MORPHOGENESIS OF ROOT NODULES IN WHITE CLOVER.
III. THE EFFECT OF MUTATION IN *nod IJ* GENES
OF THE MICROSymbiont UPON THE DNA LEVEL IN THE HOST TISSUE

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ABSTRACT

On the basis of cytophotometric measurements a slightly increased DNA level in the nuclei of curled root hairs containing infection threads was observed in white clover inoculated with wild and mutant strains of *Rhizobium leguminosarum* biovar. *trifolii*, as compared to normal root hairs of the same plants. Cells of the root nodule primordia in 72 h after the inoculation, as compared to the root primary cortex, demonstrated an increased level of the nuclear DNA. No differences were observed in the nuclear DNA contents in individual layers of the cortex of the 28 day-old nodules. Generally it was low, varying from 2c to 4c. The meristematic and bacteroidal tissues in the effective nodules were characterized by a higher DNA level, as compared to the respective zones in ineffective nodules induced with the strains ANU261 (*nod I*) and ANU262 (*nod J*). The DNA level in the effective bacteroidal tissue varied from 4c to 32c, while in the tissue containing the strain ANU261 only the 2c-8c nuclei could be found and in the tissue with the strain ANU262 – the 4c-16c nuclei.

KEY WORDS: Endoreplication; cytophotometry; *Rhizobium leguminosarum* biovar. *trifolii*; bacteroidal tissue; *Trifolium repens* L.

INTRODUCTION

In clover, after inoculation its roots with an appropriate strain of symbiotic bacterium *Rhizobium leguminosarum* biovar. *trifolii*, some of the mitotically inactive cells of the pericycle and root primary cortex start to divide again and form a root nodule primordium (Łotocka et al., 1997a). Some of the primordium cells become populated with bacteria which enter them through the infection threads originating from the characteristically deformed – curled – root hair. These cells comprise the oldest part of the nodule bacteroidal tissue. Other primordium cells remain in the meristematic state for the whole period of nodule morphogenesis and produce the derivative cells differentiating into multilayered nodule cortex, vascular bundles and bacteroidal tissue. The latter originates from the penetration by the infection threads from the primarily infected primordium cells to the meristem derivatives and the release of bacteria from the threads with the simultaneous formation of symbiosomes. A symbiosome is formed of the rhizobium cell surrounded by the membrane produced by the host cell. Bacteria inside the symbiosomes undergo transformation into much bigger cells called bacteroids which, under the conditions created by the host plant, are able to fix atmospheric nitrogen.

Although the root nodule morphogenesis is coded in the plant genome, that information is only realized in response to

the proper signal(s) sent from the bacteria symbiotic cell, thus resulting in the formation of a root nodule – a structure which tissues are characterized by various patterns of the nuclear DNA contents depending on the specialization of particular cells originating from the nodule meristem. The investigations of the karyological anatomy of the root nodule were started already in 1938 (cited after Libbenga, Bogers, 1974) by Wipf and Cooper. While investigating the size of the cell nuclei and spontaneous mitoses in the root primary cortex in pea, these authors noted the presence of disomatic cells there. Since they also observed disomatic mitoses around the growing end of the infection thread at the initial stage of the root nodule formation and monosomatic nuclei in the neighbourhood of the abortive threads they put forward a hypothesis that the nodules are initiating only then when the thread, by chance, came near the polyploid cortex cell. That hypothesis greatly influenced the opinion of many authors on the nodule ontogenesis and only lately its inaccuracy has been demonstrated (Yang et al., 1995). A more detailed picture of the karyological anatomy of the pea nodules was presented by Mitchell (1965). He demonstrated that the nodule meristem is composed of di- and tetraploid cells with diploid ones mainly observed in the surface part. It gave the basis for a suggestion that the nodule cortex is formed by the proliferation of the diploid part of the nodule meristem (outer and lateral cells) while the bacteroidal tissue – by the proliferation of the central part of

the mixoploid meristem. Mitchell also observed that the cells of the nodule outer cortex are usually 2c, inner cortex – 4c, and the bacteroidal tissue is made of cells 8c and 16c. These observations were confirmed by Olszewska and Legocki (1989) for nodules in yellow lupin.

The present research, carried out on white clover inoculated with strains of *Rh. l. bv. trifolii* with mutations in genes *nod I* and *nod J* (probably connected with transport of the Nod factor), aimed at investigating, whether the pattern of the nuclear DNA contents in the tissues of the nodule was affected by the symbiotic effectiveness of strains.

MATERIAL AND METHODS

The seeds of white clover 'Astra' were surface-sterilized, pregerminated in the sterile conditions and then, after the inoculation with rhizobium strains transferred to the test tubes on agar slopes containing nitrogen-free medium (Łotocka et al., 1997a). The following strains were used in the experiment: 24 of the wild type inducing effective nodules and ANU261 and ANU262, both inducing ineffective nodules. The mutant strains were kindly provided by Dr M.A. Djordjevic and they were obtained by the Tn5 mutagenesis with the mutations mapped in the gene *nod I* in ANU261 and *nod J* in ANU262 (Djordjevic et al., 1985). The dates of the nodule emergence were noted and the 28 day-old nodules were collected for further processing. They were fixed in 4% formaldehyde for 2 h, repeatedly rinsed in distilled water for 24 h, again fixed in the mixture 1:3 (v/v) of glacial acetic acid and 100% ethanol. Material was stored in that mixture. After rinsing the samples in distilled water for 0.5 h, they were hydrolysed in 5 N HCl for 1 h at room temperature, rinsed in distilled water for 1 min and stained in the darkness with the Feulgen reagent for 1 h. Next, the material was rinsed in the freshly prepared sodium bisulfite solution, transferred to distilled water and the squash preparations were made. Together with each batch of material the root tips of white clover 'Astra' were fixed and stained in order to determine the 2c telophase level characteristic for that plant.

In the meantime the test of the early reaction of the clover root to the inoculation with rhizobium strains was carried out. Apart from the above mentioned strains, another wild type strain was used – DJS2411, provided by Dr L. Skot from IGER, Aberystwyth. The pre-germinated and inoculated clover seedlings with the root tip position marked, were grown on agar plates in the sterile conditions. In 72 h after inoculation the parts of roots below the root-tip mark, which had grown after the transfer to the agar plates, were removed while the upper parts, which were inoculated at the beginning of the test, were fixed. At the same time and in the same way, the roots non-inoculated with bacteria (control) and root tips were also collected and fixed. After staining with the Feulgen reagent, root fragments from each inoculation were carefully torn up with needles under the dissecting microscope into, at least, 6 longitudinal strips and then squashed in 50% glycerol. Before squashing, root tips were fragmented into single cells with a brass taper.

The 28 day-old root nodules were embedded in paraffin and they were cut with a rotary microtome into serial sections 15 µm thick. Paraffin sections were used for general anatomical observations only, as they proved to be useless for the microdensitometric measurements – they were too thick for the measurements of the nodule tissues with small cells (cell nuclei covered each other) and at the same time they were too thin to measure the bacteroidal tissue (most of the cell nuclei

were cut). Examination of paraffin sections confirmed the structural consistence between the effective and ineffective nodules obtained in the present investigations with the observations by Łotocka et al. (1997a, 1997b). For the microdensitometric measurements the fixed and stained 28 day-old nodules were cut longitudinally and then one of the halves was hand-cut into transverse sections. Twelve to fourteen sections were obtained from one effective nodule of about 3 mm in length. Sections containing the nodule meristem were fragmented with brass taper and squashed in 50% glycerol, while the other sections were squashed only, in order to preserve the anatomical arrangement of cells.

Measurements of the nuclear DNA contents were carried out with the scanning microdensitometer M86 (Vickers Ltd, Vickers Instruments) at the wave length of 680 nm, band width of 50, spot size 2 and the scanning frame 2, using the immersion objective of 100x magnification. For the each combination the measurements were done in 3 replications for 20 nuclei each (in some combinations the number of measured nuclei was different). The DNA contents in particular nuclei was calculated as the mean of two measurements rejecting one, the most contrasting measurement. Statistical analysis was done in the Department of Statistics, Warsaw Agricultural University – SGGW. The Tukey's HSD test for unequal *n* was applied to compare the means.

RESULTS AND DISCUSSION

1. The DNA contents in the root cells of the clover seedlings inoculated with rhizobia (the root early reaction test)

In that part of the experiment the measurements were taken of the following cell populations: 1) normal root hairs and 2) deformed root hairs including 2a) curled root hairs and 2b) root hairs deformed in other way, 3) primary root cortex cells and, in case of plants inoculated with wild-type strains, 4) root nodule primordium cells. Additionally, among the curled root hairs and primordium cells the cells containing the infection threads were distinguished, however, these cells could not be included in the statistical analysis because their number was too low. For the purpose of the statistic analysis it was necessary to combine group 2a and 2b together because of big differences in particular combinations between the number of the curled root hairs and the root hairs deformed in other way.

Root hairs

The reaction of the clover root hairs fixed in 72 h after inoculation with mutant rhizobium strains was not delayed as compared to the seedlings inoculated with the wild type strains, however, the number of deformed root hairs was much higher with the simultaneous decrease of the percentage of hairs curled in a way which made the infection possible (Łotocka et al., 1997b). Also, the percentage of the root hairs with the infection threads decreased. The mutant strains nodulated less plants and with delay, as compared to the wild type strains, the number of nodules per plant did not differ in the effective and ineffective symbiosis.

In the accessible literature there are no data concerning the effect of the presence of rhizobia in the host plant rhizosphere on the ploidy pattern of the epiblem cells. In the discussed experiment, it was not noted that the presence of rhizobia in

TABLE 1. Nuclear DNA level in the roots of white clover 72 h after inoculation with *Rhizobium* strains.

means of 3 replications in relative c units, where c is a minimal DNA amount in haploid cells of a particular species.

* cell populations statistically analyzed.

Rhizobium strains	DNA level in the cells of:							
	rot normal hairs*	deformed root hairs				primary root cortex*	nodule primordia	
		curled		other	total*		with infection threads	total*
		with infection threads	total					
effective								
24	3.6	5.0	4.2	3.4	3.9	5.9	7.6	7.2
2411	3.7	4.3	4.1	3.8	3.8	5.0	6.9	6.6
mean	3.6	4.6	4.1	3.6	3.8	5.4	7.2	6.9
ineffective								
ANU261	3.6	4.1	3.7	3.2	3.6	4.5	none	none
ANU262	3.5	6.5	4.1	3.6	3.8	4.9	none	none
mean	3.6	5.3	3.9	3.4	3.7	4.7	—	—
control (not inocul.)	4.0	none	none	none	none	5.1	none	none

the rhizosphere affected the DNA level in the root hair cells (Table 1). Independently from the strain, both the cells with normal root hairs and with deformed hairs had the mean DNA contents similar to that in the root hair cells of the non-inoculated control (3.6c, 3.8c and 4.0c, respectively) and similar percentage of cells of various DNA contents. Only the cells with the curled root hairs which formed infection thread showed a higher DNA level (on the average 5.0c for the strain 24 and 6.5c for ANU262). The observed difference did not appear for the second wild-type strain, which can attest to its accidental character resulting from a very small number of samples. The root hairs with the thread comprised a small fraction of the population (2% in plants inoculated with ANU261, 4% in ANU262, 13% in control inoculated with wild-type strain 24 (Łotocka et al., 1997b) and their statistical comparison with the remaining ones was not possible. Thus it is still doubtful that the metabolic mobilization observed in the infected hair cells which manifested itself with, among others, the thickening of the cytoplasm, accumulation of small amounts of starch in the amyloplasts, synthesis of large amounts of the wall material (not demonstrated) was correlated with the increase of the cell ploidy level.

Primary root cortex

According to Libbenga and Bogers (1974) the legumes are various as to the root cortex ploidy pattern, thus including plants with diploid cortex (*Trifolium alexandrinum*, *Astragalus sinicus*) and mosaic one (*Vicia faba*, *Pisum sativum*). In this work roots of the control plants of white clover were characterized by the mixoploid primary cortex containing 4c-12c cells with the average DNA level amounting to 5.1c (Table 1). The observed level 12c could be represented by cells at the phase S of the cell cycle, passing from the level 8c to 16c. After inoculation with the wild type strains, the average DNA contents in the primary cortex cell nuclei was slightly higher and after inoculation with the mutant strains —

lower than in the control (Table 1). These differences were not statistically significant, thus no increase of the DNA endoreplication level in the primary root cortex of clover was found during the initial stages of symbiosis (with the exception of the dedifferentiated cortex cells forming the nodule primordium). In that respect the clover plants reacted differently than yellow lupin inoculated with a wild strain of *Bradyrhizobium* sp. (*Lupinus*) (Olszewska, Legocki, 1989), which can be connected with the fact that the first phases of the root nodule development in these plants take different course (Łotocka et al., 1997a; Golinowski et al., 1987).

The comparison of root hairs and primary root cortex

The cytological dissimilarity of the parenchyma cells of the primary root cortex and root hair cells in clover was proved by the significance of differences in the average nuclear DNA contents between these tissues, both in the control plants and those inoculated with the wild type strains (Table 1). It seems interesting that this significant difference characteristic for the control roots and roots inoculated with the effective strains disappeared in plants inoculated with mutants. Both in the roots inoculated with the ANU261 and ANU262 strain, the primary cortex and root hairs had the same average nuclear DNA contents. That fact may indicate that the inoculation with ineffective strains of *Rhizobium* inhibited endoreplication in examined fragment of root cortex (see Material and Methods), typical for the process of cortex differentiation. The interpretation of this phenomenon is difficult because, although it is known that the rhizobial Nod factor (the transportation of which is probably impaired in the investigated ineffective strains; Schultze et al., 1994) modifies the cell cycle of cells within the root in pea and alfalfa, it was not observed that it affects any other cells of the primary cortex except those which participate in the formation of the nodule primordium (Yang et al., 1995).

Root nodule primordia

The cells of the nodule primordia, especially those with the infection thread, contained more nuclear DNA than the primary root cortex cells which are their mother cells (Table 1). Nodule primordia induced by the wild type strains were made of cells with the DNA level 2c-8c and more and, as compared to cortex, the population of higher DNA levels was bigger. What is interesting, in nodule primordia the 2c cells appeared, which are practically absent in the primary cortex. Several dividing 8c cells were observed in the primordia. The obtained results correspond with the observations in pea by Torrey and Barrios (1969, quoted after Libbenga, Bogers, 1974), where the authors noted that most of the initial mitoses in the primordium was tetraploid. In white clover fixed in 72 h after the inoculation (the present paper) the infected cells, i.e. the cells containing the infection thread, not numerous at that stage of development, showed the DNA level near to 8c. In case of one preparation, exactly in the radial plane of the primordium, it was possible to observe that a group of cells with the DNA contents of 4c and higher was situated close to the root stele and was covered with the layer of the 2c-4c cells from the side of the epiblem. It reminded the arrangement of the initial cells in the meristem of the mature clover nodule.

2. The DNA contents in the clover root nodule cells

While performing the cytophotometric measurements on the effective nodules the following groups of cells were recognized: 1) nodule meristem, 2) outer cortex, 3) tannin cells layer in the nodule cortex (the, so called, nodule endodermis), 4) inner cortex, 5) differentiated infected bacteroidal cells adjacent to the nodule cortex, 6) differentiated bacteroidal cells deep inside that tissue. The cells 5) and 6) were measured independently since it was known that the differentiation of the bacteroidal tissue was quicker in its peripheral layers (Łotocka et al., 1997a) and that could result in the various number of the endo-cycles in those regions.

In order to investigate changes in the nuclear DNA contents originating from the differentiation of the cells (related with the increasing distance from the meristem), 8 subsequent sections taken from each effective nodule were statistically analysed. The remaining sections, closer to the basal part of the nodule contained numerous degenerating cells thus they were rejected. Similar procedure was not possible in case of ineffective nodules because of their decidedly smaller size and impaired anatomical structure (Łotocka et al., 1997b), which made it impossible to obtain cross-sections containing the

cells of the same differentiation stage. For this reason the ineffective nodule zones were not taken into analysis.

Root nodule cortex

The obtained results are presented in Table 2. Statistical analysis allowed a separation of two regions in the nodule: diploid (nodule meristem and all three cortical layers) and polyploid (outer and inner cells of the bacteroidal tissue). Similarly as in pea (Mitchell, 1965), the clover nodule cortex was diploid, although in both the inner and outer cortex the 4c cells predominated (they could be also the tetraploid cells at the stage G1), while in the tannin layer – the 2c cells. The tannin cells were characterized by small cell nuclei, which were losing their characteristic granular structure and turning greyish in colour (not demonstrated) at a relatively small distance from the nodule meristem. Such nuclei were treated as degenerating and they were not measured. The average DNA level in the cortex cell nuclei of both effective and ineffective nodules was the same (the difference was not statistically significant).

Nodule meristem

Significant differences between effective and ineffective symbiosis were observed in the nodule meristem and infected cells of the bacteroidal tissue (Table 2). The meristem of effective nodules was characterized by an average DNA level of 5.0c and contained the 2c-8c cells with a distinct predominance of the 4c cells (Fig. 1a). A similar to the nodule meristem the ploidy level and the localization of the diploid (the meristem peripheries) and polyploid mitoses (the inside of the meristem) were observed in the nodule primordia investigated in the 1) part of the present research, however, under the stipulation that in the meristem the percentage of cells of a lower DNA level (2c-4c) was higher. Similar results concerning the ploidy of the nodule meristem were earlier obtained by Mitchell (1965) in *Pisum sativum*, Bhaskaran and Swaminathan (1958, cited after Libbenga, Bogers, 1974) in *Trifolium alexandrinum* and Kodama (1970) in *Astragalus sinicus*. The 28 day-old *nod I⁻* and *nod J⁻* nodules in white clover did not contain a typical meristem (Łotocka et al., 1997b). Their distinguishable meristematic region was built mainly of the 4c cells, however, due to the inhibition of the cell divisions it was impossible to state whether they were the di- or tetraploid cells. Unfortunately, in the 1) part of the present experiment no primordia of ineffective nodules were obtained, so it was impossible to say whether the meristem of those nodules

TABLE 2. Nuclear DNA level in the root nodules of white clover 28 days after inoculation with *Rhizobium* strains. means of 3 replications in relative c units, where c is a minimal DNA amount in haploid cells of a particular species.

Rhizobium strains	DNA level in the cells of:							
	nodule meristem	nodule cortex				bacteroidal tissue		
		outer	tannin cells layer	inner	mean	outer	inner	mean
effective								
2411	5.0	4.1	2.8	4.0	3.8	17.7	19.6	18.6
ineffective								
ANU261	2.9	not measured	not measured	not measured	3.5	not measured	not measured	4.8
ANU262	3.7	not measured	not measured	not measured	3.7	not measured	not measured	7.8

Fig. 1. DNA content in the cell nuclei of nodule meristem (A) and infected bacteroidal tissue cells (B) in root nodules induced by wild-type strain DJS2411 and mutant strains ANU261 and ANU262.

originated as diploid or whether the polyploid cells were lost from it.

Bacteroidal tissue in effective root nodules

The average DNA contents in the effective bacteroidal tissue amounted to 18.1c (Table 2) and varied from 4c to 32c with the highest percentage of the 16c cells and single cells close to 64c (Fig. 1b). The average nuclear DNA contents was a little higher in the infected cells situated inside the bacteroidal tissue although these cells began to differentiate a little later, as compared to the cells situated close to the border of that tissue with the nodule cortex. However, statistical analysis did not confirm the significance of the difference in the average nuclear DNA contents between the surface and inner regions of the bacteroidal tissue. The bacteroidal tissue in clover was characterized by a higher endoreplication level than in pea, in which Mitchell (1965) demonstrated the DNA level in the bacteroidal tissue of 8c-16c with a few, not numerous, cells of 4c. Statistical analysis of the average DNA contents in the nuclei from successive sections of the same clover nodule (performed only for the effective nodules) revealed that the average DNA contents in the bacteroidal cells increased with their distance from the meristem, i.e. with the cell age. The differentiation of cells was rapid (Fig. 2) because already the second section from the meristem (corresponding approximately to the zone of mature symbiosis) significantly diffe-

red from it in the average DNA level (5.0c and 14.8c, respectively). In the successive sections the increase of the average DNA contents was slower and in the seventh section that value reached its maximum amounting to 22.7c. The average nuclear DNA contents in the eighth section was a little lower – 21.4c. Although the difference in the average DNA contents between the seventh and eighth section was not significant, the comparison of the DNA contents distribution in those sections (Fig. 2) suggests that the successive stage of the DNA endoreplication in some nuclei (especially in the outer layers of the bacteroidal tissue) was completed in the eighth section and they advanced to a higher ploidy class.

Bacteroidal tissue in ineffective root nodules

All the investigated tissues in the *nod I* nodules: the nodule meristem, cortex and bacteroidal tissue did not differ significantly as to the average DNA level (2.9c, 3.5c and 4.8c, respectively; Table 2). It means that in those nodules the process of endoreplication during the differentiation of the permanent tissues appeared only sporadically. In the ineffective bacteroidal tissue infected with the strain ANU261 only the nuclei of the DNA contents amounting to 2c-8c were observed, as well as some single 16c nuclei (Fig. 1b). Comparing to the *nod I* nodules, the *nod J* nodules showed the smaller decrease of the average DNA contents in the bacteroidal tissue in comparison with the wild-type nodules (4.8c and 7.8c,

Fig. 2. DNA content in the cell nuclei of nodule meristem and infected bacteroidal tissue cells in some transverse sections of nodules induced by wild-type strain. o – outer bacteroidal cells adjoining to the nodule cortex, i – inner bacteroidal cells. Section 1i not shown – no nuclei accessible for measurements.

respectively; Table 2), and, what is more, the bacteroidal tissue in those nodules was characterized by a significantly higher DNA level than the nodule meristem and cortex. However, the endoreplication level was evidently lower than in the effective nodules. In the cells populated with the ANU262 strain the 4c-16c nuclei were predominant and also there appeared some single nuclei of even higher DNA contents (Fig. 1b).

SUMMARY

Statistical analysis demonstrated a significant effect of the rhizobium strain on the average DNA level in the whole population of the nodule cells which in the nodules induced with the wild type strain amounted to 9.1c. Out of two investigated ineffective strains, the strain with the mutation in the *nod I* gene more strongly affected the nodule morphogenesis causing the inhibition of endoreplication and thus the arrest of the mean DNA level in the nodule at the average 3.7c level (5.0c for the *nod J* strain, respectively). That effect resulted from the reaction of the bacteroidal tissue because independently from the strain, the nodule cortex and meristem did not differ significantly as to the average level of the nuclear DNA contents. The comparison of the average DNA contents in the population of the meristematic cells and in the population of the bacteroidal cells in the effective and ineffective systems, with a three-fold increase of the mean DNA contents in the effective system and only two-fold in the ineffective one, clearly shows that the sign of ineffectiveness caused by the mutation in the gene *nod I* or *nod J* of the microsymbiont is the inability of the host bacteroidal tissue for normal differentiation characterized by the cell ploidy level increase.

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MORFOGENEZA BRODAWEK KORZENIOWYCH KONICZYNY BIAŁEJ.

III. WPŁYW MUTACJI W GENACH *nod IJ* MIKROSYMBIONTA NA ZAWARTOŚĆ DNA W TKANKACH GOSPODARZA

STRESZCZENIE

U koniczyzny białej inokulowanej dzikimi i zmutowanymi szczepami *Rhizobium leguminosarum* biovar. *trifolii* stwierdzono na podstawie pomiarów cytofotometrycznych nieznacznie podwyższony poziom DNA w jądrach skrzyżowanych włókien zawierających nici infekcyjne w porównaniu do normalnych włókien w tych samych roślinach. Komórki primordiów brodawki, w porównaniu do dających im początek komórek kory pierwotnej korzenia, wykazywały zwiększony poziom DNA jądrowego. Nie stwierdzono różnicy w wartości DNA jądrowego w poszczególnych warstwach kory brodawek. Ogólnie był on niski i wahał się od 2c do 4c. Tkanka merystematyczna i bakteroidalna w brodawkach efektywnych charakteryzowała się wyższym poziomem DNA w porównaniu do analogicznych stref brodawek nieefektywnych indukowanych szczepami ANU261 (*nod I*) i ANU262 (*nod J*). Poziom DNA w efektywnej tkance bakteroidalnej wahał się od 4c do 32c, podczas gdy w tkance zawierającej szczep ANU261 znajdowano tylko jądra 2c-8c, a w tkance ze szczepem ANU262 – jądra 4c-16c.

SŁOWA KLUCZOWE: endoreplikacja; cytofotometria; *Rhizobium leguminosarum* biovar. *trifolii*; tkanka bakteroidalna; *Trifolium repens* L.

LITERATURE CITED

- BHASKARAN S., SWAMINATHAN M.S., 1958. (title not quoted in Libbenga and Bogers, 1974). Nucleus, 1: 75.
- DJORDJEVIC M.A., SCHOFIELD P.R., ROLFE B.G., 1985. Tn5 mutagenesis of *Rhizobium trifolii* host-specific nodulation genes result in mutants with altered host-range ability. Mol. Gen. Genet., 200: 463-471.
- GOLINOWSKI W., KOPCIŃSKA J., BORUCKI W., 1987. The morphogenesis of lupine root nodules during infection by *Rhizobium lupini*. Acta Soc. Bot. Pol., 56: 687-703.
- KODAMA A., 1970. (title not quoted in Libbenga and Bogers, 1974). J. Sci. Hiroshima Univ., 13, ser. B, div. 2.: 223.
- LIBBENGA K.R., BOGERS R.J., 1974. Root-nodule morphogenesis. In: The Biology of Nitrogen Fixation. (ed.) Quispel, A., Amsterdam, pp. 431-472.
- ŁOTOCKA B., KOPCIŃSKA J., GOLINOWSKI W., 1997a. Morphogenesis of root nodules in white clover. I. Effective root nodules induced by the wild type of *Rhizobium leguminosarum* biovar. *trifolii*. Acta Soc. Bot. Pol., 66: 273-292.
- ŁOTOCKA B., KOPCIŃSKA J., GOLINOWSKI W., 1997b. Morphogenesis of root nodules in white clover. II. The effect of mutation in genes *nod IJ* of the microsymbiont upon the nodule structure. Acta Soc. Bot. Pol., 67: 5-22.
- MITCHELL J. P., 1965. The DNA content of nuclei in pea root nodules. Ann. Bot., 29: 371-377.
- OLSZEWSKA M.J., LEGOCKI A.B., 1989. Changes in DNA content during rhizobial nodule development in *Lupinus luteus* L. I. Cytophotometry and autoradiography. Biol. Zentralbl., 108: 221-230.
- SCHULTZE M., KONDOROSI E., RATET P., BUIRE M., KONDOROSI A., 1994. Cell and molecular biology of *Rhizobium*-plant interaction. Int. Rev. Cytol., 156: 1-75.
- TORREY J.G., BARRIOS S., 1969. Cytological studies on rhizobial nodule initiation in *Pisum*. Caryologia, 22: 47-62.
- WIPF L., COOPER D.C., 1938. Chromosome numbers in nodules and roots of red clover, common vetch and garden pea. Proc. Natl. Acad. Sci. USA, 24: 87-91.
- YANG W.-C., BLANK DE C., MESKIENE I., HIRT H., BAKKER J., KAMMEN VAN A., FRANSEN H.J., BISSELING T., 1995. *Rhizobium* Nod factors reactivate the cell cycle during infection and nodule primordium formation, but the cycle is only completed in primordium formation. Plant Cell, 6: 1415-1426.