there is a negative correlation between the phtalideisoquinoleine and the morphine quantity, while there is no correlation between benzylisoquinoline and morphine, which lead to the idea that if phtalideisoquinolinic alkaloids could be excluded, a higher morphine production could be obtained.

Enzymological and biochemical studies led to the conclusion that morphine biosynthesis takes place only by the conversion of the enolic ether of thebaine into the keto group of neopinone and then by codeinone (Antoun and Roberts 1975).

The relations between L-dopa decarboxylase and alkaloid formation have also been established (Roberts and Antoun 1978). The increase of morphine production was also the concern of those occupied with poppy cultivation under field conditions. It has been noticed that the application of glycine solution with ^{14}C-phenyloalanine and urea on leaves and stems three weeks before plant harvesting increased the morphine and codeine content (Wold 1977). Urea is absorbed very fast.

Alkaloid accumulation is closely connected with the plant's ontogenetic stage. Stamens a day after anthesis, when pollination was at its maximum, showed a high morphine content (El Khneir 1975). Analysing alkaloid storing, metabolism and translocation in *P. somniferum*, modified thebaine (containing an ethoxy group and M-methyl-^{14}C in place of the methoxy group) was administered (Fairbairn et al. 1974, Brochmann-Hanssen et al. 1975). The substrate so administered was changed (transformed) into morphine 3-ethyl-ether and morphine. The dealkylation process of aromatic ethyl-ether is the same as the biotrasformation of codeine into morphine. The enzyme behavior in latex and especially the emergence of metyltransferase is well known (Antoun and Roberts 1975). Some specific chemical compounds helped in analysing morphine deterioration as well as preservation in fresh latex (Ramanathan 1979).

There are many attempts being made to modernize the methods of extraction and to determine the morphine concentration by either gas chromatography (Grove 1976) or methanol treated with activated charcoal where morphine is determined colorimetrically from among the major alkaloids (Vaidya et al. 1980).

CELL AND TISSUE CULTURES

Papaver somniferum, as other medicinal plants, has been analysed in respect to the conditions of callus inducement, alkaloid production etc.

Unlike other species, such investigations of *P. somniferum* are only at the initial stages. Callus from eleven species of *Papaveraceae*, among which are *P. somniferum*, *P. bracteatum* and *P. orientale*, has neen obtained (Ikuta 1974). The callus was similar, though each of these species in anture differs in its benzophenanthridine, protophyne, aporphyne and alkaloid production. In cell cultures, reticuline and morphine biotransformation and morphine conversion to N-oxides take place (Furuya et al. 1978). The varying of light and darkness could uphold a high rate of photosynthesis in cells originated from mesophyll (Paul and Bassham 1977). At first, great stress was noted for 20 hrs, after which normal processes were recovered; when mesophyll cells were exposed for 30 min to 20 mM sulfite, a 30% increase of the CO_2 incorporation rate and stimulation of ammonium incorporation into glutamine were noticed. The glycolate metabolism were inhibited, hence the conclusion that glycolate represents a precursor of glycine. Exposition for 24 h to 10—20 mM sulfite had an inhibiting effect while 1 mM sulfite stimulated sucrose and amidine synthesis although it inhibited ammonium incorporation (Paul and Bassham 1978).

Addition of ammonium to a mesophyll cell suspension quantitatively alters the C metablism (Paul et al. 1978). However, when 2,4-D was added to isolated mesophyll cells, an increase of CO_2 incorporation and of glutamine synthesis was obtained. If 2 mM ammonium ions were added to the cell culture, the ^{14}C fixation rate was 3 times higher, while 0.1 mM doubled this rate. NH_4^+ stimulates phosphoenolpyruvate carboxylase but has no detectable effects on the pyruvate kinase reaction in darkness (Hammel et al. 1979).

In the cell suspension cultures, the potential for morphine alkaloid production is preserved; on the Murashige-Skoog medium (2% sucrose, 0.1 $mg \cdot dm^{-3}$ 2,4-D, 0.0025 $mg \cdot dm^{-3}$ zeatin or 1 $mg \cdot dm^{-3}$ 2,4-D, 0.1 $mg \cdot dm^{-3}$ kinetin) callus grew for a year. The tissues that were appearing were cream-colored and contained differentiating tracheal elements. If 2,4-D is replaced by 1 $mg \cdot dm^{-3}$ NAA, growth is more evident, but active proliferation coincides with a decrease in alkaloid synthesis. In any case, the alkaloid production is low — under 0.1% alkaloids in dry substance. The alkaloids narcotine and narceine (an isoquinolic alkaloid) and 3 unknown alkaloids, have been found. The addition of ammonium to a mesophyll cell suspension altered the C metabolism by limiting the amino acid synthesis stages (Paul et al. 1978). By adding 2,4-D to mesophyll isolated cell cultures, the CO_2 incorporation and glutamine synthesis were increased (Paul et al. 1978).

The ultrastructure of the callus obtained from the hypocotyl of plantlets or from mature stems is identical with that of parenchyma from intact plants (Nessler and Mahlberg 1979).

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Papaver somniferum — dokonania, wątpliwości, oczekiwania

Streszczenie

Mak (*Papaver somniferum* L.) miał, ma i zapewne będzie miał w przyszłości duże znaczenie jako roślina lecznicza. Obecnie obserwujemy wzrastające zainteresowanie makiem, związane z jego właściwościami użytkowymi, estetycznymi, pokarmowymi i farmaceutycznymi. Obecnie dysponujemy wieloma informacjami o tej roślinie z dziedziny biochemii, morfologii, genetyki i farmacji.