Biosafety of the herbicide resistant GM soybean 40-3-2: experience in the Russian Federation

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Soybeans are cultivated in two regions of the Russian Federation: the Far East along the Chinese border, and the area south of the European part of Russia. The Far East is also an area where wild relatives of soybeans occur naturally. Cytological, morphological, and molecular data and data on cross-pollination suggest that *G. soja* is the probable ancestor of *G. max*. *Glycine gracilis* is considered to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. *Glycine gracilis* may be an intermediate in the speciation of *G. max* from *G. soja* or it may be a hybrid between *G. soja* and *G. Max*. Thus there is a potential risk in introducing genetically modified (GM) cultivars to the Far East region. (Fig. 1 – 5)

Fig. 1 *G. max.*

Fig. 2 *G. soja*

Fig. 3 *Glycine gracilis*

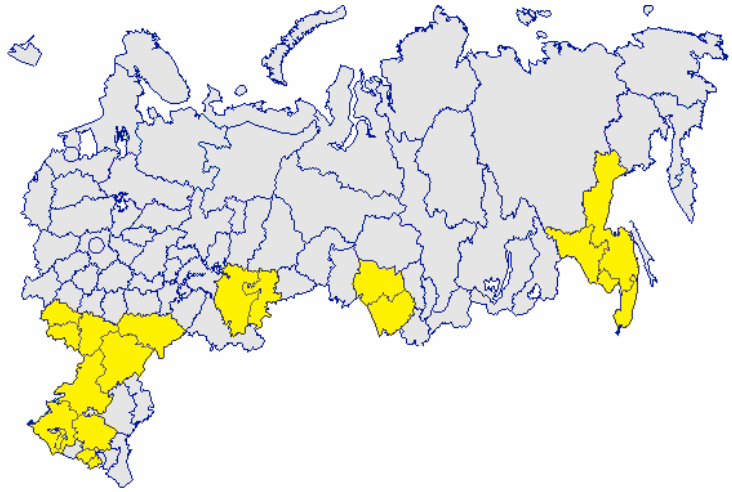

Fig. 4 Distribution of cultivated Soy in Russia

Fig. 5 Distribution of Wild Soy in Russia

Several field expeditions aimed at collection of wild soybean plants in its native areas were organized during 1998-2000 by the Centre of "Bioengineering" (RAS, Moscow), Institute of Cytology and Genetics (Siberian Branch of RAS, Novosibirsk), with participation of the Pacific Institute of Biorganic Chemistry (Far Eastern Branch of RAS, Vladivostok) and the Institute of Soybean (Far East Branch of RAAS, Blagoveschensk). Plants were collected in the Amurski and Primorski regions, in the Hasansky district; and on the islands of Gulf of Peter the Great. (Fig. 6)

Fig. 6 Field expedition aimed on collection of wild soy plants in its native areas in Russian Far East

Collections were made during the last half of September, when soybean seeds were maturing. (Fig.7) Geobotanical descriptions of phytocenoses where wild soybeans occurred were made according to previously published procedures (Dymina et al., 2001). Based on the analysis of 100 phytocenoses, four major phytocenosis groups for wild soybeans were found. The first group, the pioneer phytocenosis group, is characterized by plants from early stages of plant succession. The second group is composed of plants growing near water barriers and terraces. The third group is composed of plants inhabiting wet meadows. The fourth group is composed of plants inhabiting marshy meadows. The last phytocenosis group was found in the Primorsk region only.

Fig 7. Maturing of wild soy seeds

215 plants representing 40 wild soybean populations and 2 cultivated soybean varieties (*G. max*) were used to estimate genetic polymorphisms between genotypes and inter-population diversity by means of RAPD markers. The genetic distances between samples of wild soybean varied from 0.27 to 0.69. The genetic distance between wild and cultivated soybeans varied from 0.47 to 0.51. The genetic distance between cultivated soybean varieties “Severnaya 4” and “Stine 2254RR” was 0.22. A cluster analysis indicated that most of the wild soybean accessions were grouped according to their geographical locations. It was clear from our data that wild soybean populations vary according to the regions in which they occur. (Seitova AM., et al., 2004; Dorokhov D. et al., 2004) Fig. 8 - 9.

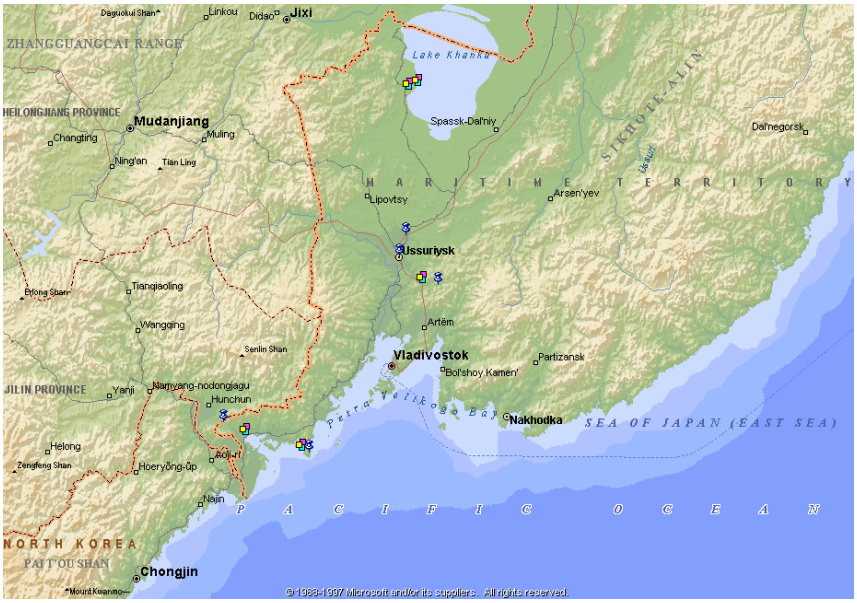

Fig. 8 Map of wild soy collection sites at Far East Region of Russia

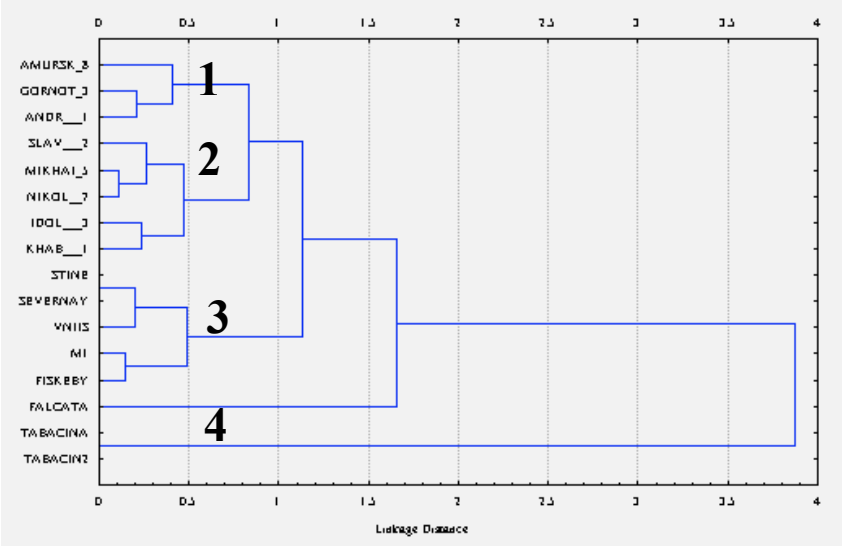

Fig. 9. Cluster analysis (Wards method) of wild and cultivated soybean accessions based on genetic distance revealed with RAPD markers.
Clusters: 1, Amurskaya region, Gornotaezhnoe, Andreevka; 2, Slavyanka, Mikhailovka, Nikolaevka, Idol, Khabarovk; 3, cultivated soybean. Outgroup: *G. falcata* and *G. tabacina.*

It is not clear whether soybean is a weedy (invasive) plant or not. *G. max* is not found outside of cultivation in most countries. In managed ecosystems, soybeans do not effectively compete with cultivated plants or become primary colonizers. *G. max* is not

listed as noxious weed in the Weed Seed Order (1986). It is not reported as a pest or weed in managed ecosystems, nor is it recorded as being invasive of natural ecosystems. In summary, there is no evidence that *G. max* has weed or pest characteristics anywhere in the world. Skvortsov (1927) mentioned wild soy as a plant of abandoned fields. Komarov (1958) described wild soy as a semi-weed in wheat stands. *G. soja* is more a ruderal plant than it is a field weed in that it occupies disturbed soil left without further cultivation. Biological traits of wild soy do not satisfy minimal qualifications for being defined as a weed in that it has long growing season, around 4 months; it is late in flowering and late in producing mature seed; and is has few seeds (100-250 per plant). If soy plants cover up to 2.5% of an area, they have no means to spread their seed over long distances (Dymina et al., 2001). Spring flooding is probably the most important means by which soybean seeds are of spread over long distances. Riverbanks and wet meadows are the most common niches for wild soy in the Russian Far East region. Soy is also common in disturbed phytocinoses, but relative rare in farmed fields.

Potential crossability of these closely related soy species was evaluated at field condition and in greenhouse. Several local cultivars of soybean were successfully fertilized by pollen from wild soy. However, when GM soybean cv. “Stine 2254 RR” was used as a pollen "donor" and plants of *G. soja* were the pollen "trap", no any herbicide-resistance plants were obtained during two growing seasons. Thus, natural cross-pollination between plants of the soybean species was extremely rare event, with possible frequency below sensitivity of the experiment. Detectable hybridization rate in artificial pollination between G. *max* and G. *soja* was demonstrated in controlled conditions of greenhouse. In average, 3.7% of cross-pollination gave fertile F_1 seeds. These seeds were of variable color and size and differed from the seeds from their parents. Plants obtained from the hybrid F_1 “Stine 2254 RR” x G. *soja* possessed RAPD and ISSR complex patterns typical of from both parents. Transgenic DNA was detected by PCR analysis in the F_1 hybrids; however, the target DNA fragment was not detected in F_2 and F_3 plants. It testifies that in the case of “RoundUp Ready” soybean the transgenic DNA could be eliminated already in F_2 progeny of hybrids between GM and wild plants of soybean. (Fig. 10)

Fig. 10 Study potential crossability of the GM soybean cv. “Stine 2254 RR” and *G. soja*

Outcrossing (gene flow) refers to the transfer of genetic material from one crop to another or from a crop to a weed. The implications of gene flow from a crop to a weed depend on many factors. In the presence of selection pressure, the crop-weed hybrids may or may not have a greater adaptive advantage compared to their parents. If hybrids are more competitive, there can be in increase in weediness (Cherve et al., 1999). The chances of the transfer of glyphosate resistance to wild soy in regions of Asia and Russian Far East are limited by other factors such as flowering asynchrony between soybean and its relatives; extent of sexual compatibility; abundance, method, and distance of pollen spread; and environmental conditions pertinent to cross-pollination. Transfer of genetic material from GM soybean to wild relatives or other soybeans is small. Our results completely support this point of view. However, more data is needed to fully evaluate the extent of transfer of herbicide tolerance genes from widespread cultivation of soybeans to wild soy that might occur in region Far East of Russia.

For this reason, now we carry out special researches whenever possible naturally hybridizations between G. *max* and G. *soja* in native condition of the Far East of Russia. The wild soy is involved in experiment collected from natural populations which settle down near to agronomic fields of with cultivated soybean. During two years, hybrid plants have not been found out.

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