Genetic engineering of plants for diseases and pest resistance

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Chemical resistance to rice blast, *Magnaporthe grisea*

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Abstract. The interaction between rice and the blast pathogen, *Magnaporthe grisea*, includes the production of anti-fungal compounds by the host. The role of these compounds in resistance to blast is being investigated. Thus, the differences in constitutive and induced anti-fungal compounds (phytoalexins) between blast susceptible and resistant rice cultivars is being determined. The five cultivars studied have been obtained from the International Rice Research Institute, Los Baños, Philippines. A method using an abiotic elicitor (irradiation by shortwave ultraviolet light) has been developed in order to determine the response of the host while unaffected by the presence of the pathogen. For this method, detached rice leaves taken from six weeks old plants are used. The phytoalexins elicited in this way have been mainly found to be the rice flavanone (sakuranetin) and the diterpenoids (momilactones and oryzalexins) which are formed by separate biosynthetic pathways. As sakuranetin was absent in the untreated leaves, it was therefore shown to be an induced compound.

By HPLC analysis, it has been shown that the susceptible cultivar IR50 produced only a negligible amount of sakuranetin and none was produced by the susceptible cultivar B40. In contrast, the resistant cultivars, Tetep and Ta-poo-cho-z , produced 17 to 20 ppm sakuranetin, which could account for their resistance. However, the resistance in the Carreon cultivar cannot be accounted for by sakuranetin production alone, as the levels produced are comparable to those recovered from the susceptible cultivars. Sakuranetin, although in low level, has also been recovered from Tetep plants punch-inoculated with *Magnaporthe grisea*.

Momilactone A and oryzalexin D, as well as sakuranetin, have been detected by TLC by *Cladosporium herbarium* bioassay and sulphuric acid/vanillin indicator in Tetep, Carreon, Ta-poo-cho-z and IR50. Sakuranetin, nomilactone A and oryzalexin D were not produced by the rice cultivar B40.

Rice breeding and blast prevention in Italy

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Abstract. Blast (*Pyricularia grisea*) is a very important constraint in the Italian monocultural rice system, especially because it appears as a limiting factor for varieties response to high nitrogen rates. Periodically, depending on meteorological cultural conditions, severe blast epidemics occur that cause widespread losses in some rice areas. Environmental conditions (climate, soil, treatments) have a strong influence on the development of this disease. The damage caused to the varieties most commonly used at the moment is increasingly important because of the long tradition of rice cultivation in our region.

Climatology

From the point of view of climatic conditions, the main area of Italy under rice cultivation, located in the Po Valley, has a climate with hot summers and generally high air humidity. Fortunately, high temperature conditions and insufficient sources of primary blast inoculum seldom result in early epidemic attacks of *P. grisea* at seedling and vegetative stages during the growth season.

More frequently, rotten neck (neck blast) and panicle symptoms develop after heading stage, in August, wih the change in the climatic conditions, due principally to a varying daily range of temperatures and increased air humidity (fog, dew or rain). Generally, the yield losses for a variety are more influenced by the contemporary development of leaf blast and neck blast symptoms.

The influence of cultivation techniques

In addition to the very important role played by climatic conditions, the nitrogen rates and timing of application dramatically influence the blast susceptibility of rice cultivars. High soil fertility due to increasing rates of nitrogen application (about 200 kg/ha) or natural organic components (peaty soil) generally increase the severity of blast attacks.

Split nitrogen application can greatly determine rice cultivars vulnerability and generous amounts of nitrogen fertilizer applied during the vegetative period, i.e. at the mid-tillering stage, favour early epidemics. Our experiments on rice field fertilization have allowed us to prove that late nitrogen topdressing at reproductive stage, at panicle initiation, did not generally increase blast symptoms. This technique is therefore strongly recommended for reducing the damaging effects of blast.
Plant stress caused by crucial cultivation conditions, like the water stress in dryland cultivation, can increase the susceptibility of rice varieties.

This method of rice cultivation is receiving great attention in some non traditional rice areas in Italy. The prolonged soil moisture stress under upland conditions in general produces heavy infections in the presence of adequate sources of blast inoculum.

Breeding for blast resistance

Although attempts have been made to introduce chemical prevention by spraying crops with fungicides, the use of tolerant varieties is still considered the most feasible and economical strategy to control rice blast. The commercial seed is generally dressed with various products in order to prevent spores dissemination, even if the role of rice seed as a possible source of primary blast infection is rather questionable.

Because of the prolonged selection pressure on the Italian rice germplasm, several varieties can be used as sources of resistance in breeding programmes. For the identification and assessment of the resistant varieties, we have used a modified International Blast Nurseries Test, based on the varietal response to natural infections from different races of the pathogen. Scoring of plant reaction to blast is made later, at panicle stage, in order to evaluate the neck blast resistance. An experimental screening device repeated several years allowed us to identify a very promising line with high blast resistance, ISC 403 ([RB x Nano)] x [Bajang x Allorio]). Later, this line has been released as a commercial indica type variety which was named *Panda*.

There is evidence that in farm conditions the presence of a large number of cultivars, with one or more resistance genes for rice blast, cropped in the same area, is a good means to prevent evolutionary adaptation of pathogen population to new varieties.

Screening for blast resistance was also carried out by *in vitro* culture of calli using phytotoxins for selection of resistant material. In this case calli obtained from immature embryos were treated in an *in vitro* comparative test with the picolinic acid (the main component of the blast phytotoxin) produced synthetically, and the sterile filtrate of the pathogen. The results of this first experiment showed a good accordance between the effects of the phytotoxin and pathogen filtrate and with the *in vivo* test.

The use of phytotoxins for screening rice varieties resistant to blast has appeared to be a promising technique, even if it has obvious limitations.

Genetic engineering of plants for diseases and pest resistance

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Abstract. The aim of this work was to demonstrate the possible biotechnological value of plants transformed with some proteinase inhibitor genes and antibacterial polypeptides genes for engineering pest and diseases resistance in plants. In the case of proteinase inhibitors among the different gene sources, we used soybean because: (i) soybean proteinase inhibitors are the most studied antinutritional factors; the metabolic disturbance caused by these inhibitors in man and in several monogastric animals is well known; methods to quantitate soybean inhibitors are simple, sensitive and reliable; their inactivation by heat is already applied at an industrial scale during the preparation of soy products, (ii) The soybean Kunitz factor is the most powerful trypsin inhibitor found in nature, (iii) the C-II inhibitor is able to inactivate both chymotrypsin and elastase, (iv) The soybean PI-IV inhibitor is supposed to have a biological significance that exceeds the limits of the weak inhibition of mammalian trypsin, (v) the soybean cystatin is a protein active against papaine and bromelain of coleoptera. A wide array of different antibacterial polypeptides in organisms ranging from insects to mammalians have been isolated and charactarized; we are interested in cecropins, a family of antibacterial polypeptides originally found in the hemolynph of insects.

In this seminar we present the results obtained by engineering tobacco plants with some of the mentioned genes.

Rice cultivation and varietal improvement in Hungary

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Abstract. Hungary is situated on the northern part of the European rice growing zone. Rice cultivation extends to 48° N latitude. The main environmental constraints of production are related to the climatic situation of that area. Low temperature and the short growth period are the main limiting factors for rice cultivation. Varieties which are highly adapted to this special environment are considered as a very important need for successful production.

Since the possibilities for introducing foreign rice varieties to the local conditions are very limited, the national variety improvement program has been very much involved in increasing the genetic variability of the existing cold tolerant and early type, photoperiodically insensitive materials, which dominantly belong to the japonica group. Basically, the germplasm has come from Russia, Italy, Korea and Japan. Besides the crossings, induced mutations and biotechnological methods were also successfully adapted in rice varietal improvement.

As a result of the breeding program started during the 1950s, 17 Hungarian rice varieties were released between 1964 and 1993, with a yield potential of 4-8 t/ha. Rice breeding in Hungary is presently focusing on developing very early, cold tolerant, blast resistant varieties with good yielding ability on irrigated lowlands. High milling yield and good cooking and eating quality are also required. In the framework of a research project dealing with the improvement of a sprinkler irrigated rice production technology, drought tolerant lines were also developed for irrigated uplands.

Phylogenetic relationships among *Magnaporthe grisea* isolates from different host plants

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Abstract. *Magnaporthe grisea* is a fungal pathogen of monocots, including rice. We estimated the genetic diversity of *M. grisea* species and its structure using rDNA and single copy genes RFLP. We analyzed a collection of 75 isolates from diverse geographic origins and 20 host plants. *M. grisea* isolates were classified into ten distinct groups differing by their rDNA restriction sites map. Variations of rDNA unit length, located in the IGS, were detected within three of these groups. We sequenced the rDNA intergenic transcribed spacers (ITS1 and ITS2) of a subset of isolates with different rDNA restriction maps. The ITS phylogenetic tree revealed four highly divergent populations within *M. grisea,* which could be considered as subspecies using molecular (> 7% nucleotidic divergence in ITS) or biological criteria (crosses without viable progeny), despite a lack of morphological differences. Three populations (II, III and IV) were monomorphic when considering their ITS sequences and each corresponded to isolates from only one host plant (*Digitaria* spp. or *Pennisetum* spp.). Population I was subdivided into 3 related groups differing by their ITS sequences and rDNA restriction maps. Each of these groups had a low level of polymorphisms revealed by single copy RFLPs and corresponded to isolates from a limited number of host plants, such as rice and *Panicum repens* (group 1), *Eleusine* spp. and wheat (group 2) or *Eleusine* spp. and *Eragrotis* (group 3). Cluster analysis using single-copy gene RFLPs confirmed the relationships deduced from ITS phylogeny. Repeated and dispersed sequences such as transposons pot3/MGR586 or retrotransposons Grasshopper and Fosbury/Maggy, were detected in high copy number in specific groups of isolates sharing the same or related alleles at rDNA and single copy gene loci. This discontinuous distribution of repeated sequences strongly supported the existence of genetically isolated populations within *M. grisea* species. Such populations were structured mostly according to their host plants and not to their geographic origins. Therefore, selection by different host plants must have played a significant role in the evolution of this fungal species.