ЛИТЕРАТУРА

1. *Гамалей Ю.В.* Природа пищевого тракта сосудистых растений // Цитология. 2009. Т. 51. № 5. С. 375–387.
2. *Гамалей Ю.В.* Роль симбиогенеза в происхождении сосудистых растений и колонизации ими суши // Ранняя колонизация суши. М.: ПИН РАН. 2011, в печати.
3. *Гамалей Ю.В.* Происхождение сосудов и сосудистых растений // Бот. журн. 2011. Т. 96. № 7.
4. *Гамалей Ю.В., Шереметьев С.Н.* Филогения биоморф двудольных в кайнозое // Бот. журн. 2010. Т. 95. № 6. С.777–800.
5. *Дьяков Ю.Т.* Грибы и растения // Природа. 2003. № 5. С. 73–78.
6. *Каратыгин И.В.* Коэволюция грибов и растений. СПб.: Гидрометеоиздат, 1993. 116 с.
7. *Козо-Полянский В.М.* Новый принцип биологии. Очерк теории симбиогенеза. Л., М.: Пучина, 1924. 84 с.
8. *Мережковский К.С.* Теория двух плазм как основа симбиогенезиса и нового учения о происхождении организмов. Казань, 1909. 102 с.
9. *Пиневич А.В., Аверина С.Г.* Оксигенная фототрофия. СПб.: Изд-во СпбГУ, 2002. 250 с.
10. *Розанов А.Ю.* Когда появилась жизнь на Земле? // Вестник РАН. 2010. Т. 80. № 5–6. С. 533–541.
11. *Фаминцын А.С.* О роли симбиоза в эволюции организмов // Тр. Имп. Акад. наук, физ.-мат. отд. 1907.Т.20. С.3–35.
12. *Gamalei Yu.V.* Evolution of cell systems and plant life forms // Paleontol. J. 2010. Vol. 44. № 12. P. 1540–1551.
13. *Margulis L.* Symbiosis in cell evolution. San Francisco: Freeman, 1981. 328 p.
14. *Margulis L.* Serial endosymbiotic theory (SET) and composite individuality. Transition from bacterial to eukaryotic genomes // Microbiol. Today. 2004. Vol. 31. P. 172–174.
15. *Müller J., Dulieu H.* Enhanced growth of non-photosynthesizing tobacco mutants in the presence of a mycorrhizal inoculum // J. Exper. Bot. 1998. Vol. 49. P. 707–711.
16. *Smith S.E., Read D.J.* Micorrhizal symbiosis. London: Acad. Press. 1997. 240 p.

THE THICKENED UNDERGROUND SYSTEM OF *JACARANDA OXYPHYLLA* AND THE MAINTENANCE OF GENETIC DIVERSITY IN PRESERVED AND DISTURBED HABITATS IN BRAZILIAN SAVANNA

Guimarães, E[1]., Bortolotto, T[2]., Marino C. L[2]., Machado S. R[1]., Marina Aparecida de Moraes Dallaqua[1], Rita de Cássia Sindrônia Maimoni-Rodella[1]

[1]Departament of Botany and [2]Departament of Genetics, Institute of Biosciences, UNESP - Univ Estadual Paulista, Botucatu 18618-970, SP, Brazil,.

Introduction. Global changes, specially the landscape human modifications, have transformed continuous habitats into small and isolated remnants that can show a decrease of population size and disruptions in mating system and gene flow, resulting in a reduction in genetic diversity within populations [11, 1].

The Brazilian savanna is an important centre of vegetal diversity [15] and is included among the 25 hotspots considered a priority for the conservation of global biodiversity [14]. This has been one of Brazil's most threatened ecosystems during the last few decades [5] due to deforestation, introduction of monocultures and pastures, and fire. However, several disturbed areas of Brazilian savanna show an intense and rapid regeneration when the causative agents of disturbance are removed or minimized.

This is particularly due to the strong bud-forming potential of many plants that provides the ability to re-sprout after unfavourable or disturbed periods [16, 6] and to spread by vegetative propagation [10, 2], which is a characteristic of several herbs, subshrubs and trees of Brazilian savanna. These capabilities can lead to important long-term effects on population genetic diversity, including preventing local extinction of several species.

Based on this, we selected *Jacaranda oxyphylla*, a non-arboreal heliophyte, with developed underground system, which occurs in preserved [9] and in disturbed savanna habitats, as a model. We hypothesize that the thickened underground system may have an important role in maintaining the genetic process in a repeatedly and intensely disturbed habitat. So, here we investigated the morphology of underground system and the occurrence of vegetative propagation and compared the genetic structure of populations occurring in two distinctive habitats.

Material and Methods.

Study populations and morphology of underground system of adult plants

We compared two populations of *Jacaranda oxyphylla* Cham. (Bignoniaceae), one situated in a fragment of savanna (22º57'38"S and 48º31'22"O), herein named *savanna area*, and another in one area of a recently cut eucalyptus culture, which was originally covered by savanna vegetation (22º57'02"S and 48º26'24"O), called *disturbed area*. The two populations are located in the municipality of Botucatu, state of São Paulo, South-eastern, Brazil and are about 12 kilometres apart. Voucher specimens were included for taxonomic documentation in the «Irina Delanova Gemtchujnicov» Herbarium (BOTU) at the UNESP – São Paulo State University, Institute of Biosciences of Botucatu, Botany Department (record nos. 24408, 24409, 24410, 24411, 24412).

Holes were dug around 10 specimens to analyse the morphology of the underground system. The occurrence of vegetative propagation and re-sprouting of aerial branches from these structures was evaluated.

Molecular analysis

Total genomic DNA extraction was performed on fully extended young leaves from 46 plants, 23 from each of the populations under study, using the CTAB method described by Doyle & Doyle [7], with modifications. Eight primers were tested (Operon Technologies – OPH04, OPH11, OPH19, OPA12, OPA16, OPM10, OPM16, and OPR12). The DNA samples of each genotype were used in the amplification reactions and the fragments were separated by horizontal electrophoresis in 1.5 % agarose gel, following standard methods. The results were viewed under ultraviolet light and the images were captured with a video camera using the EagleSightII© software program (Stratagene® v.3.2). The gels were interpreted and each individual was genotyped according to the presence or absence of a band for each analyzed primer. These data were employed to build the binary matrix used in the statistical analysis.

Statistical analysis of the molecular data

To analyze the distribution of genetic variability among and within populations of *J. oxyphylla*, only the amplified DNA fragments of greater intensity and reproducibility were used. The percentage of polymorphic loci, with a 95 % probability, was estimated and the distribution of the genetic variation among ($\hat{\Phi}_{ST}$) and within (1 - $\hat{\Phi}_{ST}$) populations was evaluated by analysis of molecular variance – AMOVA [8], using the GENES software program (Bioinformática, UFV, [4]). In addition, the genetic similarities between the populations and among the individuals of each population were calculated with the help of the TFPGA (Tools for Population Genetic Analysis) version 1,3 software program [13]. A dendrogram was built by the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) algorithm developed by Sokal & Michener [17], using Jaccard's similarity coefficient.

Results.

Morphology of underground system of adult plants

The underground system of young plants is an orthotropic axis that consists of the elongated, sinuous hypocotyl, the stem-root transition region and a primary root that is tuberous in their proximal region. Lateral plagiotropic roots arise below the tuberous region.

In the adult plants, the stem portion of the underground system develops in a woody, strongly thickened perennial organ (up to 13cm) with numerous adventitious buds, morphologically characterized as xylopodium. These resting buds are localized on the up region of the xylopodium and possess the ability for sprouting after disturbance. Plants displaying carbonized vestiges of branches at ground level produced new aerial twigs, indicating the species' ability to re-sprout from the xylopodium after being burned and losing its aerial portion. The root system of these adult plants is formed by a thickened, orthotropic tap root that presented around 10 cm diameters at 2 meters deep indicating it can grow several meters deep. Thickened, woody, lateral plagiotropic roots were observed 30–40cm deeper in the soil and presented adventitious buds, which were observed sprouting only after root mechanical injury. Thus, adult plants of *J. oxyphylla* show an underground cauline system (xylopodium), which originates aerial stems and a well-developed primary root system. No connection was observed among neighbour dug up plants, indicating absence of vegetative propagation in the *J. oxyphylla* studied populations.

Genetic diversity

The seven selected primers were OPH04, OPH11, OPH19, OPA12, OPA16, OPM16, and OPM10. A total of 138 bands were found, of which only 73 were suitable for analysis. The percentage of polymorphic loci was 65,7 %, considering the loci whose frequency of the most common allele did not exceed 0,95. The number of polymorphic fragments per primer varied from 4 (OPH04) to 14 polymorphic bands (OPA16).

No clones were detected based on RAPD analysis corroborating the previous findings in plants dug up. The analysis of molecular variance (AMOVA) revealed that most of the genetic diversity occurred within populations. RAPD revealed significant genetic subdivision ($\hat{\Phi}_{ST}$ = 0,1011; P<0,001) and a moderate differentiation between savanna and disturbed area. The *J. oxyphylla* populations exhibited considerable levels of genetic diversity, 89 % of it within populations. The mean similarity (Jaccard coefficient) among the 23 plants representing the savanna population was 0,4224 and that of the 23 plants representing the population of the disturbed was 0,4087, while the magnitude of similarity between the populations was higher, showing a mean value of 0,8288, revealing a little mean distance between populations.

Discussion. The underground structures for storage or vegetative propagation are common in several Brazilian savanna species [10]. Peculiarities of this environment favour species with renewing buds protected at the level of the soil surface, as the hemicryptophytes, or under the ground as the cryptophytes [3], as is the case of *J. oxyphylla*.

The subterranean buds of *J. oxyphylla*, mainly located on xylopodium, allow the reiteration of aerial organs after disturbances, like fire or vegetation cut, or even under natural conditions, in the absence of mechanical injury. On the other hand, the root-buds appear to be stimulated only after serious damage to the plagiotropic roots, considering that we have just found root-sprouting plants in disturbed areas. Similar findings are referred by Klimesová and Klimes [12] for other disturbed communities.

According to parameters established by Wright [18], the populations of *J. oxyphyllla* evaluated here showed the most of the genetic variability within populations.

It is therefore possible to suppose that, the maintenance of interpopulation genetic divergence at moderate levels can be due to the maintenance of original genotypes present in a continuous population existing in the past, which was supported through a perennial bud bank, which persisted in the underground system of this species, notwithstanding anthropic pressures. In this context, we suggest that xylopodium and root buds in *J. oxyphylla* can be considered as a potential germplasm bank, allowing the maintenance of original population genetic variability in rigorously and repeatedly disturbed savanna habitats.

LITERATURE

1. *Aguilar, R. et al.* Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches // Molecular Ecology. 2008. 17. P. 5177–5188.
2. *Alonso, A.A., Machado, S.R.* Morphological and developmental investigations of the underground system of *Erythroxylum* species from Brazilian cerrado // Austr. J. Bot. 2007. 55. P. 749–758.
3. *Batalha, M.A., Martins, F.R.* Life-form spectra of Brazilian cerrado sites // Flora. 2002.197. P. 452–460.
4. Bioinformática, UFV. *Bioinformática* como Ferramenta nas Pesquisas Atuais. In: < http://www.genmelhor.ufv.br/materiais. *2004.*
5. *Carvalho, F.M.V. et al.* The Cerrado into-pieces: habitat fragmentation as a function of landscape use in the savannas of central Brazil // Biological Conservation**.** 2009. 142. P. 1392–1403.
6. *Coutinho, L.M.* O bioma do cerrado // In: Klein, A.L. (Org.) Eugene Warming e o cerrado brasileiro: um século depois. Ed. UNESP, 2002. P. 77–91.
7. *Doyle, J.J., Doyle, J.L.* Isolation of plant DNA from fresh tissue // Focus. 1987.12. P. 13–15.
8. *Excoffier, L. et al.* Analysis of molecular variance inferred from metric distances among DNA halophytes: application to human mitochondrial DNA restriction data // Genetics. 1992.131. P. 479–491.
9. *Gentry, A., Morawetz, W.* Bignoniaceae. Tribe Tecomae // Flora Neotropica Monographs. 1992. 25. P. 51–105.
10. *Gottsberger, G. and Silberbauer-Gottsberger, I.* Life in Cerrado: origin, structure. Dynamics and plant use. Reta Verlag. 2006
11. *Jump, A.S. and Peñuelas, J.* Genetic effects of chronic habitat fragmentation in a wind-pollinated tree // Proc. Natl. Acad. Sci. 2006.103. P. 8096–8100
12. *Klimesová, J., Klimes, L.* Bud banks and their role in vegetative regeneration: a literature review and proposal for sample classification and assessment // Perspective in Plant Ecology, Evolution and Systematics. 2007. 8. P. 115–129.

13. *Miller, M.P.* TFPGA: Tools for population genetic analysis, 1.3 A Windows program for the analysis of allozyme and molecular population genetic data. Logan: Utah State University. In: http://www.marksgeneticsoftware.net. 1997.
14. *Myers, N. et al.* Biodiversity hotspots for conservation priorities // Nature. 2000.403. P. 853–858.
15. *Ratter, J.A. et al.* The Brazilian cerrado vegetation and threats to its biodiversity // Annals of Botany. 1997. 80. P. 223–230.
16. *Rizzini, C.T., Heringer, E.P.* Underground organs of plants from some southern Brazilian savannas, with special references to xylopodium // Phython. 1961.17. P. 105–124.
17. *Sokal, R.R, Michener, D.* A statistical method for evaluation of systematic relationships // University of Kansas Scientific Bulletin. 1958. 38. P. 1409–1438.
18. *Wright, S.* Evolution and the genetics of populations, variability within and among natural populations // Univ. Chicago Press. 1978. Vol. 4.

EFFECTS OF INCLINATION ANGLE OF STEM ON GROWTH STRESS AND ANATOMICAL CHARACTERISTICS IN THREE WOODY ANGIOSPERMS FORMING REACTION WOOD WITHOUT GELATINOUS FIBERS

Hiraiwa T.[1,2], Takashima Y.[1,2], Ishiguri F.[2], Iizuka K.[2], Yokota S.[2], Yoshizawa N.[2]

[1] United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183–8509, Japan
[2] Faculty of Agriculture, Utsunomiya University, Ustunomiya 321–8505, Japan

In woody angiosperms, reaction wood is formed usually on the upper side of inclined stems or branches where excessive tensile growth stress causing large promotion of radial growth generates. In general, it can be characterized by the presence of gelatinous (G) layer being formed in wood fiber cell walls and the reduction in the number and diameter of vessels. On the other hand, in some species which possibly belong to the primitive angiosperms, the G-layer is not always formed in reaction wood fibers. The detailed nature of reaction wood without G-fibers in angiosperms has not been much investigated. In addition, there are less information concerning the effects of the inclination angle of stem from the vertical on the growth stress and anatomical characteristics in reaction wood. In the present study, the reaction wood was artificially formed with inclination angle of 30, 50, and 70 degrees from the vertical in the stems of *Paulownia tomentosa* Steud., *Liriodendron tulipfera* L., and *Osmanthus fragrans* Lour. var. *aurantiacus* Makino, of which reaction wood does not form G-layer in wood fibers. Vertically straight stems were used as normall wood in each species. The effects of inclination angle of stem from the vertical were investigated on the longitudinal-released strain and the change of anatomical characteristics.

In each species, generation of large negative longitudinal-released strain was confirmed on the upper side of all the inclined stems compared to the control stems having formed normal wood. Xylem formed with inclination angle of 30 degrees showed the largest tensile stress on the upper side of the inclined stem of *P. tomentosa*, whereas, in *L. tulipfera* and *O. fragrans*, inclination angle of the 50 degrees gave the largest tensile growth stress on their stems.

In all the three species examined here, radial growth was promoted on the upper side of inclined stems. The number of vessels in all the reaction woods formed significantly decreased in comparison with that of normal wood, suggesting that the influence of stem inclination angle is greater rather than growth promotion. On the other hand, the changes in the diameter of vessels and the length of wood fibers and vessel elements were different among species and among their different inclination angles. Reaction wood of *P. tomentosa* increased vessel diameter, whereas that of *O. fragrans* decreased it compared with normal wood, and *L. tulipfera* showed no difference in vessel diameter between normal and reaction woods. The length of wood fibers and vessel elements significantly increased in reaction wood of *P. tomentosa* and *O. fragrans*, while both of them decreased significantly in reaction wood of *L. tulipfera*.

The typical three-layered structure (S1+S2+S3) was comfirmed in the secondary wall of normal wood fibers in three species. On the other hand, reaction wood fibers of *L. tulipfera* and *O. fragrans* lacked the S3 layer, whereas reaction wood fibers of *P. tomentosa* showed a three layered structure. In these species, reaction wood formed with the inclination angle of 30 and 50 degrees showed smaller microfibril