General defense system in the plant kingdom II.

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Summary: In addition to successes achieved in certain varieties in resistance breeding based on a defense reaction of host plants involving hypersensitive tissue destruction, resistant varieties putting a very strong selection pressure on pathogens have selected more and more aggressive types of pathogens. The never-ending race between plant and pathogen resulting from this can only be controlled by a defense system characterised by a different strategy. In each of the plant species that we bred a defence system was found, which contrary to hypersensitive reaction strives to keep the tissues at all costs and is not pathogen specific. This is implied in the term *general defense system.*

Introduction

Plants are forced to develop defense systems against different pathogenic microbes causing disease. The first defense reaction was discovered by Stakman in relation of wheat and rust as host and pathogen *(Stakman* 1915). Then Holmes described the defense reaction against the tobacco mosaic virus concomitant with fast tissue destruction *(Holmes* 1929, 1938). Klement observed the same reaction called "hypersensitive tissue destruction", or HR as a consequence of bacterial infection, similar to those described in relation of fungus and virus infections *(Klement* 1964). Resistance breeding so far has been based on these specific defense reactions efficient particularly against one particular pathotype of the pathogen. These discoveries shaped the technique applied by breeders endeavored to develop disease resistant varieties.

The resistance to one pathogen based on the hypersensitive tissue destruction (HR) of the host plant exerted strong selection pressure on the population of the given pathogen species. Meanwhile breeders owe to its spectacular practical though temporary results. Consequently, more and more aggressive (or virulent) pathotypes of the pathogen species were selected and in this way a boundless competition began between evolution of the pathogen and breeders of the host plant. This problem created the demand to find another type of disease-resistance with a different strategy. The most desired feature required from that disease resistance is to ensure a less specific, wide defense spectrum against different pathotypes arising in the future. The purposeful search for a new types of resistance over several decades was justified as the most aggressive (or virulent) pathotypes could not be controlled by the resistance genes known to act by the mechanism of hypersensitive tissue destruction *(Szarka & Csillery,* 1995). The most important feature of this new resistance mechanism is that it hinders the invasion of microbes by thickened and closely packed tissues and not by hypersensitive tissue destruction. In addition, its reaction speed is faster than that of the hypersensitive reaction, it is less subject to environmental impacts and even susceptible plants contain features which can enrich this defense system and works efficiently both in the case of compatible and incompatible plant-microbe relations. On this basis, this reaction is called general defense system (*gds*).

Observation

The *general defense system* tries to preserve the cells and tissues attacked by microbes instead of fast tissue destruction and this is achieved by tissue packing. Cell and tissue packing against environmental stress is common in the plant kingdom. The hyphae of *Sclerotinia sclerotiorum* are packed into sclerotium under unfavorable environmental conditions *(Figure 1)*. The myceliums of *Agaricus bisporus*, mushroom, in the case of lack of water and nutrient and unfavorable changes in the carbon dioxide contents of the air will only pack into carpophore primordia if they are affected by some foreign protein *(Figure 2)*. Carpophores cannot differentiate under microbe-free sterile conditions. The saprohyte *Pseudomonas fluorescens* bacterium also may attack the carpophore, the hyphae developing from primordium at over 90 percent humidity of the air, which significantly decrease mainly caused by epidemic bacterium infection. In the case of *Xanthomonas* epidemic, the bacterium spotted leaves and the flowers will fall greatly, which has sensitive effect on the quantity of the produce. Quality reduction resulting from the loss of first fruit stet is even more serious than the decreasing quantity. Produces, which arose from the earliest (first) fruits set had longer period of time to develop suitable color and aroma. Saving these valuable first fruits, resistance to *Xanthomonas* bacterium may improve the quality of the ground pepper.

Resulting from the host-pathogen relation of the spice pepper and the *Xanthomonas campestris pv. vesicatoria* bacterium, the most efficient way to prevent epidemics is the development or restoration of the natural defense system of spice pepper, the resistance to bacterium. In this way seed cultivation without infection and one of the most serious plant protection problems of spice pepper production are solved.

In course of breeding spice pepper resistant to *Xanthomonas campestris pv. vesicatoria* bacterium, out of the well-known genes providing protection, the efficiency of only the *Bs-2* gene was found satisfactory against the Hungarian pathogen population. In addition to special resistance gene, the *Bs-2*, we have been working for almost a decade on incorporating the *gds* gene into spice pepper. We discovered and described this gene, which develops a defense system *(Figure 1)*.

Water soaked spots of 3–4 mm diameter develop on the leaves of susceptible pepper plants infected by bacterium under natural conditions. Later the spots desiccate, become white and wear into holes as the leaves grow. The plants containing *Bs-2* resistance gene have at most 1 mm, quickly drying purple-brown lesions, while plants having *gds* gene localize the bacterium in a hardly visible, tiny pin point-like spot under natural conditions.

In the case of the three reaction types, the differences of the changes in the tissues are shown convincingly by the injection of bacterium suspension into leaf. The injected tissues of the susceptible single organisms will become water soaked in 5–6 days and later become brown and dry. The infected tissues of the plants with *Bs-2* resistance will become purple-like in 2 days and will dry only in some days. The injected tissue on the leaves of the plants containing *gds* gene will turn to yellow (chlorotic) only in 7–8 days, but the tissue overwhelmed with the bacterium is not seen any more, but will thicken and harden already on the first day and will not dry even over weeks. An important criterion is, that not slow but, on the contrary very fast plant reaction is observed as changes detectable later in the tissues injected.

Resistance gene *Bs-2* provides satisfactory defense for the spice pepper during the whole cultivation period. The spice pepper varieties **Kaldom** *(Figure 2)* and **Kalorez** *(Figure 3)* produced as first results during the breeding work

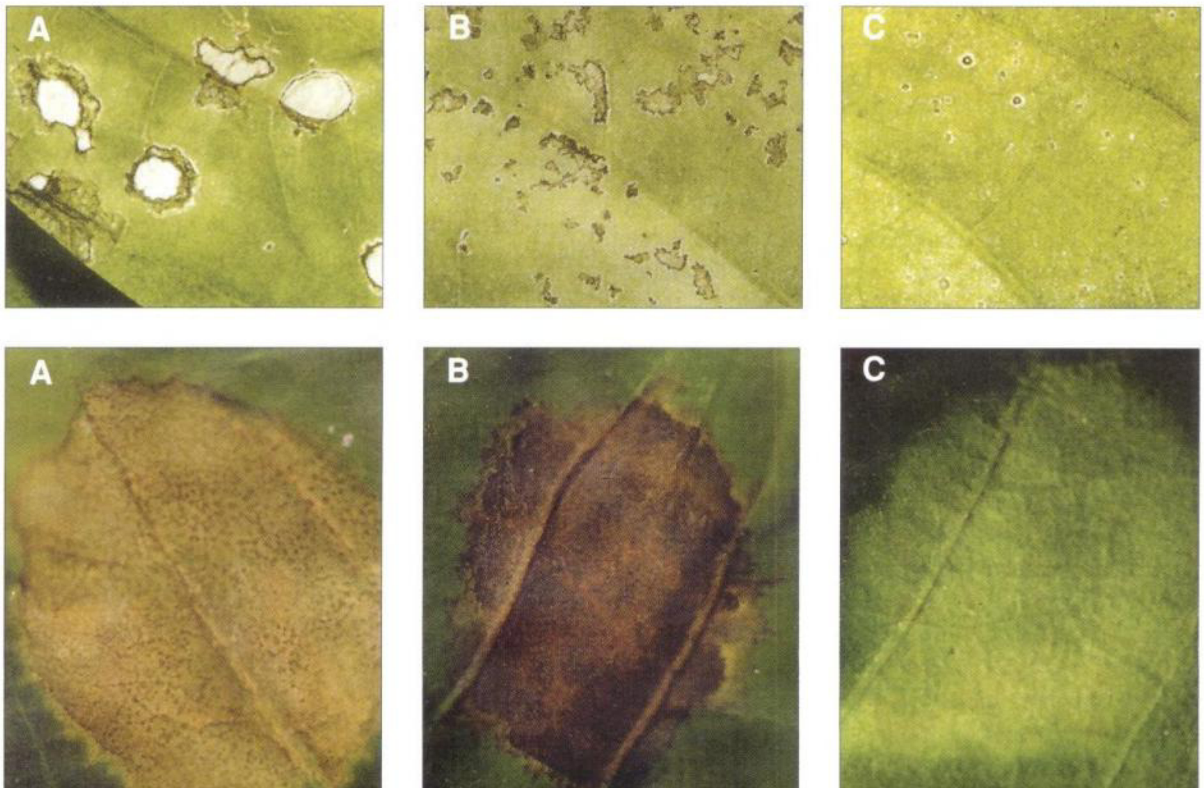

Figure 1 Symptoms of spice pepper leaves of different genotzpes, (A) susceptible, (B) *Bs-2* specific resistance gene and (C) *gds* gene creating general defense system, following inoculation technique by painting (brush) – upper row and injection – lower row

at the Kalocsa institute of the Red Pepper Research-Development C.B.C. contain the *Bs-2* resistance gene.

Kaldom is a semi-determinate growing variety candidate without pungency with upright fruit position. Its bush is 35–45 cm high, its stem is rigid with loose leafage and short offshoots of stalks. Its leaves are thickened like leather, slightly wavy and dark green. The fruit is 12–15 cm long, tapering gradually, slightly bent and pointed. The color is ripe dark red. Its cultivation period is short, grows ripe early and is recommended especially for direct seeding. Excellent color, taste and flavor are characteristic features and it is suitable for producing high quality ground pepper. It is resistant to *X. c. pv. vesicatoria* bacterium and may be cultivated with an environmental-friendly technology.

Kalorez is a continuously growing variety candidate without pungency with hanging fruits. Its bush is 50–55 cm high with closed leafage, growing fast. Its leaves are medium green. It grows ripe in medium term-early, the fruits are picked easily. It is highly productive, recommended mainly for transplanting into the field (no direct seeding). Good coloring capacity, pleasant taste and flavor characterize the fruit. Excellent basic material to produce quality ground pepper being resistant to *X.C. pv. vesicatoria* bacterium and may be cultivated with environmental-friendly technology.

Figure 2 Spice pepper variety **Kaldom** carries *Bs-2* gene providing resistance to the bacterium *Xanthomonas campestris pv. vesicatoria*

Figure 3 Spice pepper variety **Kalorez** is protected against the bacterium *Xanthomonas campestris pv. vesicatoria* by *Bs-2* gene

The features ensured by the *gds* gene are favorably fitted to the spice pepper varieties, since they provide defense not only against *X. c. pv. vesicatoria* bacterium but also ensures a biological condition for the plant, which gives efficient defense against other pathogens and environmental factors. The features defined by the *gds* gene basically differ from the resistance reactions localizing pathogens by fast tissue destruction called hypersensitive, which has been widely utilized so far in plant breeding. The basic difference is that the system ensured by the *gds* gene wants to preserve the infected tissues by all means is not willing so to say to sacrifice the tissue to exclude the pathogen (see *Figure 1.)*. It is achieved by far much faster localization of the pathogens than the reaction known so far. Besides the resistance of the bacterium, the tolerance of plants to environmental factors is also better as a result of the *gds* gene, because their roots are stronger and the leafage is dark green. Excellent tolerance to environmental factors comes from this feature.

The goal of the team working together for several years is the development of "isogenic" spice pepper varieties, which contain *Bs-2* and *gds* resistance genes alternatively.

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