III.8.1.1. METHODS TO SCREEN FOR RESISTANCE AGAINST FOLIAR BACTERIAL PATHOGENS

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The most effective control method for bacterial pathogens is unquestionably varietal resistance. In order to successfully detect useful levels of resistance in crop species, the methods used for assessing resistance are of primary importance. At the very least these methods must expose the plant to adequate levels of inoculum under conditions likely to be encountered in nature at a growth stage of the plant in which differences in susceptibility can be measured.

Screening methodologies may vary depending upon objectives. For example, methodologies developed to identify primary sources of resistance may be very detailed and highly controlled, while those used for screening large breeding populations known to be segregating for resistance can usually be simpler. In either case a number of key points must be be addressed to develop a successful methodology. These include appropriate environmental conditions, adequate and good quality inoculum, plant material at the proper developmental stage, inoculation and incubation methods that yield dependable symptom expression and rating methods that clearly separate resistant from susceptible. These points are considered in more detail in this chapter.

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8.1.1.1. Environment

Appropriate environment is necessary for successful infection by any pathogen. Temperature and relative humidity are among the most important environmental factors for bacterial plant pathogens and, with light intensity, are among those most easily controlled (Kiraly et. al, 1974). These may directly influence pathogen survival prior to infection, determine its ability to enter the plant, or influence key processes in the plant directly related to infection, such as stomatal behavior and exudation at hydathodes, or directly affect physiological susceptibility (Colhoun,1973).

Screening in glasshouses or screenhouses compared to the field has a number of advantages and disadvantages. Certainly a main advantage is one of convenience. The researcher is not confined to the warm growing season in the temperate zones or to the rainy season in the tropics. As well, there is generally more precision, since environmental conditions can be precisely controlled in a growth chamber and to some extent in glasshouses. Growing conditions are more uniform, can be controlled and can be carefully monitored to assure that the conditions permitting expression of symptoms are continuously maintained. Likewise, sterile soil may be used to avoid interference by soil pathogens.

In cases where environmental conditions determining symptom expression exceed normal, expected, or predicted field conditions, controlled conditions are warranted. For example, for symptoms caused by Xanthomonas campestris pv. phaseoli var. soyensis (bacterial pustule of soybeans) to develope normally, daytime temperatures should be at least 30°C (Chamberlain, 1962). Day/night temperatures of 25°C/17°C favor rice sheath brown rot development (caused by Pseudomonas fuscovaginae) over temperatures of 29°C/23°C. The latter pathogen also

requires at least 15 hours 100% RH for infection, when inoculation is by simple spraying (Miyajima, 1983). Where a number of pathogenic races are thought to exist [such as in the X. campestris malvacearum (Brinkerhoff, 1970) and X. campestris pv. oryzae (Mew, 1987)] screen or glasshouse inoculation offers complete control over inoculum source, permitting a careful analysis of resistance genes to each race. This can be very useful when attempting to combine, or "pyramid" resistance genes.

Where these relatively sophisticated facilities are unavailable, such as in many developing countries, or inappropriate, such as for screening large segregating populations, manipulation of the field environment is best. Semi-controlled field evaluations offer a number of advantages as well, many of which respond to the disadvantages of screen or glasshouse evaluation. There is usually abundant space and the flexibility to permit the plant to mature while monitoring disease progress, both within the individuals and among individuals of a given line or population. The former permits the researcher to distinguish lines which "recover" to produce some yield from those which do not, while the latter may give some comparison of the relative impact of different resistances on disease progress. If these evaluations occur under conditions which approximate the production environment, they may provide a more accurate picture of the usefulness of the resistance than that obtained in the glasshouse.

Site selection is the most important factor determining the success or failure of a field screening method. Choosing a site where the pathogen is endemic and causes serious losses will assure that during some periods of favorable environmental conditions there will be disease development. By carefully manipulating planting time a researcher can

increase the probability that the appropriate growth stage will coincide with favorable environmental conditions. Other factors such as soil fertility, irrigation, plant density may also be adjusted to favor disease development. However, rarely can a field method alone be expected to yield uniform and repeated disease levels; for these reasons most researchers prefer to use some level of controlled inoculation methods. While the environment tends mainly to influence successful infection, a number of methods have been developed to enhance success and reduce environmental effects. These will be presented in more detail under "Inoculation methods." Of course if post-inoculation disease development is highly dependent on the environment, the researcher assumes a certain risk that a given trial will yield a "no test."

It should be emphasized that when the precision and convenience of growth chamber or screen/glasshouse testing determine the primary screening sites, release of a variety should never be contemplated before thorough, adequate field confirmation of resistance has been completed. This should be conducted under a range of realistic production environments.

8.1.1.2. Inoculum

Information on this topic is also given in Chapter I.5 "Inoculation of plant tissue" by Kement and Chapter II.8.3. "Resistance screening with mixtures of strains and races or with individual bacterial isolates", by Kennedy in this book.

For pathogens where several races are known to exist mixed inoculum maybe adequate to assure evaluating resistance against the different races. Using race mixtures of X. campestris pv. malvacearum in controlled inoculations permitted selection of plants with resistance to

several races (El-Zik and Bird, 1970). However, prior research should be done to determine that the isolates do not interfere with one another when they are mixed. Such interference could occur when one isolate is capable of inducing a resistant reaction in the host plant or decreasing the aggressiveness of the other strain. For example, in the case of X. campestris pv. oryzae race mixtures compared over different compatible and incompatible race-resistance combinations gave different results on different cultivars (Reddy and Kauffmann, 1974). Thus, before using race mixtures their effectiveness must be proved on a range of different, and known, combinations of resistance sources.

In producing inoculum care must be taken to use pure isolates of the pathogen with known aggressiveness and virulence. For small-scale screening the pathogen can be raised on solid medium; however, for large-scale screening a liquid medium may be more convenient. The medium chosen should permit the most rapid growth of the pathogen with a low risk of loss of virulence. Isolates must be periodically checked both for purity and virulence. Appropriate inoculum concentration will be determined in part by the inoculation method to be used. The actual concentration will depend upon the pathogen, the method of inoculation, and the environment. In general, concentrations vary from 10^5 to 10^9 cfu/ml, most commonly determined by colorimetic absorbance (Kiraly, et. al, 1974). The lower concentrations are used for vacuum infiltrations or atomizing inoculum. The overriding objective is to use a concentration that will produce typical symptoms on a known susceptible cultivar, or line, but permit expression of resistance on a known resistant line. A general discussion on the effectivity titration with bacterial plant pathogens was presented by Ercolani (1984) which gives detailed information on the topic.

When a large amount of inoculum is prepared there is the risk that virulence may decrease, particularly when there is delay between preparation and inoculation. Virulence of the bacteria to be inoculated can be stabilized by buffering the inoculum (e.g. as is done with P. phaseolicola in 0.01 M $MgSO_4$). Inoculum may be kept as infected seed, dried plant parts, agar cultures with calcium carbonate, or under sterile water. The virulence of some pathogens such as P. glumae which are notoriously unstable in culture can be maintained by routine passes through susceptible cultivars. Stock agar-cultures of all isolates should be lyophilized and maintained as reference strains to monitor the accuracy and stability of screening over time.

8.1.1.3. Plant material

It is important that plant material be pathogen-free prior to screening. This is true particularly in the case of seed-transmitted pathogens. The nutritional status of the plant is very important for most bacterial pathogen-plant interactions. High levels of N and P increase maize susceptibility to E. stewartii while elevated level of Ca and K increase resistance (Pepper, 1967). However, increasing fertilization rates has also been reported to increase resistance to P. campestris pv. hederae. (Chase and Poole, 1987). Micronutrients also may affect the development of bacterial diseases; Mg and Cu were found to be important in the development of bacterial blight symptoms on rice (Phillip & Devadath, 1984). It is in general advisable to investigate the effect of macro-and-micro-nutrients on disease severity when screening for resistance to a bacterial pathogen in order to enhance results.

Plant tissues may differ in the susceptibility to bacterial pathogens according to their maturity or morphological stage of

development. Leaf sheaths of rice seedlings spray-inoculated with the rice sheath brown rot pathogen (P. fuscavaginae) do not develop symptoms. However, as plants approach flowering, the sheath enveloping the fluorescence is readily infected when inoculated in the same manner (Miyajima, 1983; Zeigler and Alvarez, 1987). Stem tissues of cassava become resistant to X. campestris pv. manihotis with age as lignification progresses (Lozano, 1986). The phenomenon of different responses at different developmental stages is very common and should be a foremost consideration when devising a resistance screening methodology.

The age of the plant can also affect the interpretation of the manner of inheritance of resistance, and consequently, breeding strategies. In rice, resistance to one isolate of X. campestris pv. oryzae was judged recessive when plants were inoculated at the boot stage, but dominant when inoculated at flowering (Sidhu and Khush, 1978).

The conditions under which the plants are grown normally should closely mimic the environment under which the crop is produced. Stressed plants may yield unreliable results when inoculated with a weak pathogen. Even though the growth stage of the plant may be important, for the sake of convenience most researchers prefer seedlings when an accurate assesment of resistance is possible. Obviously when the pathogen only attacks the influorescence or fruits these have to be obtained to develop a practical screening methodology.

8.1.1.4. Inoculation methods

The inoculum methodology must be considered when comparing results of different experiments. This has been cited as a cause of the disparate conclusions regarding the pathogenic variability of X.

campestris pv. oryzae (Mew and Veracruz, 1979; Buddenhagen and Reddy, 1972).

The leaf clip method has been used for inoculating many pathogens such as X. campestris pv. oryzae (Kauffman et. al, 1973) and X. campestris pv. manihotis (CIAT, 1975); seed infiltration and tissue puncture are commonly used for screening for resistance to P. phaseolicola (Frazier, 1970); carborundum mixture with the inoculum has been commonly used to increase inoculum efficiency such as in cucurbits inoculated with P. syringae pv. lachrymans, causal agent of bacterial angular leaf spots (Sitterly, 1973).

The above methods are very suitable for large-scale screening; however pressure or syringe injections may be useful for small scale-screening. Atomizing inoculum over the tissue may be appropriate for the bacteria that infect directly through the stomata and when injury is not required for infection as is the case with P. syringae pv. syringae on rice (Zeigler et. al, 1987) and P. glycinea on soybeans (Jones and Hartwig, 1959). Mild injury can be obtained by inoculating with a cotton swab or dusting the plant with carborundum. However, care should be taken to avoid confusing necrosis due to injury from the inoculation method with symptoms caused by the pathogen. When field conditions permit, and rapid plant to plant infection is possible, uniform disease pressure can be obtained by planting spreader rows of a susceptible cultivar. These are usually planted prior to the test material and may be artificially inoculated to initiate the epidemic (Lozano and Laberrry, 1982). Planting of the test material is usually delayed until symptoms are clear in the spreaders.

Other points related to this Chapter are discussed in Chapter I.5 by Klement in this book.

8.1.1.5. Incubation

For most bacteria high relative humidity after inoculation favors disease development. Under controlled conditions this is easily obtained; however, under field conditions only through judicious site selection (favorable microenvironment) and planting time can success be assured. Since there are bacterial disease reactions sensitive to temperature changes and extremes (Takatsu, 1979; Chamberlain, 1962; Miyajima, 1983) this factor must be considered during the incubation periods.

For bacteria that penetrate directly via stomatas or hydathodes, pretreatment of plants under saturated humidity will open these structures and facilitate infection (Schaad, 1980).

8.1.1.6. Disease rating

The rating is the crucial point on the identification of resistance to any pathogens. This is particularly true for many bacterial pathogens which unlike many fungi and viruses do not elicite absolute resistance responses. The difficulties in quantifying and analyzing disease resistance has led to considerable confusion and controversy over the existence, distribution and the importance of pathogenic races of bacterial pathogens. Two important examples of these are X. campestris pv. malvacearum on cotton (El-Zik and Bird, 1970; Inn, 1965; Bird, 1973) and X. campestris pv. oryzae on rice (Mew and Veracruz, 1979; Buddenhagen and Reddy, 1972), where some researchers report discrete "races" and others describe only continuously varying virulence.

The rating method used must accurately reflect differences in resistance among plants. Consequently, specific methodology must be developed for each pathosystem and method utilized. No evaluation

scheme will be of practical use without the inclusion of appropriate checks. These checks should include highly susceptible plants of a local commercial cultivar, and the highest known resistance available.

In case of foliar pathogens in which a specific wound was made, the length of lesion development from the point of inoculation after a given time is often measured to quantitatively compare individuals or lines (see next Chapter on bacterial blight of rice by Mew et al.). Where inoculum was sprayed on the plant the percentage of leaf area affected or number of lesions per leaf area is usually measured (Chand and Walker, 1964). The kind of lesion produced (e.g. water-soaked) and the presence of bacterial exudate and/or streaming can be used to assess the level of susceptibility (CIAT, 1975). A large halo can indicate susceptibility to a toxin and may be more significant than the size of the actual lesion (Frazier, 1970). Under field conditions overall plant vigor should be considered and compared with the check varieties.

The time of evaluations may be critical particularly under a field situation and should coincide with maximum disease expression to permit the separation of levels of resistance. The evaluation timing can be determined from the checks or the physiological development stage of the plant.

8.1.1.7. Conclusions

There are numerous examples of resistance to bacterial pathogens successfully incorporated into commercial crops. The following are some few examples which bibliography cited can add more information related to this topic: P. syringae, P. andropogonis and Corynebacteria nebraskense (Shurtleff, 1980); C. michiganense (Boelema, 1980); X. campestris pv. manihotis (Lozano, 1986); X. campestris pv. phaseoli, (Schuster et al., 1983a; Schuster et al., 1983b); P. phaseolicola

(Innes et al., 1984; Webster et al., 1983); X. campestris pv. phaseoli var. soyensis, P. glycinea (Dunleavy, 1973); X. campestris pv. vignicola (Gitaitis, 1983); X. campestris pv. vesicatoria (Dahlbeck, 1979; Scott and Jones, 1986); P. lachrymans (Sitterly, 1973; Chand and Walker, 1964); X. campestris pv. malvacearum (Brinkerhoff et al., 1984; Bird, 1973); X. campestris pv. oryzae (Mew, 1987). Additionally, a specific methodological case is presented in the next chapter by Mew et al.

Since varietal resistance offers the most promising method of bacterial disease control, both inoculation and evaluation methodologies are critical for a successful resistance screening program. Sustainable and long-term advances can be achieved only through careful and precise development of techniques specifically designed for the pathosystem, the crop, the environment, and the economic and logistic facilities available to the researcher. However, it should not be forgotten that sustainable control of a plant disease will be obtained by managing the disease, through the use of all effective control measures (cultural, biological, chemical, etc.) in addition to varietal resistance as has been demonstrated in Cassava bacterial blight (Lozano, 1986).

8.1.1.8. Literature cited

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