EVOLUTIONARY ASPECTS MICROSPOROGENESIS AND MICROGAMETOGENESIS INTERSPECIFIC HYBRIDS WITHIN THE GENUS *Glycine* L.

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Abstract*. A cytogenetic study of interspecific • Glycine max × • G. soja hybrids was made. The F_1 – F_2 meiosis and pollen formation was studied on preparations stained by Felgen and by acetokarmin. The range in pollen diameter variability of interspecific hybrids proved significant: 9, 6-31, 2 mkm, and giving evidence of some meiotic disturbances in the process of microsporogenesis. Chromosomes of interspecific hybrids are from desynaped bivalents univalents during diakinesis - metaphase-I. The interspecific hybrid chromosome composition arises because of chromosome dissimilarity caused by the taxonomic differences in soja bean species under study. Genome isolation in hybrids and chromosome conjugation within each of the genomes confirmed the hypothesis on the leading role of polyploidy in the phylogeny of G. max and G. soja.*

Keywords: *Glycine max, G. soja (G. ussuriensis),* wild soybean, phylogeny, Interspecific hybrid, Microsporogenesis, meiosis, Microgametogenesis, pollen.

Introduction. Wild soybean, *Glycine soja* Sieb. and Zucc. (*G. ussuriensis* Regel. and Maack), it is connected with a cultural kind (*Glycine max* (L.) Merr. The origin (Fucuda, 1933; Herman, 1962; Komarow, 1961; Zolotnitsky, 1962; Zhao, et al. 2009 *Glycine soja* has been a source of many valuable genes, which are absent in domesticated soya. High adaptability to adverse environment conditions of *G. ussuriensis* soya represents genetic potential of this species. Unfortunately, because of reduced area of natural cultivation, *G. ussuriensis* has a reduced genetic polymorphism in natural populations. One approach to wild species preservation through artificial hybrid between cultivated and wild species. ot only maintaining genetic variety of the initial material for selection through interspecific crosses, but also studying of interspecific hybrids in the Genus *Glycine*. Through genetic and cytology the analysis of interspecific hybrids, research on phylogenetic relationships can be established.

Materials and Methods. Objects of research were to create an interspecific F_1 study and F_2 hybrids from crossing of *G. max* with wild soybean *G. soja (G. ussuriensis*). All parents and interspecific hybrids were sowed in comparable conditions. The female parent was the cultivated soybean '*Amur brown 57'* (AB-57), and the father was always the wild *G. ussuriensis* soya. Plants and seeds G. ussuriensis were collected by the author and were hybridized in places of its growth on *Zeya rive*r coast at its merge to the *Amur* near to *Blagoveshchensk* city. Flowers from the F_1 and F_2 hybrids at different stages of development were fixed in 3:1 acetic acid: ethyl alcohol, respectively. Viability of pollen was defined by a method of colouring by acetokarmin with preliminary etch (by mordanting) the material in a water solution containing Fe $(NH_4)_2$ alum, and also by germinating the pollen a 1 % an agar-agar and 20 % of sucrose with traces of a pine forest. Studying the process meiosis in

interspecific hybrids in comparison with parental forms utilized Felgen staining the chromosome preparations with additional painting *«lichtgr• n»*, and a microscope at 900 ×.

***Results and Discussion*.** First generation hybrids from crossing *G. max.* × *G. soja* had the biggest quantity of pollen grains in its anthers, Sterile pollen grain for (F_1) interspecific hybrids ranged from 5,5 to 48,5 %. Sterile pollen were not painted with acetokarmin, was deformed. Diameter of cells of pollen F_1 of interspecific hybrids substantially varied from 3 to 39,5 micrometers. In the second generation (F_2) the quantity of viable pollen in anthers has increased and varied within the limits of 77.3 ÷ 89.5 %, however the significant variation of pollen on diameter was kept (Fig. 1).

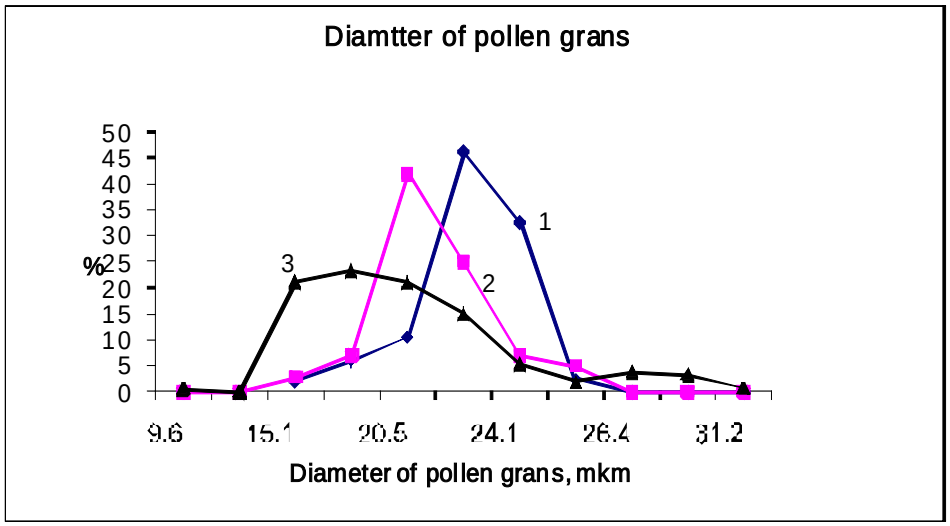

Fig. *1. Distribution of mature pollen grains of hybrids on diameter: 1 • B -57 (G. max); 2.* Wild soybean *(G. soja); 3. Interspecific hybrids*.

Earlier (Williams, 1948) it was revealed that, there are 50 % aborted pollen and eggs at F_1 plants from the majority of 15 hybrids *G. max.* × *G. ussuriensis*. On other hand, plant of the first generation of such hybrids had normal fertile pollen (Hadley, Hymowitz, 1973).
The average size of pollen varieties Amur brown 57 was 20.02 mkm, Ussuri wild soybean - 20.51 micrometer; the second-generation hybrids average pollen diameter was 18.63 micrometer with a coefficient of variation 21.5%. Pollens of the interspecific hybrids F2 was, on average, smaller than the parental forms pollen. The limits of variability of diameter of pollen varieties Amur brown 57 and wild soybean are identical and are as follows: 15, 1-25, 1 micrometer (micrometer, mkm). Much of the pollen cells of interspecific hybrids of F_2 was nonstandard. Thus, the diameter of hybrid pollen varied within the range: 9, 6 to 31, 2 micrometer. Coefficient of variation of pollen diameter (MVG) was 21,5%, Ussuri wild soybean 11,7% and variety Amur brown 57 was 13,2%. Consequently, pollens of the second generation hybrids were not aligned. About 25,5% of the pollen cells of the hybrids had a lower diameter than the minimum diameter of the parental forms and 7,9% of the pollen cells had a diameter exceeding the maximum size of the representative sources pollen. 10.0 % is the proportion of "small pollen" for wild soybean, the plants of soybean varieties: Amur brown 57 - 2, 4%, grade Amur yellow -41 - 0%, Beltskaja 636 2.9%, F2 25 interspecific hybrids was 2-48,4%. The formation of the small pollen "from Interspecific hybrids in F_1 - F_2 was preceded by a violation of the meiosis process during microsporogenesis: education (along with the tetrad) pentades (pentads), hexahedron (hexadiene) octads (octahedron) and the other polyhedron (polyads) microspores (microspores). Among the "very large pollen"

interspecific hybrids, the diameter of which ranged from 26 4 to 31.2 micrometer, found multicore pollen from many "small nucleus" from 2 to 6, in addition to a core nucleus. Among the "makroyader» macronucleus (such cells was less than one percent) are very large with a large number of chromosomes, probably "restitution" nucleus. Major violations are causing polymorphism microspores and reduce pollen viability revealed in the process of meiosis in microsporogenesis and are associated with deviations from the usual mechanism of its occurrence.

Microsporogenesis, pollen formation in soya interspecific hybrids. At early stages of sporogenous development the fabric is presented by weight of many-sided cells with large kernels. Till the moment of end of formation of an external wall of microsporangium nondirectional cell fissions sporogenous fabrics are characteristic. As a result of it, in jack's pollen sack of anther soya parent cells of pollen (parent cells of pollen) which developments at early stages are densely closed are formed. During meiosis they stand apart one from another. Their form is approximated, and cells appear surrounded by a thick jellylike media. The beginning microsporogenesis at a soya occurs at very early stages of development young green anthers. During meiosis in soya anthers and hybrids quickly increases in sizes. Prophase-1 the longest phase meiosis at a soya. At this time soya microsporocyte reaches the maximal size.

At representatives' cultural and wild-growing ussuriensis soya in diakinesis meiosis are formed on 20 short bivalent with one terminal chiasm. Length bivalent from 1, 43 up to 2, 88 micrometers. In cells at a stage anaphase-1 at representative's cultural and wild ussuriensis a soya to poles moved on 20 chromosomes. Telophase-1 at representatives' sort *Glycine* L comes to an end with formation of a diad cells. The second division meiosis passes without appreciable deviations. In four sisterly kernels contained on 20 chromosomes. Both in the first and in the second division of meiosis at representatives cultural and wild a soya (from 3 to 5 % anaphase) it is observed backlog of chromosomes at their divergence in anaphase-1 and anaphase-2, that, can be connected from polyploidy by an origin. It is not reflected noticeably in viability of pollen which remains high, especially at wild ussuriensis a soya. Viability of pollen of plants of parental forms, at definition in the morning (*city of Khabarovsk*), was: *Amur brown 57* (AB-57) - 98.3 %, wild ussuriensis soya-100%. Already during the first meiosis division around of parent cells microdispute is observed characteristic callosal an environment incrassate callosal tunics. This environment is kept some time, and after formation early microdispute.

Research of processes chromosomes conjugation in prophase-1 of soya meiosis is complicated owing to the fine sizes of chromosomes: (0,650 - 1,038 micrometers). In diplonema - diakinesis meiosis at interspecific hybrids (F_1) it is revealed 18 precise bivalent, differing on the length, and 4 univalent. During pachitene univalent to observe it was not necessary. There are bases to believe, that they arise later, in a difrinese-metaphase-1 owing to premature break chiasm, and formed univalent are pseudo-univalent.

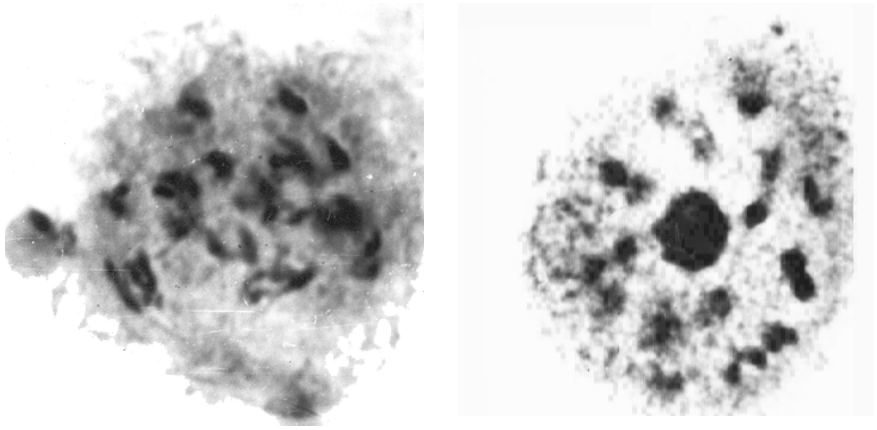

Fig. 2. Soya interspecific hybrids Bivalents at different stages of prophase-1 meiosis

In the metaphase-I, hybrids attracted attention to the fact of a cyclic (circular) chromosomes arrangement (fig. 3, at the left - a kind of metaphase from equator). In the same phase, but in position «a kind of a metaphase from a pole» (fig 3, on the right) it is visible, that chromosomes are located in two groups or chains in which on two bivalent are located separately. Paired chromosomes in two combinations were observed in early anaphase-1 (fig. 4). During this moment on preparations three lines of section of chromosomes are looked through: main - between two pair and on one - between pairs of homotype in each of them. Except for divided of bivalents, in anaphase -1 are visible univalent in an equatorial plane and one - outside a spindle. As a rule, univalent early anaphase-1 at hybrids of the first generation met in quantity of four.

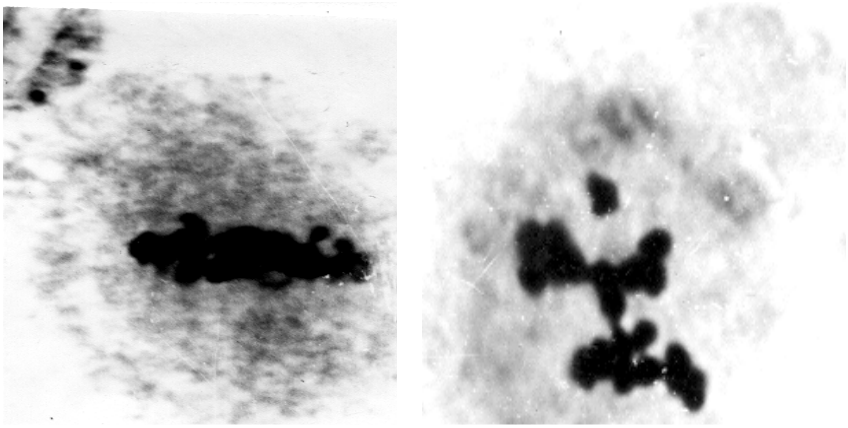

Fig. 3. **Metaphase-I hybrids:**
« A kind from equator » (at the left) and « a kind from a pole » (on the right).

Reduction the number of paired chromosomes from pachitene to a metaphase-1 testifies that their genetic information differs. In this case takes place genetically induced desynapses effect which reason, obviously, display in meiosis homeologie genome is. In a metaphase-I of hybrids the cyclic (circular) arrangement of chromosomes is observed. Pairing chromosomes in two combinations is observed in early anaphase-1. At parental forms *G. max. and G. soja* in diakinesis, and also other researchers it is revealed twenty precise bivalent.

Parental species of a soya: *G. max and G. soja*, in karyotype which set is available in 40 chromosomes, are in the evolutionary attitude stable tetraploid.

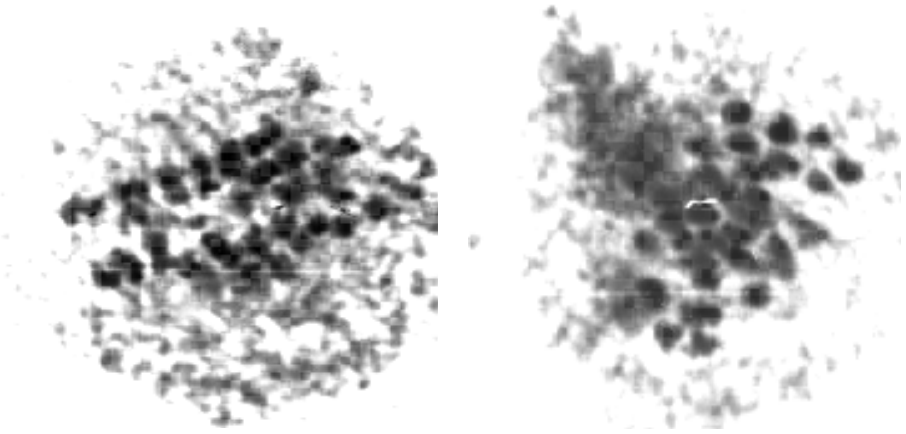

Fig. 4. Anaphase-1 (At the left) and anaphase-2 (on the right) in soya microsporogenesis interspecific hybrids

Synopsizing chromosomes in chains and rings, noted by us in the first, reduce the meiosis division, hybrids repeats at, as educational, in the second division. In a metaphase-2 (fig. 5, on the right) connection of each two chromosomes chains at the terminal pole is visible. In a metaphase-2, pictures of chromosome segregation, similar to an arrangement of two groups in the metaphase-1 also are observed. Obviously, also, there is isolation in groups of chromosomes of each of crossed kinds. Chromosomes pairing in the isolated groups in the first and second meiosis division testifies that they are a part of observed groups from the whole separate genomes. Thus among chromosomes of genome each species there are more homologous chromosomes, than in the crossed partner. This fact is the argument confirming a hypothesis of polyploidy origin (Ramanathan, 1950; Herman, 1962, Hymowitz, Singh, 1987, Zelentzov, 2002 and others) of each crossed species and, besides significant genomic structure divergence of crossed species.

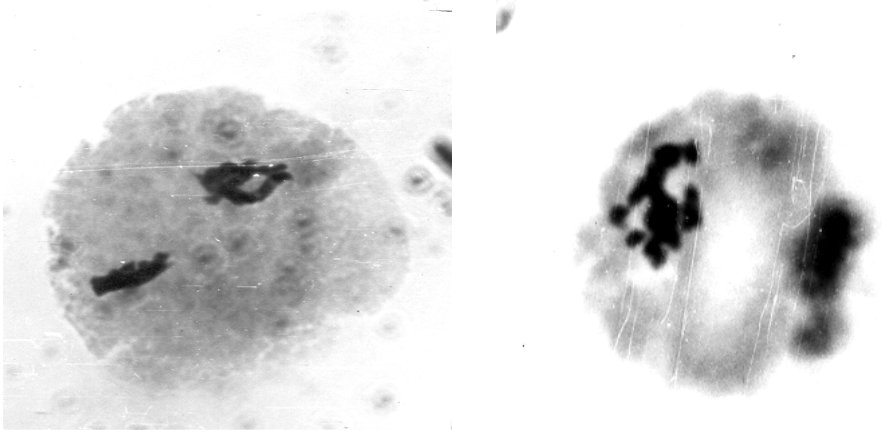

Fig. 5**.** *chromosomal pairing complements in groups and a cyclic (circular) shape of soya interspecific hybrids chromosomes in metaphase-2 meiosis*

Researches on wheat interspecific hybrids (Sapegin, 1971), and other authors, generalization of literary data show, that coupling of chromosomes in chains or a ring underlies the mechanisms limiting a free chromosomes combination in meiosis and independent inheritance of pairs of alternative traits at interspecific hybrids. The similar phenomenon occurs and in posterity of hybrids between *G. ussuriensis* (wild) soya and a cultural soya which we repeatedly marked. The phenomenon of chromosomes pairs formation in meiosis in the form of chains and rings at the species having a polyploidy origin, and the cytological mechanisms of this phenomenon are discussed in works of various authors on various objects. A chromosome conjugation at interspecific hybrids in soya as autosyndesis bivalent formation with each parental species not only confirms hypothesis polyploidy origins of crossed species, but also absence of chromosomes homology in these species. Quite often chromosomes distribution process in meiosis is accompanied with formation of «chromosomal bridges» and «loops» (fig .6). As a result of it are formed «polyad» microdispute as a result microsporogenesis (alongside with tetrad), and also aneuploidie pollen grains.

As a whole, during the first division meiosis at hybrids of the first generation following deviations from normal process meiosis are found out: a chromosomes premature divergence in metaphase (9,4 % of cells), a premature and chromosomes non-uniform divergence in anaphase-1 (21, 5 % anaphase). Meiosis process in Microsporogenesis at soya interspecific hybrids in the second division is broken. As a result of it in anaphase - telophase the second division arises multipolar meiosis, chromosomal «loops» and «bridge» is formed. As a result at interspecific hybrids of the first generation up to 48, 5 % 1-2-• nuclear pollen was sterile: were not painted acetokarmin (and on Felgen, were deformed, diameter of cells).

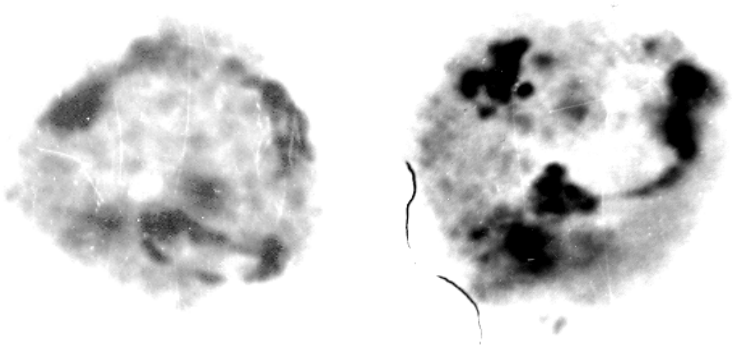

Fig.6. *Anomalies in distribution process of chromosomes in •••• •••-2 in soya interspecific hybrids (• G. max. × • G. soja)*

Two-nuclear pollen strongly varied from 3 to 39,5 micrometers. In the second generation the quantity of viable pollen at hybrids has increased to 77.3-89.5 % (against 99-100 % fertility pollen at parental forms). Thus, in the second generation there was some stabilization of distribution processes of chromosomes in meiosis, but the significant variation of pollen on diameter was observed.

Microgametogenesis Young pollen grains in a jack pollen sack of anther are surrounded tapetum by a fabric and in the beginning vigorously grow. To formation of fibrous thickenings in cells endothecium in pollen grains, there is the first nuclear fission. Pollen grains in soya anther, as a rule, during the moment of pollination are in *two-nuclear* stages (fig 7).

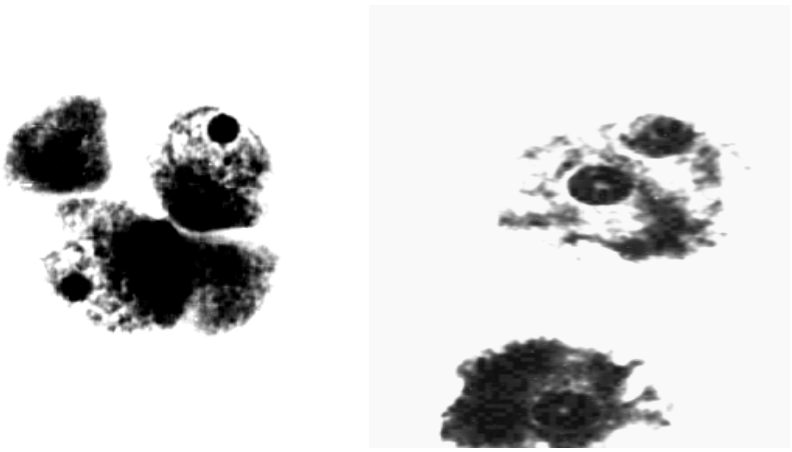

Fig. 7. *Tetrad microdispute (at the left), formation of bicellular pollen (on the right).*

Generative nucleus division occurs in the pollen tube at germination of pollen in anther or on pistil snout. Mature single pollen grains, one - and *two-nuclear pollen*, three aperture. At very early stage of development of a «young» green bud when white or slightly violet petals are still latent in a bud, soya pollen cells already are morphological mature 3-4 pore. The first stage *microgametogenesis* at a soya occurs after formation (fig.7) morphological is standard in a bright-yellow pollen bag in the sizes and is well painted acetokarmin, and also on Felgen. Anthers at this time crack, also there is a process of pollination. In 6-7 hours after pollination pollen tubes reach of soya ovules.

During the tetrads formation the aperture is not detected. Mature pollen grains are provided with three apertures (prorostkovymi pores or furrows) - this is the place where ekzina very thin, and Intini well developed. During the germination of pollen grains of pollen tubes pass through the aperture. The number of apertures in wild and cultivated types of soybean equally. Mature pollen viability in soybean round, on its surface has many protuberances, so all the grain looks rough. However, during the ontogeny of pollen grain shape varies depending on age, physiological state and stage of development of pollen grains from triangular to oval and round.

Conclusion. The meiosis (pollen and genomic) analysis in microsporogenesis and pollen formation of genus *Glycine* L., carried out by using the remote hybridization, has given an additional experimental material for the decision the matter of genetic relationship degree of representatives of kinds of *G. max* (L.) Merr. *(G. hispida* Max.) decor wild soybean (G. soja Sieb. & Zucc. Or *G. ussuriensis* Reg. & Maack). Investigated kinds of a soya during *phylogeny* have reached significant genome divergence. Genome isolation in hybrids and chromosome conjugation within each of the genomes confirm the hypothesis on the leading role of polyploidy in the phylogeny of *G. max and G. soja.*

References

Komarow, *• . • ., 1961. (• •• ••••, • . • .)* •••••••• ••••• ••••• •••• • •••• ••••. • . :• ••-•• • • • • • • . *138 •. (Russia)*

Sapegin, *• . • ., 1971.(• ••••i• , • . •).* ••••: • • • • • • *i* • • • • *i. • . 18•—211. (Ukraine)*

Hadley, • . • ., Hymowitz, T., *1973. // Soybeans: Improvement, production and uses. American Society of • agronomy. Inc. Publisher, Madison. Wisconsin. (USA). P. 97-116.*

Herman, F. J., *1962. A revision of the Genus Glycine and its immediate allies //U. S. Dep. Agr. Techn. Bull.. • 1268. P. 1-79.*

Hymowitz, T., Singh, R.J. *1987. Taxonomy and speciation / In: Soybeans: improvement, production and uses /ed. by J.R. Wilcox. – Madison. – Wisconsin. –USA. – P. 23-48.*

Ohara, M., and Shimamoto, Y*. 2002. Importance of genetic characterization and conservation of plant genetic resources: The breeding system and genetic diversity of wild soybean (Glycine soja). Plant Species Biology 17 : 51 - 58 .*

Ramanathan, K., *1950. Addendum to list of chromosome numbers in economic plants // Curr. Sci.. 19. • 5. • . 1515-1516.*

Ru Zhao, Hanbing Xia, and Bao-rong Lu*., 2009. Fine-scale genetic structure enhances biparental inbreeding bi promoting mating events between • • re related individuals in wild sojabean (Glycine soja; Fabaceae) populations // American Journal of Botany 96(6): 1138–1147.*

Weber, • . R., *1950. Inheritance and interrelation of some agronomic and chemical characters in a interspecific cross in soybeans, Glycine max. × G. ussuriensis //Jowa Agr. Exp. St. Res. Bull. -. • 374. P. 767—816.*

Williams, L. F., *1948. Inheritance in a species cross in the soybean // Genetics. - 33, • 1. P. 131 - 132.*

Zolotnitsky, • . • ., *1962. (••••• ••••••, • . • .) ••• •• ••••••• •••••••. •••••••••: 1962. 247 •.(Russia)*

Zelentzov S.V., *2002 (••••••• • .• ., 2002). ••••••••••• ••••••••••• •••••• ••• •••••• ••••••••••••••• • ••••• ••••••••. // •••••••••• • ••••• «•••••••••• ••••••». • . 357 - 365 http:// zhurnal.ape.relarn.ru/articles/2002/035.pdf 369 (Russia).*

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